A Disease of *Phlox paniculata* Caused by Alfalfa Mosaic Virus

Gordon E. Holcomb,¹ Rodrigo A. Valverde, and Dina L. Gutierrez

Department of Plant Pathology and Crop Physiology, Louisiana State University AgCenter, Baton Rouge, LA 70803-1720

Additional index words. alfamovirus, perennial phlox, ornamental plants, virus diseases

Phlox paniculata L., commonly called garden, summer or perennial phlox, is native to the eastern U.S. from New York and Georgia to Arkansas and Illinois. It is a popular flowering plant, with >100 cultivars and seed races available, and is widely cultivated in the U.S. and Europe (Griffiths, 1992). Flowers are produced in large showy panicles in colors of blue, lavender, orange, pink, purple, red, salmon and white. *P. paniculata* is susceptible to the foliage diseases powdery mildew, caused by the fungus *Erysiphe cichoracearum* DC., and leafspot caused by the fungus *Septoria phlogis* Sacc. & Speg. in southern Louisiana (Holcomb and Witcher, 2003). A trial garden containing 28 *P. paniculata* cultivars propagated from cuttings was established at the Louisiana State University Burden Center in Baton Rouge in 2000. During routine disease surveys in 2001 and 2002, virus-like disease symptoms were observed on several phlox cultivars. We report here the identification of the virus that caused this phlox disease, its partial host range and its homology with other viral strains.

The cultivar ‘Robert Poore’ showed the most severe virus-like symptoms from year to year in a trial garden and was therefore selected for this study. Healthy plants for virus transmission tests were either grown from seed in the greenhouse or purchased from local garden centers. Virus inoculum was prepared by grinding freshly collected leaves of infected ‘Robert Poore’ in a mortar in cold buffer [1:2 wt/vol, in 0.01 M sodium phosphate, plus 0.01 M sodium diethyl dithiocarbamate (DIECA), pH 7.0] with 50 mg of 600-mesh silicon carbide abrasive. Inoculum was rubbed on leaves of experimental host plants with the end of a pestle and inoculated leaves were rinsed with tap water. Noninoculated plants served as controls and all plants were maintained in a greenhouse at ambient temperatures (25 to 30 °C) for symptom development.

Double-stranded RNA was extracted from 3.5-g leaf samples from infected plants using a modification of the Morris and Dodds (1979) CF-11 cellulose column chromatography (Valverde et al., 1990). Hosts used for dsRNA extraction included pepper (*Capsicum annuum* L.) ‘Jalapeño M’, cowpea (*Vigna unguiculata* (L.) Walp. subsp. *unguiculata* (L.) Walp.)

‘Tvu 612’, and phlox ‘Robert Poore’. Purified dsRNAs were analyzed by electrophoresis in 6% polyacrylamide gels at 100 V for 3 h. Electrophoretic analysis of dsRNAs obtained from virus infected phlox, pepper and cowpea yielded a profile similar to that of *Alfalfa mosaic virus* (AMV) (Valverde et al., 1990).

ELISA tests were performed (PathoScreen Kit; Agdia Inc., Elkhart, Ind.) for AMV on samples from infected ‘Robert Poore’ phlox and all inoculated and noninoculated plants. Positive control (sap extracts from AMV infected tobacco provided by AGDIA) and negative controls (sap from healthy plants) were included in the tests. The green peach aphid, *Myzus persicae*, was used in transmission experiments as described by Dijkstra and de Jager (1998). Pepper leaves infected with AMV were used as virus source. Six healthy seedlings of ‘Jalapeño M’ pepper were used as the transmission host. The exposed seedlings

were placed in the greenhouse and the number of seedlings that developed symptoms was recorded and tested for AMV by ELISA.

