# High Incidence of Transmission and Occurrence of a Viroid in Commercial Seeds of *Coleus* in Canada

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**ABSTRACT**

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A survey of 16 ornamental plants indicated widespread infection of *Coleus scutellarioides* with a viroid similar to that reported in *Coleus* from Brazil and of goldfish (*Hypocyrta mummularia*) plants with a new viroid. Because *Coleus* plants in Canada originated from seeds obtained from Japan, the United States, and European countries, attempts were made to determine if the viroid was transmitted through the *Coleus* seed. The viroid from *Coleus* was transmissible to *Ocimum sanctum* plants by mechanical and graft inoculation but not through the seeds. Seeds produced by infected *Coleus* plants tested by return-polyacrylamide gel electrophoresis (R-PAGE) contained the viroid, however. The viroid was detected in dormant (about 325 μg) as well as in sprouted single seeds. Transmission rates were 71.4% in sprouted seed and 67.3% in seedlings. The viroid was present in the flower parts and endosperm of *Coleus* but not in the seed coat. The viroid could have been introduced into Canada by infected seeds imported from the United States, seeds that probably were produced in Costa Rica. Infection rates in seed lots from four commercial sources of *Coleus* ranged from 16 to 68%. The *Coleus* viroid was not transmissible to potato (*Solanum tuberosum*) or tomato (*Lycopersicon esculentum*) plants by mechanical or grafting inoculation.

Additional keywords: seed germination, seed parts, symptomless carrier, viroid spread

The potato spindle tuber viroid (PSTVd) (9) naturally infects only potato plants. Experimentally, however, potato plants are susceptible to several viroids (1,4,8,9,14). A viroid that is carried symptomlessly in the ornamental plant *Columnea erythrophaea* Decne ex Houll. has been shown to cause symptoms in potatoes similar to those incited by PSTVd (1). Because PSTVd has been eradicated from seed potato crops of New Brunswick (15) and Prince Edward Island (16) in Canada, it is essential to determine if ornamental plants grown and multiplied at establishments dealing with disease-free potato seed production are free from viroidlike pathogens. If the plants do carry viroids, can the viroids infect potato plants?

Recently, a viroid present in most of the plants of a cultivar of *Coleus (C. scutellarioides* (L.) Benth.) (5) was found in Brazil (3). Since *Coleus* is a common ornamental plant in Canada and the tenth most important bedding ornamental plant in the United States (17), we examined *Coleus* plants for viroid infection. A viroid identical in its mobility on polyacrylamide gel to that of the Brazilian isolate was discovered from four of the five cultivars tested. Although *Coleus* plants were multiplied for a year by vegetative propagation, initially most were grown from seeds obtained from the United States. Our objectives in this study were to obtain some information about its transmission and origin in Canada and to determine if the *Coleus* viroid would infect potato plants.

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## MATERIALS AND METHODS

**Collection of plant samples.** Leaves of 16 ornamental plant species grown in a greenhouse at the Fredericton Research Station were collected. Plants were either raised from seed or multiplied by cuttings. All plants were of flowering age and growing vigorously. About 1 g of leaves collected from each plant was used for nucleic acid extraction. Two samples were collected over a period of 1 yr.

**Nucleic acid extraction and viroid detection.** Nucleic acids were extracted from leaves as described for PSTVd (11,12). Briefly, tissues were homogenized in extracting buffer containing phenol. The aqueous layer was separated by centrifugation, and nucleic acids were precipitated from the aqueous layer with 2.5 vol of ethanol. The nucleic acid precipitate was dissolved in STE buffer (0.1 M sodium chloride, 0.05 M tris-HCl, 0.001 M ethylenediaminetetraacetate [EDTA], pH 7.2) containing 35% ethanol and subjected to CF-11 chromatography (6) to remove pigmented material. The nucleic acid was recovered by ethanol precipitation and dissolved in a high salt buffer (89 mM Tris, 89 mM boric acid, 2.5 mM EDTA, pH 8.3) and used for return-polyacrylamide gel electrophoresis (R-PAGE) (11,12). A 6-μl nucleic acid sample containing the dyes bromophenol blue and xylene cyanol and 40% glycerol was applied on each slot of a slab gel (16 × 14 × 0.15 cm). The first electrophoresis was done for 1.75–2.5 hr at 46 mA at 25 C. The buffer was then replaced with boiling low-salt buffer (1:8 dilution of high-salt buffer), and electrophoresis was done in reverse direction for another 1.5 hr at the same current but at 70 C.

