

Dalmatian Toadflax (*Linaria dalmatica*): New Host for Cucumber Mosaic Virus

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To date, there have been no reports of Dalmatian toadflax serving as a host for cucumber mosaic virus (CMV). Infestations of Dalmatian toadflax may serve as a reservoir of CMV, thereby facilitating aphid transmission of CMV to both agricultural crops and native plants. The goal of this study was to determine whether Dalmatian toadflax is a host for CMV. Dalmatian toadflax seedlings were randomly assigned to two treatments (18 replicates/treatment): no inoculation (control) and inoculation with CMV (Fast New York strain). The Dalmatian toadflax seedlings were inoculated by standard mechanical methods and tested for the presence of CMV using enzyme-linked immunosorbent assay (ELISA). Ten of the 18 CMV-inoculated toadflax plants tested positive for the virus; 6 of the 18 displayed systemic mosaic chlorosis and leaf curling. All control plants tested negative. Transmission electron microscopy obtained from CMV-positive plants confirmed the presence of CMV based on physical properties. To verify CMV infestation, tobacco plants were assigned to the following treatments (six replicates/treatment): no inoculation (control), CMV-negative (control) inoculation, and a CMV-positive inoculation. Plants were inoculated by standard methods. Five of the 6 tobacco plants treated with the CMV-positive inoculum tested positive for CMV using ELISA. All control plants tested negative for the virus.

Nomenclature: Dalmatian toadflax, *Linaria dalmatica* (L.) Mill. LINDA; tobacco, *Nicotiana tabacum* L.; cucumber mosaic virus.

Key words: Invasive species, risk assessment, CMV Fast New York strain, Agdia Immunostrip.

Dalmatian and yellow (*Linaria vulgaris* Mill.) toadflax (Scrophulariaceae) are short-lived perennial herbs of Mediterranean origin. Both species were first imported to North America as ornamentals but have since become established through multiple introductions (Vujnovic and Wein 1997). Dalmatian and yellow toadflax displace desirable plant species in rangeland and forest habitats, diminishing grass and forb diversity in those areas. In addition to diminishing floral diversity, toadflax is unpalatable and often avoided by cattle; consequently infestations can reduce available grazing areas.

Because of their competitive ability in disturbed areas and their lack of utility, both species of toadflax are considered serious weeds in North America. Yellow toadflax is classified as a noxious weed or weed seed in nine U.S. states and four Canadian provinces. Dalmatian toadflax is classified as a noxious weed or weed seed in 11 U.S. states and three Canadian provinces (Univ. Montana 2001). Determining whether Dalmatian toadflax serves as a host to cucumber mosaic virus (CMV) and its potential as a virus reservoir for infection of agricultural and native plants would allow for a more complete understanding of the types of environmental and economic risks posed by this invasive weed.

Two of the present authors (R.K.D.P. and S.E.S.) have been conducting comprehensive environmental risk assessments for Dalmatian and yellow toadflax and their associated management strategies. To accomplish these determinations, the risk assessment paradigm has been used. Risk assessment is a formalized basis for the objective evaluation of risk in a manner in which assumptions and uncertainties are

considered and clearly presented (NRC 1983). Risk assessment represents three major phases: problem formulation, data analysis (exposure and effects assessment), and risk characterization (USOSTP 1999). As part of the risk assessment process, effects caused by toadflax plants as potential impacts on ecological receptors have been determined. The effects are competitive displacement of other plants, reservoirs of plant disease, livestock and wildlife use, livestock toxicity, human toxicity, erosion, and wildfire.

CMV, which is transmitted primarily by aphids, can be a serious plant pest and can infect a broad range of vegetable and ornamental hosts (Rist and Lorbeer 1989). Examples of vegetable hosts for CMV include cucumber (*Cucumis sativus* L.), tomato (*Lycopersicon esculentum* L.), spinach (*Spinacia oleracea* L.), celery (*Apium graveolens* L.), pepper (*Capsicum annuum* L.), beet (*Beta vulgaris* L.), lettuce (*Lactuca sativa* L.), turnip (*Brassica campestris* L.), watermelon (*Citrullis lanatus* Thunb.), pumpkin (*Cucurbita maxima* Duch.), broad bean (*Vicia faba* L.), onion (*Allium cepa* L.), potato (*Solanum tuberosum* L.), carrot (*Daucus carota* L.), dill (*Anthem graveolens* L.), and parsley (*Petroselinum crispum* Mill.). Examples of ornamental hosts for CMV include delphinium (*Delphinium* × *Elatum*), snapdragon (*Antirrhinum majus* L.), and zinnia (*Zinnia elegans* Jacq.) (Francki et al. 1979).

