

Three Stable Types of Hydrangea Virescence that Differ in Severity in Foliage and Flowers

R. H. LAWSON and F. F. SMITH, Research Scientists, Agricultural Research, Science and Education Administration, U.S. Department of Agriculture, Beltsville, MD 20705

ABSTRACT

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Hydrangea virescence produces three distinct types of symptoms. Plants with the severe type were stunted and had small leaves, vein yellowing, and dwarfed green cymes. Vegetative growth was more vigorous in plants with the intermediate than in those with the severe type, and leaves expanded normally but showed vein yellowing. Cymes varied from completely green and dwarfed florets to mixed, with both pink and green florets. Plants with mild disease produced leaves that expanded normally and cymes that had large, all green florets. Shootlike structures proliferating from the pistils developed from some of the green florets on plants infected with mild disease. Each form of the disease remained stable for more than 3 yr through repeated transfer of the causal agent by budding to the same seedling clone. The severe form remained severe, the mild form remained mild, and the intermediate form failed to become severe, although infected plants gradually declined and died 1 or 2 yr after inoculation.

Additional key words: hydrangea phyllody

Green flower (virescence) in florists' hydrangea (*Hydrangea macrophylla*) has been reported in Germany (6), Belgium (7), Italy (1), and the United States (2). Symptoms differ among plants of a single cultivar. In infected Strafford hydrangea, cymes vary from completely green to mixed, with pink and green florets, with and without foliar symptoms (3).

Naturally infected Strafford plants with severe symptoms decline, and some die during the first year after flowering when observed in the greenhouse. Surviving plants stored at 4–5 C for 6 wk and subsequently forced often show even more severe symptoms.

We previously hypothesized that the variation in symptom severity was related to the age and size of the plant at the time of inoculation and also to the length of time between inoculation, dormant cold storage, and growth of plants forced after dormancy (4). Although mortality in the year of inoculation was highest in plants budded early in the season, it was also high in those budded late in the season. Death of plants budded late in the season was delayed, however, until after the

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plants were forced following dormancy and cold storage.

Here we report three types of virescence associated with bud graft inocula that produce characteristic, stable symptoms with repeated transmission.

Table 1. Symptoms in a hydrangea seedling clone budded monthly in 1977 with tissue from a virescent hydrangea

Month budded	No. of plants budded	Plants with foliar symptoms		Dead plants		Plants with floral symptoms/floral flowers	
		1977	1978	1977	1978	1977	1978
Inocula from plants with the severe form of disease							
May	5	4	4	0	4	0/0	0/0
June	5	4	4	0	4	0/0	0/0
July	5	3	3	0	3	0/0	0/2
August	5	4	4	0	4	0/0	0/1
September	5	5	5	0	4	0/0	1/5
October	5	1	3	0	3	0/0	1/3
Inocula from plants with the intermediate form of disease							
May	5	4	4	0	4	0/0	4/5
June	5	5	5	0	2	0/0	4/4
July	5	4	4	0	3	0/0	3/5
August	5	3	4	0	0	0/0	4/5
September	5	4	5	0	1	0/0	4/5
October	5	0	1	0	1	0/0	1/5
Inocula from plants with the mild form of disease							
May	5	0	0	0	0	1/1	4/5
June	5	0	0	0	0	0/1	3/5
July	5	0	0	0	0	0/0	3/5
August	5	0	0	0	0	0/0	5/5
September	5	0	0	0	0	0/0	5/5
October	5	0	0	0	0	0/0	0/0

grower. The causal agent was transmitted by grafting with chip buds that included phloem tissue to a seedling of the cross Kunnert \times Blue Lace Cap. The budded plant showed vein yellowing, stunting, small leaves, and dwarfed green cymes. Buds were later transferred from the diseased plant to the seedling clone and were maintained by periodic transfer in this clone.

The second and third sources of inoculum were obtained from Strafford

hydrangea in decline in a propagation field on the West Coast. When received at Beltsville, foliage on vegetative stems of the second inoculum source showed mild vein yellowing. This source of inoculum induced the intermediate form of the disease. In the field the third Strafford plant was only slightly reduced in vigor and had no foliar symptoms. This plant was the source of inoculum that induced the mild form of the disease.

Cuttings 7–10 cm long from clone No.

7 were propagated starting at the end of March. The cuttings were rooted in perlite under mist and potted about 1 mo after cuttings were taken. Rooted cuttings were established in soil in 15-cm pots and budded about 2 wk after potting. Cuttings were propagated on a schedule that provided rooted cuttings from May through October, and plants were budded at about the same stage of development in each test.