Total RNA was extracted from 0.1 g of pepper leaf tissue infected with AMV with TRIzol Reagent (Life Technologies, Inc., Grand Island, N.Y.) following the procedure provided by the manufacturer. The purified RNA was resuspended in 50 µL of nuclease free water and stored at –70 °C. Reverse transcription–polymerase-chain reaction (RT–PCR) was performed using primers designed from the coat protein sequence of AMV (Martinez-Priego, et al., 2004). Synthesized primers used for the amplifications were AMV coat-F (GT GGT GGG AAA GCT GGT AAA) and AMV coat-R (CAC CCA GTG GAG GTC AGC ATT). RT-PCR was performed according to instructions of the manufacturer (Promega, Madison, Wis.). Two microliters (5.5 µM) of forward, 2 µL (6.5 µM) of reverse primer, and 15 µL of purified RNA were incubated at 70 °C for 5 min and then immediately cooled on ice for 1 min. The following reagents were added: 5 µL of reverse transcription buffer, 5 µL (10 mM) of dNTPs, 0.5 µL of RNase inhibitor, 1 µL (200 U) of reverse transcriptase, and 9 µL of water. This solution was incubated at 42 °C for 60 min. The PCR mixture was 67 µL of nuclease free water, 10 µL of 10× PCR buffer, 10 µL of 25 mM MgCl₂, 2 µL of 10 mM dNTP mixture, 1 µL of Taq DNA polymerase (2.5 U), and 10 µL of the reverse transcription sample. PCR was performed as

Fig. 1. Symptoms of severe yellow mosaic on *Phlox paniculata* ‘Robert Poore’.

Received for publication 21 Sept. 2005. Accepted for publication 1 Dec. 2005. Approved for publication by the Director of the Louisiana Agricultural Experiment Station as manuscript 05-38-0593.

¹To whom reprint requests should be addressed; e-mail gholcomb@agcenter.lsu.edu.

Fig. 2. Phylogenetic analysis of alfalfa mosaic virus (AMV) strains based on the coat protein (CP) nucleotide sequences. The unrooted tree was constructed using data obtained with Clustal W (1.81) multiple sequence alignment and generated by Phylip's Drawtree. AMV strains used included Lyh-1, Caa-1, Dac-16, and Lye-80 (France); F-430, 195-AN, and 126-A (Italy); 425 M and NY-A (U.S.); VRU, 15/64, and S (U.K.); and NZ34 (New Zealand).

described by Martínez-Priego et al. (2004): first PCR cycle at 94 °C for 2 min, 35 cycles each of 30 s at 94 °C, 30 s at 54 °C, 30 s at 72 °C, followed by a final extension at 72 °C for 10 min. PCR was performed in an Ampli-tron II thermocycler (Thermolyne, Dubuque, Iowa). The PCR products were separated by electrophoresis (1.2% agarose) and stained with ethidium bromide. Bands corresponding to the expected PCR products, for cloning and sequence analyses, were excised from agarose gels and further purified after electrophoresis using MiniElute (Qiagen Inc., Valencia, Calif.) DNA purification kit and ligated into the pGEM-T Easy vector (Promega). Recombinant plasmids were transformed into competent JM 109 *Escherichia coli* cells. The nucleotide sequences were determined at the Genomics Technology Support Facility of Michigan State University, East Lansing, using a capillary sequencer (model 3100; Perkin Elmer/Applied Biosystems, Foster City, Calif.). At least three replicate PCR-clones were sequenced to minimize error caused by Taq polymerase. Sequences were compared with others deposited in the GenBank database using the BLASTN 2.0.4 program (Altschul et al., 1998).

Foliar symptoms of varying severity, that included bright yellow mosaic, chlorotic and necrotic lines and chlorotic and necrotic ringspots, were observed on 8 of 127 *P. paniculata* plants growing at Burden Center in May 2001. Of 28 cultivars, 6 ('Darwin's Joyce', 'David', 'Eden's Crush', 'Snow White', 'Rosalinde', and 'Robert Poore') had individual plants that showed symptoms, with 'Robert Poore' showing the most severe and consistent symptoms (Fig. 1). Transmission of AMV by mechanical inoculation to healthy plants of 'David' and 'Robert Poore' was accomplished as determined by Elisha. Moderate to severe symptoms of yellow mosaic and spotting developed 9 d after inoculation. Systemic symptoms also developed on the following experimental host plants 9 d after inoculation: *Capsicum annuum* L., 'Jalapeño M' pepper, mosaic; *Catharanthus roseus* (L.) G. Don, 'Cooler Peppermint' vinca, mosaic;

Datura stramonium L., Jimson weed, mosaic; *Ocimum basilicum* L., 'Cinnamon' basil, severe yellow mosaic; *Petunia × hybrida* Hort. Vilm.-Andr., 'Rose' petunia, severe yellow mosaic and ringspots; *Torenia fournieri* Linden ex. E. Fourn., 'Clown Blue' torenia, severe yellow mosaic; *Vigna unguiculata* (L.) Walp., 'Tvu 612' cowpea, severe yellow mosaic; and *Zinnia elegans* Jacq., zinnia, mosaic.