Yellow toadflax serves as an overwintering site for CMV and broad bean wilt virus (BBWV); however, the potential for Dalmatian toadflax to serve as a reservoir for plant disease, particularly CMV, has not been identified (Rist and Lorbeer 1989). As part of our risk assessment process, an uncertainty analysis revealed several effects and exposure factors with high uncertainties. For example, there is high uncertainty associated with the ability of Dalmatian toadflax to be a host for viruses such as CMV. Therefore, the objective of this study was to determine whether CMV can infect Dalmatian toadflax.

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Materials and Methods

All plants were grown in a greenhouse or growth room at the Plant Growth Center (Montana State University [MSU], Bozeman, MT) and planted in 50 : 50 MSU : Sunshine #1¹ soil mix and fertilized with Scott's Peter Professional Peat-Lite Special 20–20–20.² The MSU soil mix consisted of equal parts of silt loam soil, washed concrete sand, and Canadian Sphagnum peat moss in addition to AquaGro 2000 G wetting agent³ blended in at 593 g/m³ of soil mix. The MSU soil mix was aerated and steam-pasteurized to 80 C for 45 min. The Sunshine Mix #1 consisted of Canadian Sphagnum Peat Moss, perlite, vermiculite, starter nutrient charge, wetting agent, and Dolomitic lime.

In a greenhouse maintained at 21 C ± 2 C with a photoperiod of 4 : 4 : 4 : 12 h (light : dark : light : dark), Dalmatian toadflax seeds were planted in a Lee Valley Self-Watering Seed Starter⁴ with premoistened 50 : 50 MSU : Sunshine soil mix. The seed starter was placed within a 61 by 61 by 61 cm Dacron Chiffon collapsible cage⁵ until seedlings were approximately 5 cm tall. Sixty-one seedlings were transplanted to 18.5-cm-diam pots in premoistened 50 : 50 MSU : Sunshine soil mix and watered biweekly and fertilized weekly. Plants were randomly assigned to two treatments (18 replicates/treatment): no inoculation with CMV (control) or inoculation with CMV cv. Fast New York. The plants were placed in a growth chamber maintained at 21 C ± 2 C with a photoperiod of 14 : 10 h (light : dark).

Using standard methods, an inoculation buffer was prepared using 50 mM of potassium phosphate buffer (pH 7.2), 10 mM of sodium sulfite, and five to six medium-sized, mottled and curled, CMV-positive tobacco leaves (cv. 'Havana 38'), which were ground into a thin pulp using a small mortar and pestle. After all plants were sprayed with carborundum, the inoculation buffer was spread along 5 to 10 leaves per plant using a Q-tip for the 18 CMV-inoculated treatment plants.

Four weeks after inoculation, plants were tested for the presence of CMV using an enzyme-linked immunosorbent assay (ELISA).⁶ Leaf areas of approximately 3 to 5 cm² were taken from young plant tissue showing symptoms and placed between the mesh linings in the extract bags filled with 3 ml of sample extract buffer. The extract bags were rubbed until all leaf tissue was ground, and an immunostrip was placed into each extract bag no higher than the fill line. Within 3 to 5 min, a control line appeared to ensure the test was valid. If the test was positive, a test line appeared below the control line; if the test was negative, only the control line appeared.

Confirmation of the ELISA with transmission electron microscope (TEM)⁷ was conducted using negatively stained, ground leaf tissue. A mottled, curled leaf of an infected Dalmatian toadflax plant was ground into a thin pulp using distilled water in a mortar and pestle. A 5- μ l drop of the thin pulp was applied to a Formvar grid (300 mesh copper) and negatively stained with a drop of 2% uranyl acetate in distilled water. The sample was analyzed under the TEM using Soft Imaging System software⁸ to identify icosahedral cucumber mosaic viruses approximately 25 to 30 nm in size.