**Transmission tests.** Extracts of nucleic acids or crude sap containing buffer (0.05 M glycine + 0.03 M phosphate, pH 9.2) were inoculated mechanically to seedlings of viroid-free *Coleus* or other plant species. The inoculation was done by rubbing the inoculum with a glass rod on leaves dusted with Carborundum (350 grit) or placing a drop of inoculum on the petiole and puncturing through the inoculum 10–15 times with a 18-gauge needle. Graft transmission was performed by grafting diseased *Coleus* scions on healthy stocks of potato (*Solanum tuberosum* L. 'Kennebec', 'Katahdin', and 'Russet Burbank'), tomato (*Lycopersicon esculentum* Mill. 'Sheyenne'), *Scopolia sinensis* Hemsl., and *Ocimum sanctum* L. plants. Plants were maintained for 2–3 mo, then retested for viroidlike bands by R-PAGE.

Seeds collected from infected *Coleus* and *Ocimum* plants were tested for viroid infection by R-PAGE (11). The usefulness of R-PAGE to detect viroid from a minimal number of *Coleus* and *Ocimum* seeds was tested by using 1, 3, 5, and 10 seeds per sample (12). Later, individual dormant or sprouted seeds or seedlings of *Coleus* were assayed for viroid. No CF-11 column chromatography was done with nucleic acids from the seeds.

**Nuclease sensitivity.** The nature of *Coleus* nucleic acids migrating in a viroid zone was determined by treatment with pancreatic ribonuclease A (100 μl, 50 mM sodium acetate buffer, pH 5.0, containing 10 μg/ml of enzyme, incubated 1 hr at 20 C) and with deoxyribonuclease (DNase) (100 μl of 150 mM NaCl, 15 mM sodium citrate, 30 mM $MgCl_2$, pH 7.0 containing 2 μl of enzyme, incubated for 10 min at 37 C). As a control, PSTVd RNA was treated identically. The content of the enzyme and

nucleic acid mixture was placed on gels and analyzed by R-PAGE.

**Detection of viroid in commercial seed lots.** Seeds of 47 cultivars or cultivar mixtures of *Coleus* were tested for the presence of viroid by R-PAGE. Three samples of 10 seeds each were used for nucleic acid extraction and the R-PAGE test as described earlier (12) for each cultivar.

## RESULTS

In a limited survey of 16 ornamental genera, two to 11 plants of each genus were sampled twice and tested for the presence of viroid. The genera tested were *Alternifolia (Cyperus), Browallia, Cactus, Calceolaria, Coleus, Crossandra, Freesia, Gloxinia, Hypocyrta, Maranta, Nephrolepis, Primula, Saintpaulia, Streptocarpus*, succulents (*Cheiridopsis*), and *Thunbergia*. Only *Coleus* and *Hypocyrta* plants consistently showed the presence of viroidlike bands on R-PAGE (Fig. 1). Of the five *Coleus* cultivars—Green, Pink, Red, Scarlet, and Velvet—only the Green plants did not contain viroid bands. Only *Coleus* viroid (CVd) was studied in detail for seed transmission.

**Transmission tests.** CVd was transmitted by mechanical and graft inoculation to 40 seedlings of *Coleus* cv. Scarlet Dragon. Viroid was detected 3 wk postinoculation, but the concentration was low. High concentrations of CVd were observed from 5 wk postinoculation onward.

CVd was also transmitted by grafting to one of the eight plants of *O. sanctum* and by mechanical inoculation to seven of the 24 plants of *O. sanctum* seedlings. In graft-inoculated plants, CVd was present in two of the six shoots on the plant, indicating uneven distribution of the viroid. Similarly, inoculation of young seedlings did not result in infected plants at 3 wk postinoculation but only after 8–10 wk postinoculation. No symptoms were discernible on infected plants.