DsRNA electrophoretic profiles obtained with extracts from infected phlox, cowpea, and pepper were similar to those reported for AMV by Valverde et al. (1990). ELISA tests were positive for the presence of AMV in 'Robert Poore' phlox showing symptoms and also in all inoculated experimental plants that showed symptoms. Noninoculated, healthy plants of each species were negative for the presence of AMV in the ELISA tests. Symptoms did not develop on the following inoculated plants: *Pisum sativum* L. (garden pea), *Diascia* sp. Link & Otto, *Cucumis sativus* L. (cucumber), *Glycine max* (L.) Merr. (soybean), and *Vigna unguiculata* subsp. *unguiculata* ('Quickpick Pinkeye' cowpea). ELISA tests for the presence of AMV in these plants were negative.

Three weeks after aphid transmission experiments, three of six 'Jalapeño M' pepper plants showed mild mosaic symptoms. Plants were tested for AMV by ELISA and all three symptomatic plants were positive while non-symptomatic plants were negative.

An about 700 bp DNA product was obtained by RT-PCR from plants showing virus symptoms. This DNA was gel purified, cloned, sequenced and submitted to the GenBank (2005) as accession number DQ124429. Analysis of the nucleotide sequences of three clones derived from three independent RT-PCR products confirmed that the sequence corresponded to the coat protein gene of AMV. Comparisons with corresponding sequences of other AMV strains in the GenBank showed various degrees of homology that ranged from 93% to 95% at the nucleotide level. The highest percentage of identity (95%) was obtained with the coat protein of AMV strains VRU and 15/64 from the U.K. and Lyh-1 from France.

The next group (94%) included strain NZ34 from New Zealand. Two AMV strains reported in the U.S., 425 Madison and NY-A, had 93% identity to AMV-phlox.

AMV is the only member of the genus *Alfavirus* in the family *Bromoviridae*. This virus is of economic importance worldwide particularly because it is transmitted by seed and aphids and has a broad host range. It is often found infecting perennial and annual crops such as alfalfa, soybean, cowpea, pepper, and tomato. Infected susceptible weeds, such as *Chenopodium album* (lambsquarters), *Phytolacca americana* (pokeweed), *Solanum nigrum* (black nightshade), and *Stellaria media* (chickweed) can serve as sources of AMV inoculum for cultivated crops (Brunt et al., 1996). Other ornamental plants, besides phlox, that have been reported as natural hosts of AMV include ajuga (Shukla and Gough, 1983), petunia, sweet pea, and zinnia (Smith, 1972). *Buddleia davidii* Franch. (butterfly bush) (Walter et al., 1985) is also a natural host of AMV and more recently Japanese peony (Bellardi and Rubies-Autonell, 2003) and lavender (Martínez-Priego et al., 2004) have been reported as natural ornamental hosts of the virus. The most common symptom caused by AMV in these plants is a yellow mosaic. AMV is known to be transmitted by at least 14 species of aphids, one of which is *Myzus persicae* (Smith, 1972). Since pepper and tomato are also grown at the Burden Center, it is possible that aphids transmitted the virus to the phlox trial planting at this location.

