

# Effect of Ribavirin on Green Ring Mottle Causal Agent and Necrotic Ringspot Virus in *Prunus* Species

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## ABSTRACT

Hansen, A. J. 1984. Effect of ribavirin on green ring mottle causal agent and necrotic ringspot virus in *Prunus* species. *Plant Disease* 68:216-218.

Ribavirin, a guanosine analogue, was applied as a foliar spray to seven 2-yr-old *Prunus serrulata* 'Kwanzan' trees infected with the non-sap-transmissible viruslike agent (VLA) of green ring mottle (GRM). Weekly applications of 500 ppm ribavirin prevented symptom development on newly developing Kwanzan foliage and gradually eliminated the infective principle from the previously infected older wood. Back-indexing confirmed that new shoots were free of GRM and demonstrated that the VLA in older wood gradually disappeared. One year after ribavirin treatments were stopped, no symptoms or VLA could be detected in shoots or limbs. Two *P. persica* 'Veteran' trees infected with necrotic ringspot virus (NRSV) and treated with weekly foliar applications of ribavirin continued to display virus symptoms throughout the growing season. Back-indexing in the fall indicated that NRSV was still present.

Additional key word: chemotherapy

During the past 25 yr, fruit tree collections free of viruses and of non-sap-transmissible viruslike agents (VLAs) have become increasingly important as major sources of budwood for nurseries and growers. In North America, starter material for state or provincial certification programs has usually been provided by the fruit tree repositories in Prosser, WA, or Saanichton, BC, respectively. These repositories obtained their first virus and VLA-free material through thermotherapy (2) or through selection of naturally healthy trees (15) or buds (3). Recently, meristem propagation and micrografting have been added as other possible methods for obtaining healthy starter material (10). The major disadvantage of all these methods is the need for extensive indexing, which is costly and time-consuming; in addition, thermotherapy and meristem propagation require special equipment and expertise.

Ribavirin (Virazole) is known to inhibit replication of several plant viruses in herbaceous hosts (5,8), meristem cultures (13), and protoplast systems (12) and therefore seemed to be a promising material for chemotherapy of fruit trees. Cost and regulatory considerations prevent large-scale use of the compound in orchards; however, these limitations do not apply to small-scale work with virus- or VLA-infected starter material

for repository use. To be effective, the material would have to be easily absorbed, evenly distributed, and active in preventing replication and upward translocation of the virus or agent in question.

This study was initiated to determine whether ribavirin chemotherapy could be used successfully against viruses and VLAs in woody hosts. Attempts were made to eliminate green ring mottle (GRM), a non-sap-transmissible VLA, from young trees of *Prunus serrulata* L. 'Kwanzan' and necrotic ringspot virus (NRSV) from *P. persica* L. 'Veteran' trees. Both hosts were selected because they react with clear symptoms to infection and therefore allow immediate evaluation of the effect of the treatment.

## MATERIALS AND METHODS

Ribavirin (obtained from Viratek Inc., Covina, CA 91722) was dissolved in water to a concentration of 500 ppm (0.002 M) and was applied with a hand-operated garden sprayer to runoff.

Virus- and VLA-free 2-yr-old *P. avium* L. 'F 12/1' and peach seedlings were grown out-of-doors and used as understocks. Cultivar buds were taken from our own indexed collection. Back-inoculations were made by budding bark patches or buds to healthy indicator trees and by rub-inoculating fully expanded leaves of *Chenopodium quinoa* Willd. under appropriate conditions (4) in the greenhouse or growth chamber.

The inoculum source for GRM was an F 12/1 stock tree that contained an isolate originally obtained from a Glohaven peach tree and the inoculum source for NRSV was a *P. avium* 'Bing' tree infected with a local field source of the rugose mosaic strain (9). Field observations, as

well as herbaceous and woody host indexing, had shown that both trees were free of other viruses and VLAs.

For the GRM experiment, 15 F 12/1 understocks were budded with healthy Kwanzan indicator buds in August 1980 and 10 of these were simultaneously inoculated by budding with the GRM-infected F 12/1 buds. The latter were then divided into two groups of five trees and treated as shown in Table 1. Group 1 received weekly applications of ribavirin from 28 August until leaf drop on 7 October 1980. Group 2 was simultaneously treated with water only. The third group of five Kwanzan trees served as the uninoculated control. In 1981, identical treatments were resumed on 7 May about 1 wk after budbreak. Inoculum buds were pruned off 1 wk later. On 25 June, the treatments of two trees each in groups 1 and 2 were reversed so that two of the trees in group 1, which had been treated until then with ribavirin, received only water for the rest of the season (1B), whereas two previously untreated trees were treated with 500 ppm ribavirin for the rest of the season (2B). The treatments for the remainder of the trees (1A and 2A) remained unchanged until leaf drop on 15 October.

For the NRSV test, two 6-yr-old NRSV-infected peach trees were sprayed weekly from 7 May to 15 October 1981. One tree was sprayed with water and the other with 1,000 ppm ribavirin. Young leaves and fruits were used for back-indexing onto herbaceous hosts (*Cucumis sativus* L. 'National Pickling' and *C. quinoa*) (4).

Data on symptom development on field trees were taken at approximately monthly intervals.