CVd was not transmissible to 22 plants of potato cvs. Kennebec, Katahdin, and Russet Burbank and to seven plants each of tomato and *S. sinensis* by graft or mechanical inoculations.

**Viroid nature.** CVd consisted of RNA because it was hydrolyzed by RNase treatment but not by DNase. It migrated faster than PSTVd and the viroidlike RNA from goldfish (*Hypocyrta mummularia* Hanst.) plants (Fig. 1).

**Distribution of viroid in *Coleus* cultivars.** Of the four coleus cultivars tested, CVd was detected consistently in Pink, Scarlet, and Velvet but irregularly in Green of the Dragon series (Table 1). A similar pattern was observed in various flower parts, shoots, and branches of different *Coleus* plants (Table 1). Seeds from Velvet, Scarlet, and Pink contained viroid (Table 1).

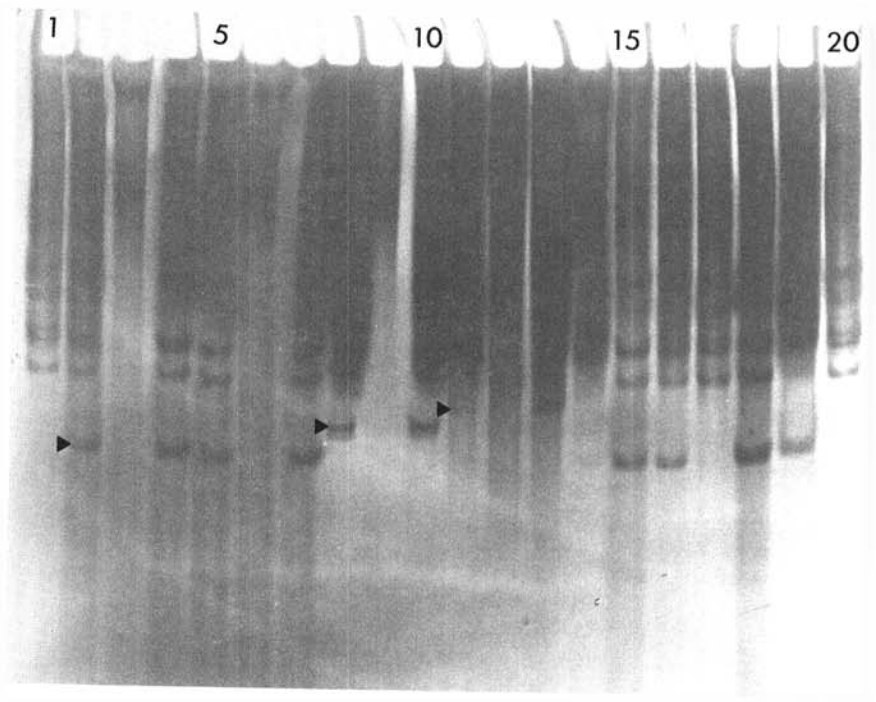


**Fig. 1.** Return-polyacrylamide gel electrophoresis of viroids with or without enzyme incubation: lanes 1 and 20, nucleic acids from healthy *Coleus*; lanes 2–4, *Coleus* viroid from cv. Velvet without enzymes, with RNase, and with DNase, respectively; lanes 5–7, same as lanes 2–4 but with *Coleus* viroid from cv. Scarlet; lanes 8–10, same as lanes 2–4 but with viroid from goldfish (*Hypocyrta mummularia*) plants; lanes 11–13, sames as lanes 2–4 but with PSTVd-MF; lane 14, *Coleus* cv. Velvet; lane 15, *Coleus* cv. Scarlet; lane 16, *Coleus* cv. Pink; lane 17, *Coleus* cv. Green; lane 18, *Coleus* viroid from Brazil; and lane 19, *Coleus* viroid from *Ocimum sanctum* plants. First electrophoresis was on 5% gels under nondenaturing conditions and second was under denaturing conditions at 46 mA for 1.75 and 1.5 hr, respectively; arrows indicate viroid bands.