Because tobacco (Havana 38) is a convenient host for replicating CMV and confirming infection through plant symptoms, tobacco seeds were planted in a greenhouse set at 21 C ± 2 C with a photoperiod of 14 : 10 h (light : dark). Eight weeks after planting, plants were randomly assigned to the following treatments (six replicates/treatment): no inoculation (control), CMV-negative inoculation (control), and a CMV-positive inoculation. The plants were then transferred to a growth chamber maintained at 21 C ± 2 C with a photoperiod of 14 : 10 h (light : dark). Using a small mortar and pestle, 50 mM of potassium phosphate buffer (pH 7.2) and 10 mM of sodium sulfite were combined with either approximately eight young, medium-sized, mottled and curled, CMV-positive Dalmatian toadflax leaves to produce a CMV-positive inoculum or with approximately eight young, medium-sized leaves from the control Dalmatian toadflax plants to produce a CMV-negative inoculum. Using standard methods, each inoculation buffer was then applied to six carborundum-treated tobacco plants. At the appearance of characteristic CMV symptoms, all plants were tested for CMV using ELISA. Data were analyzed using the Fisher's Exact test.⁹

Results and Discussion

Visual symptoms of CMV in Dalmatian toadflax were observed in young and mature vegetative tissue. Visual symptoms included systemic chlorosis and mottling in addition to leaf curling (Figure 1) on the young vegetative tissue in the early stages of infection and on the entire plant in the later stages of infection. Symptoms were observed on the young vegetative tissue of 6 of the 18 CMV-inoculated Dalmatian toadflax plants approximately 3 wk after inoculation. Not all infected plants exhibited symptoms nor were lethal effects of CMV observed in Dalmatian toadflax. Visual symptoms for CMV in tobacco included severe mottling on both young and mature vegetative tissue and slight curling on young vegetative tissue within 2 wk after inoculation.

Nine of 18 (50%) CMV-inoculated Dalmatian toadflax plants tested positive for CMV using ELISA. Inoculated plants that initially tested negative were retested; one additional inoculated plant tested positive for CMV, resulting in 10 out of 18 (56%) of the total inoculated Dalmatian toadflax plants having a CMV-positive infection rate ($P = 2.204 \times 10^{-10}$). All control plants tested negative.

Confirmation of the ELISA was conducted by viewing ground, negatively stained, mottled leaf tissue with a TEM. Virus particles were observed with an average size (\pm SE) of 26.9 ± 2.47 nm. The particles were consistent with key visual features of CMV.

Infection by CMV in Dalmatian toadflax was verified by inoculating tobacco (Havana 38) with CMV-infected Dalmatian toadflax from the initial experiment. Results using ELISA indicated that five of the six (83%) tobacco plants treated with the CMV-positive inoculum tested positive for CMV ($P = 1.616 \times 10^{-4}$). All nontreated and CMV-negative inoculated plants tested negative for the virus.

A better understanding of the environmental and economic risks posed by invasive weeds and their management strategies



Figure 1. The Dalmatian toadflax plant on the left shows the characteristic systemic chlorosis, leaf curling, and mottling associated with cucumber mosaic virus (CMV, Fast New York strain) infection. The plant on the right was not infected with CMV.

will lead to more optimal agricultural and natural resource management decisions. Our results support that Dalmatian toadflax is a susceptible host of CMV and reduce the uncertainty regarding the ability of CMV to infect Dalmatian toadflax. A key step in the risk assessment process is effects assessment, which is the characterization of the inherent ability of the stressor to affect ecological receptors. This study represents the first step in objectively determining this risk because the ability of Dalmatian toadflax to serve as a host for CMV has been determined. Data on the frequency (i.e., exposure) under field conditions is now needed to determine the risk (i.e., the product of effect and exposure) of CMV transmission to agricultural crops or desirable plants in the presence of Dalmatian toadflax. Subsequent steps would include determining whether (1) CMV is naturally occurring in Dalmatian toadflax under field conditions, (2) Dalmatian toadflax provides an overwintering reservoir for CMV, (3) CMV can be transmitted from Dalmatian toadflax to desirable plants via aphid or mechanical transmission, and (4) the frequency and location of CMV-infested Dalmatian toadflax plants covaries with the frequency and location of susceptible, desirable plants.

Sources of Materials

¹ Sunshine #1 soil mix, Sun Gro Horticulture, Inc., Bellevue, WA.

² Scott's Peter Professional Peat-Lite Special 20–20–20, Marysville, OH.

³ AquaGro 2000 G wetting agent, Aquatrol Inc., Anaheim, CA.

⁴ Lee Valley Self-Watering Seed Starter, Lee Valley Tools, Ltd, Ogdensburg, NY.

⁵ Dacron Chiffon collapsible cage, BioQuip Products, Inc., Rancho Dominguez, CA.

⁶ ELISA, Agdia ImmunoStrip™ for CMV, Agdia, Inc., Elkhart, IN.

⁷ Leo 912AB TEM, Carl Zeiss SMT, Inc., Oberkochen, Germany.

⁸ Imaging software, Soft Imaging System, GmbH, Münster, Germany.

⁹ Statistical software, SAS Institute Inc., 2002 Version. Cary, NC.

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