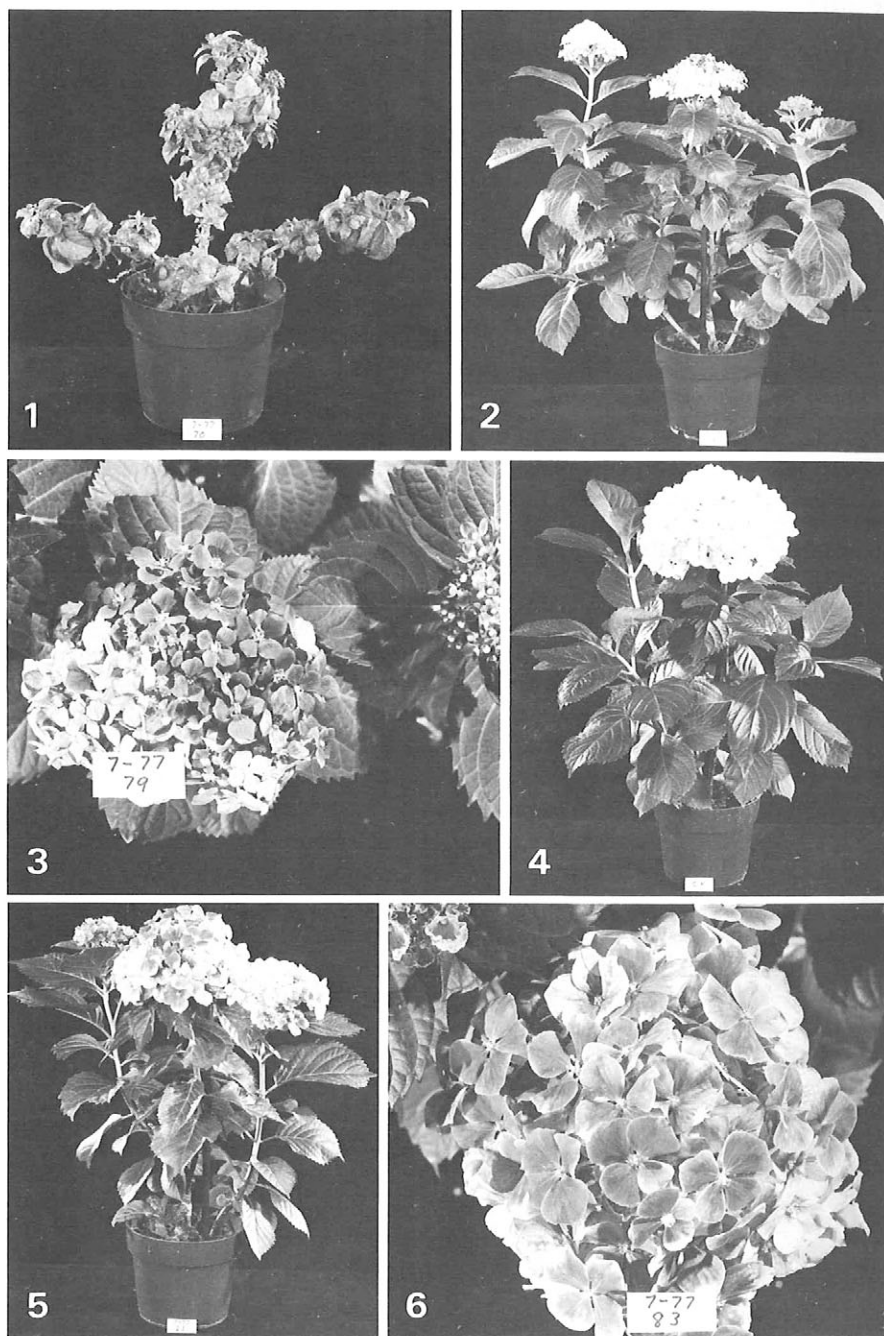
Five plants were bud-grafted monthly from May through October from each of the three inoculum sources. Buds for inoculation were taken from the leaf axils of shoots of a naturally infected Strafford hydrangea free of hydrangea ringspot virus. Foliage and flowers of budded plants and unbudded controls were observed for symptoms during the summer and fall of 1977 until the plants were stored at 4–5 C during the first week of November. The plants were returned to the greenhouse after 6 wk and forced at 22–23 C.

Plants of the No. 7 clone that were budded with the three disease sources in 1977 and showed symptoms in 1978 were used as sources of bud inoculum for healthy Strafford plants. The purpose was to determine whether symptoms in Strafford would be modified after each of the three disease types was transferred to a genetically different hydrangea stock and then back to Strafford.

RESULTS

Of the 30 plants budded from May through October 1977 with inoculum from the severe form of the disease, 21 developed foliar symptoms before the plants were stored in November. All plants that developed symptoms in 1977 also showed symptoms when forced in 1978 after storage (Table 1). In addition, two plants that were budded in October and failed to develop symptoms in 1977 before they were stored developed symptoms in 1978 when they were forced. None of the plants died in 1977 before storage, but 22 died after forcing in 1978. Only two plants survived long enough to produce flowers the second year. The most common symptoms on diseased plants were vein yellowing, small leaves, and severe stunting that intensified during the first season after inoculation and when the plants were forced the following year (Fig. 1). When floral organs developed on plants infected with the severe form of disease, only stunted, severely malformed cymes with small, green florets were produced.