**Table 1.** Distribution of viroid in vegetative plant, flower parts, and seeds of four cultivars of *Coleus*, Dragon series

| Plant and flower parts | Cultivar | | | |
|---|---|---|---|---|
| | Velvet | Scarlet | Pink | Green |
| Shoots | 2/2[a] | 4/4 | 2/2 | 0/1 |
| Branches | 7/7 | 5/5 | 12/15 | Nt[b] |
| Sepals | 1/2 | 2/2 | 2/2 | 0/2 |
| Petals | 1/2 | 2/2 | 2/2 | 0/2 |
| Anthers | 1/2 | 2/2 | 2/2 | 0/2 |
| Pistils | 1/2 | 2/2 | 2/2 | 0/2 |
| Dormant seed | 3/5[c] | 2/5 | 4/5 | 0/5 |

[a]Number of samples positive/number of samples tested by R-PAGE.
[b]Nt = not tested.
[c]Ten seeds constituted one sample for nucleic acid extraction.

**Table 2.** Detection of viroid from seeds of *Coleus* and *Ocimum sanctum*

| Treatment | No. of seeds per sample | *Coleus* cultivar | | *O. sanctum* |
|---|---|---|---|---|
| | | Pink | Green | |
| Dormant seeds | 1 | 1/3[a] | Nt[b] | 0/3 |
| | 2 | 0/3 | Nt | 0/3 |
| | 3 | 4/6 | 0/3 | 0/3 |
| | 4 | 3/3 | Nt | 0/3 |
| | 5 | 5/6 | 0/3 | 0/3 |
| | 10 | 3/3 | 0/3 | 0/3 |
| Seed coat | 5 | 0/9 | Nt | Nt |
| Endosperm + embryo | 5 | 8/9 | Nt | Nt |
| Germinated seeds | 1 | 30/42 | Nt | Nt |
| Seedlings | 1 | 66/98 | Nt | Nt |

[a]Number of replicates positive/number of replicates tested by R-PAGE.
[b]Nt = not tested.

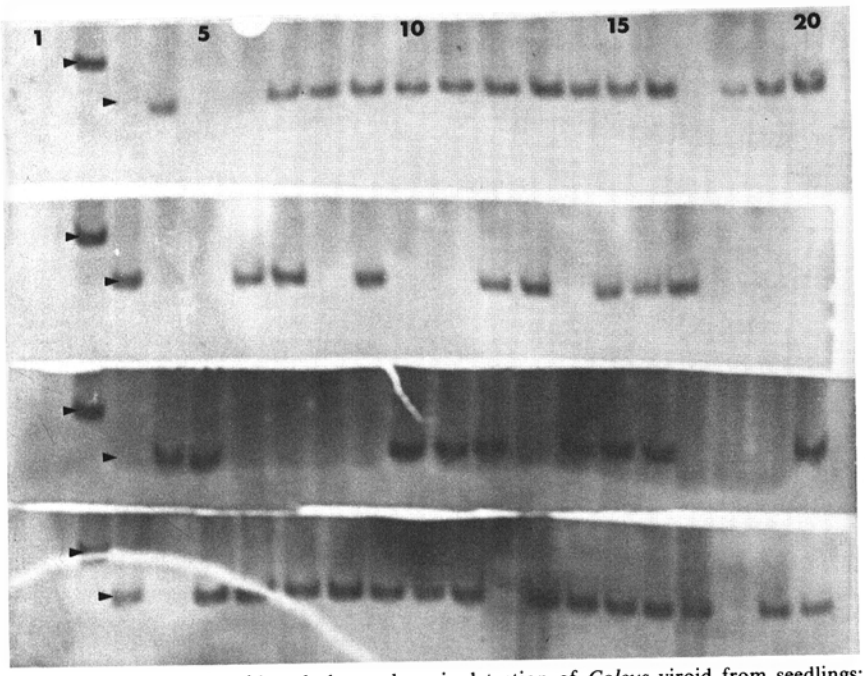


**Fig. 2.** Return-polyacrylamide gel electrophoresis detection of *Coleus* viroid from seedlings: lane 1, nucleic acids from healthy potato; lane 2, PSTVd; and lanes 3–20, nucleic acids from individual *Coleus* seedlings grown from seeds obtained from infected *Coleus* cv. Pink. Only the lower portions of four electrophoretograms are shown; top arrow in each portion indicates the viroid band of PSTVd and bottom arrow indicates the viroid band of *Coleus*.