Nucleotide sequence comparisons of the coat protein showed that AMV-phlox is a distinct strain of AMV. Parrella et al. (2000) compared the coat protein sequences of 14 strains of AMV from the U.K., France, Italy, and the U.S. These strains were 93% to 99% identical at the nucleotide level. Phylogenetic trees showed that these strains clustered in two monophyletic groups. One cluster included Italian and U.S. strains (subgroup I) and the other French strains (subgroup II). AMV-phlox was more closely related to three members of subgroup II (AMV strains Lyh-1, VRU, and 15/64) than to members of subgroup I. According to this grouping of AMV isolates and newly available coat protein sequence information in the GenBank, AMV-phlox is more closely related to AMV strains from the U.K. and France than to U.S. and Italian strains (Fig. 2). Limited host range studies suggest that AMV-phlox has a wide host range and could be a threat to other ornamentals or crops planted nearby infected phlox. AMV has been reported previously in *Phlox paniculata* from Poland (Kaminska, 1977), Czechoslovakia (Novak and Lanzova, 1979), and Lithuania (Navalinskiene and Samuitiene, 1996). This is believed to be the first reported occurrence of AMV on phlox in the U.S.

Literature Cited

- Altschul, S.F., L.M. Thomas, A.S. Alejandro, Z. Jinghui, Z. Zheng, M. Webb, and L. David. 1998. Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucleic Acids Res.* 25:3389-3402.

- Bellardi, M.G. and D. Rubies-Autonell. 2003. First report of a disease of peony caused by *Alfalfa mosaic virus*. *Plant Dis.* 87:99.
- Brunt, A.A., K. Crabtree, M.J. Dallwitz, A.J. Gibbs, L. Watson, and E.J. Zurcher (eds.). 1996-. *Plant viruses online: Descriptions and lists from the VIDE database*. Alfalfa mosaic *alfamovirus*. 16 Jan. 1997. <http://image.fs.uidaho.edu/vidé>.
- Dijkstra, J. and C.P. de Jager. 1998. *Practical plant virology*. Springer-Verlag, Germany.
- GenBank. 2005. Accession number DQ124429. Natl. Center for Biotechnology Information. Bethesda, Md. <http://www.ncbi.nlm.nih.gov>.
- Griffiths, M. (ed.). 1992. *The Royal Horticultural Society dictionary of gardening*. vol. 3. The Stockton Press, New York.
- Holcomb, G.E. and A. Witcher. 2003. Evaluation of perennial phlox cultivars for reaction to powdery mildew and leaf spot, 2002. *Biological and cultural tests for control of plant diseases* (online.) Rpt. 18:0010. DOI.10.1094/BC18. Amer. Phytopathol. Soc., St. Paul, Minn.
- Kaminska, M. 1977. Identification of alfalfa mosaic virus infecting *Phlox paniculata* L. (in Polish). *Zesz. Probl. Postępowe Nauk Roln.* 195:165–171.
- Martínez-Priego, L.L., M.C. Córdoba, and C. Jordá. 2004. First report of *Alfalfa mosaic virus* in *Lavandula officinalis*. *Plant Dis.* 88:908.
- Morris, T.J. and J.A. Dodds. 1979. Isolation and analysis of double-stranded RNA from virus-infected plant and fungal tissue. *Phytopathology* 69:854–858.
- Navalinskiene, M. and M. Samuitiene. 1996. Viral diseases of flower plants. VII. Identification results of viruses affecting *Phlox paniculata* L. *Biologija* 1996:52–57.
- Novak, J.B. and J. Lanzova. 1979. New natural hosts of alfalfa mosaic virus (in Czech). *Ochrana Rostlin* 15:303–304.
- Parrella, G., C. Lanave, G. Marchoux, M.M. Finetti Sialer, A. Di Franco, and D. Gallitelli. 2000. Evidence for two distinct subgroups of *Alfalfa mosaic virus* (AMV) from France and Italy and their relationships with other AMV strains. *Arch. Virol.* 145:2659–2667.
- Shukla, D.D. and K.H. Gough. 1983. Tobacco streak, broad bean wilt, cucumber mosaic, and alfalfa mosaic viruses associated with ring spot of *Ajuga reptans* in Australia. *Plant Dis.* 67:221–224.
- Smith, K.M. 1972. *A textbook of plant virus diseases*. 3rd ed. Academic Press, New York.
- Walter, B., J. Kuszala, M. Ravelonandro, and L. Pinck. 1985. Alfalfa mosaic virus isolated from *Buddleia davidii* compared with other strains. *Plant Dis.* 69:266–267.
- Valverde, R.A., S.T. Nameth, and R.L. Jordan. 1990. Analysis of double-stranded RNA for plant virus diagnosis. *Plant Dis.* 74:255–258.