Symptoms developed in 20 of the 30 plants budded with the intermediate form of the disease before plants were stored in 1977 (Table 1). None of the plants budded in October developed symptoms in 1977 and only one had symptoms in 1978; the late budding and short interval between budding and storage probably resulted in the infection of only one of the five plants.



Figs. 1–6. Seedling clone No. 7 of *Hydrangea macrophylla* with symptoms of virescence 8 mo after bud inoculation and a control plant: (1) Severe virescence with small cymes, stunted florets, and small leaves with vein yellowing. (2) Intermediate virescence with mixed pink and green cymes and expanding leaves with vein-yellowing. (3) Enlarged view of cyme on plant in Fig. 2. Dark florets are green and light florets are white to pink. (4) Control plant with large cyme and normal pink florets. (5) Mild virescence with large green florets in cymes and large leaves without symptoms. (6) Enlarged view of cyme on plant shown in Fig. 5. Compare the size of the florets with those shown in Fig. 3 (both photographs were taken at the same scale).

The disease agent was apparently not translocated out of the bud graft before the plant was transferred to storage. Because the buds did not survive the low temperature storage, the disease agent could not translocate into the budded seedling after storage. Nine of the 11 plants budded with the intermediate inoculum that died in 1978 were budded during May, June, and July (Table 1). Only two of the plants budded in August, September, and October died in 1978, although 10 plants budded during those months developed symptoms in 1978. Although leaf expansion was much greater in plants infected with the intermediate form of the disease than in plants infected with the severe form, vein yellowing was obvious in many leaves of plants with the intermediate form. Cymes were composed of florets of varying size (Figs. 2 and 3), but most were smaller than florets in a control plant (Fig. 4). Many cymes in plants inoculated from the intermediate source were mixed, with some normal pink and some green florets (Fig. 3) or with pink and green in the same floret.

Plants inoculated with buds with mild virescence had no foliar symptoms in 1977 or when forced in 1978 (Table 1). The plants produced large leaves that expanded normally, without vein yellowing. One plant budded in May 1977 and one budded in June flowered during the first season. The plant budded in May produced a cyme with large, all green florets and mixed cymes with some normal pink florets (Figs. 5 and 6). Twenty of the 25 plants budded in 1977 flowered in 1978. Again, no symptoms were observed in the leaves of any plants with green flowers. Florets in many cymes were larger than those in cymes of normal control flowers or green florets in cymes of plants infected with the intermediate type of virescence. Leafy, shootlike structures proliferated from the pistils of the large green florets in cymes of plants infected with the mild virescent disease.

Unbudded control plants were main-

tained with each group of budded plants of the same age from May through October 1977. All control plants produced normal pink cymes without evidence of abnormalities and produced normal leaves when forced in 1978.

Strafford plants budded in 1978 from the No. 7 clone infected with the mild, intermediate, or severe form of the disease all produced the same form as that in the No. 7 plants that provided the inoculum.

DISCUSSION

When the intermediate and mild forms of the disease were first observed, we speculated that the severity of disease would increase with repeated transfer of infected bud tissue from plant to plant in the seedling clone that proved to be highly susceptible to the severe disease. Instead, the intermediate and mild forms of the disease remained stable with repeated transfer of the causal agent by bud inoculation to the No. 7 clone for more than 3 yr. In addition, the symptoms produced from each of the three sources of inoculum in budded Strafford plants were the same as those produced in the No. 7 clone.

Mycoplasmalike organisms have been observed in ultrathin sections of hydrangeas with virescence (3,5). They were more easily detected in Strafford plants with severe symptoms than in those with mild symptoms (3). Vigor progressively declined as symptoms increased in severity, and few plants survived more than 2 or 3 yr. Different stable forms of the disease were not recognized in this earlier work.

Hydrangea virescence was previously attributed to hydrangea ringspot virus, based on the observation of virus particles in ultrathin sections and the mechanical transmission of the virus from virescent hydrangea to *Gomphrena globosa* and the absence of mycoplasma-like organisms in the phloem of affected plants.

We have observed mycoplasma-like organisms in association with severe,

intermediate, and mild virescence (*in preparation*). However, mycoplasma-like organisms were in low concentration in plants budded with inoculum from the hydrangea with mild virescence. The indication that green flower in hydrangea is caused by hydrangea ringspot virus (8) may be explained by the difficulty in detecting mycoplasma-like organisms in the mild form of the disease.

Based on our findings, the variation in expression of hydrangea virescence in naturally infected plants may be attributed to differences in pathogenicity of strains of mycoplasma-like organisms. Relationships among the mycoplasma-like organisms from different sources of virescent hydrangeas are being sought by investigating the biological interactions among the isolates.

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