**Table 3.** Distribution of viroid in commercial seeds of *Coleus* from four seed suppliers

| *Coleus* cultivars | Seed suppliers[a] 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Rainbow series | | | | |
| Bronze | 3/3[b] | ... | ... | ... |
| Multicolor | 1/3 | 0/3 | ... | 0/3 |
| Red | 1/3 | ... | ... | ... |
| Rose | 0/6 | ... | ... | ... |
| Scarlet | 5/5 | ... | ... | ... |
| Velvet | 0/3 | ... | ... | ... |
| Rainbow mix | 3/5 | ... | 0/3 (Netherlands) | ... |
| Rainbow Fringed mix | 1/3 | ... | ... | ... |
| Ball Rainbow mix | ... | 0/3 | ... | ... |
| Wizard series | | | | |
| Golden | 0/3 | 3/3 | ... | ... |
| Jade | 6/6 | ... | ... | ... |
| Pastel | 2/3 | 2/3 | ... | 0/3 |
| Pineapple | 8/8 | ... | ... | ... |
| Pink | 0/3 | ... | ... | ... |
| Red | 2/3 | ... | ... | ... |
| Rose | 5/5 | ... | ... | ... |
| Scarlet | 1/6 | ... | ... | ... |
| Sunset | 2/3 | ... | ... | ... |
| Velvet | 6/6 | ... | ... | ... |
| Wizard mix | ... | 2/3 | 2/3 (USA) | ... |
| Others | | | | |
| Color Pride | 0/3 | 0/3 | ... | ... |
| Fashion Parade mix | 0/3 | ... | ... | ... |
| Festive Dance | 0/3 | ... | ... | ... |
| Carefree mix | 3/6 | 0/3 | ... | 0/3 |
| Fiji mix | 2/3 | 2/3 | ... | ... |
| Red Poncho | 1/3 | 0/3 | ... | ... |
| Scarlet Poncho | ... | ... | ... | 0/3 |
| Volcano | 0/3 | ... | ... | ... |
| Saber mix | ... | 0/3 | 2/3 (USA) | 1/3 |
| Old Lace Fringed mix | ... | ... | 0/3 (France) | ... |
| Seven Dwarf mix | ... | ... | 0/3 (Japan) | ... |
| Hybrid *Coleus*—Bellevue blend | ... | ... | 0/3 (Denmark) | ... |
| Fairway Ruby | ... | ... | ... | 0/3 |

[a] 1 = Jack Van Klaveren Ltd., 2 = Ball Superior Ltd., 3 = Stokes Seed Ltd., 4 = F. C. Gloeckner & Company Inc.

[b] Number of samples positive/number of samples tested. Three samples were tested from each cultivar; when sample number is more than three, the cultivar originated from more than one source.

**Transmission through *Coleus* seed.** When nucleic acid extracts of 1, 2, 3, 4, 5, and 10 seeds of infected *Coleus* and *O. sanctum* plants were analyzed by R-PAGE, CVd was detected frequently from groups of three to 10 seeds of *Coleus* cv. Pink but not in the cv. Green or in *O. sanctum* (Table 2). In one case, CVd was detected from a single seed from cv. Pink (Table 2). Use of a germinated individual seed for CVd detection improved the diagnosis in cv. Pink, and viroid was detected in 71.4% of the seeds. This level of detection was comparable (67.3%)) to CVd detection from 8-wk-old seedlings (Table 2) of the same cultivar.

When single germinated seeds were used for R-PAGE analysis, the viroid bands were visible on electrophoretograms illuminated from underneath but were too weak to be photographed. However, the viroid bands from 8-wk-old individual seedlings were very distinct (Fig. 2). In a typical test of seed of cv. Pink, 47 of 72 seedlings contained CVd (Fig. 2).

CVd was apparently present inside the *Coleus* seed because the seed coats from 45 seeds, tested in groups of five, did not contain viroid, whereas the endosperm plus embryo of the same seeds, also tested in groups of five, contained viroid in eight of nine samples (Table 2).

**Effect of viroid on *Coleus* seed germination and plant growth.** In one germination test of *Coleus* seed from healthy and viroid-infected lots planted in a greenhouse soil mix, 10 and 75% germination, respectively, was observed. In subsequent tests in which seeds were incubated in petri dishes, the germination rate was 65.5 and 87.3% for healthy and viroid-infected lots, respectively. Seeds from viroid-infected lots began sprouting 3 days earlier than those from healthy lots.

Presence of the viroid had no effect on the number of leaves, size of leaves, and height of plants in 33 healthy and 65 viroid-infected seedlings examined for 4 mo. There were no diagnostic symptoms related to viroid infection.

**Survey of commercial sources for viroid.** Coleus seed distributed by four companies was screened for the presence of viroidlike RNA by R-PAGE. Thirty seeds of each cultivar (three samples of 10 seed each) were used for nucleic acid extraction and R-PAGE. The presence of viroid was demonstrated in seed lots from each distributor. Cultivars belonging to Carefree, Fiji, Poncho, Rainbow, Saber, and Wizard series contained viroid infection (Table 3). Seed lots originating from Denmark, France, Japan, and the Netherlands did not contain viroid (Table 3).

## DISCUSSION

No registries or *Coleus* grower associations publish cultivar lists. Seed com-

pany catalogues appear to be the only source of such information (5). Commercial cultivars are characterized on the basis of color, leaf shape, and branching and flowering habits, and they vary widely because of genetic and environmental factors. Therefore, recognizing symptoms of viroid infection was not simple, but none were observed. However, presence and transmission of a viroidlike RNA from infected *Coleus* to uninfected seedlings of *Coleus*, as well as to *O. sanctum* plants, confirms the infectious nature of CVd.

The high rate of viroid transmission through seeds of certain cultivars of *Coleus* is similar to that observed for PSTVd (2,13) and avocado sunblotch viroid (ASBVd) (18). CVd is well adapted to *Coleus* plants because its presence did not impair seed germination or subsequent growth of the plants in certain cultivars. In other *Coleus* cultivars or hosts, however, CVd replicates unevenly (Table 1). In this respect, it is also similar to ASBVd (7).

CVd did not infect potato, tomato, or *S. sinensis* plants that are common to several PSTVd-group viroids (9,10,14), so there is no danger of its spread to potatoes. However, the high rate of seed transmission and high proportion of commercial *Coleus* seed infected with the viroid are of concern, particularly when propagation is made vegetatively, which would result in 100% infection of cuttings obtained from infected seedlings. This could have been the case in Brazil, where no healthy plants of a cultivar were found (2).

*Coleus* seeds are produced in Costa Rica and supplied to various seed companies in North America (F. Kwong, *personal communication*). Therefore, testing of parent plants at the source could reduce the extent of viroid infection, provided viroid-free plants are available. Detection of viroid in individual *Coleus* seed, weighing about 325 μg, indicates that the viroid must constitute a major portion of seed nucleic acids. The viroid bands from seed can be detected by R-PAGE without the additional step of CF-11 column chromatography, thus making seed testing a simple operation.

The faster electrophoretic migration of CVd than that of other viroids (2) as well as the identical migration of Canadian isolates and Brazilian isolates (Fig. 1) indicate similarity of size. Because the seedborne nature of CVd of Canadian isolates compared favorably with the occurrence of the viroid in commercial seed supplies, which may have come from Costa Rica (F. Kwong, *personal communication*), they could be of common origin and possibly were introduced in North American gardens through the seed produced in Central America. This would be in accordance with the nature of viroids, which are warm-climate pathogens (9,10).


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