## RESULTS

At the beginning of the 1981 season, none of the Kwanzan trees treated during the previous fall (group 1) showed any symptoms and the VLA could not be recovered by back-indexing, with one exception (24 June, Group 1A, Table 1). All five trees in the untreated group (group 2) displayed typical GRM epinasty by 1 June. On the trees treated only during the summer and fall of 1981 (group 2B), typical GRM symptoms persisted on leaves that had started to expand before the beginning of the treatment, whereas foliage that began to expand during treatment remained free of symptoms (Table 1, Fig. 1). Back-

Contribution No. 581, Agriculture Canada, Research Station, Summerland, BC.

Accepted for publication 29 August 1983.

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indexing demonstrated that no GRM VLA could be detected in the Kwanzan parts that were free of symptoms after treatment. Back-inoculations made with axillary buds of leaves visibly affected by GRM, taken from the lower part of the 1981 shoots (group 2B), gave positive results when taken on 23 July but gave negative results on 28 August. All trees treated during part or all of the 1981 season were still free of GRM epinasty and other symptoms during the summer of 1983.

Of the nine back-inoculations from the untreated, GRM-infected controls (group 2A), only one failed to detect GRM (group 2A, 28 August, base). All uninoculated control trees remained healthy. On NRSV-infected peach trees, there was no difference in symptom expression between treated and untreated trees, and all back-inoculations were positive.

The only phytotoxic symptoms observed consisted of a transient, slightly yellowish discoloration of the first leaves in 1982 on some of the Kwanzan trees that had been treated weekly throughout 1981.

## DISCUSSION

Visual observations of infected and treated Kwanzan trees indicated that foliar treatment with ribavirin completely prevented development of GRM symptoms. Back-inoculations confirmed that the newly developing symptom-free shoots were free of VLA (group 1). When treatment was discontinued, trees remained healthy and were still symptom-free in the summer of 1983. I have shown previously (5) that the direct inhibitory effect of foliar applications of ribavirin on apple chlorotic leaf spot virus in *C. quinoa* lasts only about 36 hr. It is probably safe to assume that the direct inhibitory effect against GRM in Kwanzan trees would last for a similar period of time because the mechanism of inhibition is apparently the same

regardless of host and affected virus (7,14). The continued freedom from symptoms in treated Kwanzan trees therefore does not appear to be due to a persistent action of ribavirin but rather to permanent elimination of the VLA from treated trees.

Results obtained with two subgroups in treatment 1 give a rough indication of the time course of VLA inactivation. In 1A and 1B, nine weekly fall applications were sufficient to eliminate the VLA, as shown by the lack of symptoms and the negative back-inoculations the following season. The one exception, one positive back-inoculation using tissue from older wood of a single tree in group 1A indicates that small pockets of active VLA may remain in older tissues of otherwise healthy trees. In group 2B, where treatment began on 24 June 1981, the first effect of the treatment was apparent within 1 wk; newly emerging leaves remained symptom-free (Fig. 1). When bark patches from near the base of these leaves were back-indexed 4 wk later, GRM was not detected. This indicates that ribavirin prevents VLA replication and upward translocation almost immediately after application and therefore effectively protects new growth from GRM.

Various speculative explanations are possible regarding the gradual disappearance of infectivity from the older parts of these trees, which were infected when the treatment began. Infectivity may, for instance, require a continuous production of the (still hypothetical) VLA; once ribavirin prevents this replication, existing VLAs gradually disintegrate and infectivity ceases. Under the conditions of this experiment, this process would have taken up to 9 wk, 24 June to 28 August.

In all experiments aimed at eliminating viruses or VLAs from systemically infected tissue, the question arises whether the observed effect is permanent

or whether infectivity has only been reduced to an undetectable level. The maximum time span during which such infections may remain undetectable is difficult to determine and would probably vary from experiment to experiment, depending upon host-virus combination, type and duration of treatment, sensitivity of detection methods, and environmental conditions. Some indications may be obtained from thermotherapy, where viruses and VLAs are frequently undetectable for several months after treatment. In a few exceptional cases, back-indexes from treated trees remained negative for as long as 2 yr and then became positive (M. F. Welsh, *personal communication*). A specific case of an undetectable, yet persistent infection after chemical treatment has been reported by Secor and Nyland (11) for ribavirin-treated ring pattern in roses; the time span between the seemingly successful single treatment (slow injection of 10 ml of 200 ppm ribavirin) and the first positive back-inoculations from the symptomless



Fig. 1. (Right) Green ring mottle-affected foliage and (left) healthy foliage on Kwanzan branch treated with ribavirin from 25 June onward (group 2B). Foliage to the left developed while branch was being treated.

Table 1. Effects of ribavirin treatment: symptom development and results of back-inoculations from green ring mottle (GRM)-infected ribavirin-treated Kwanzan trees

Date	Group 1 (treated fall 1980)				Group 2 (not treated fall 1980)				Group 3 (not treated) 5 Trees (symptoms)
	A (3 trees, treated throughout 1981)		B (2 trees, treated until 25 June 1981)		A (3 trees, not treated in 1981)		B (2 trees, treated after 25 June 1981)		
	Symptoms	Back-index	Symptoms	Back-index	Symptoms	Back-index	Symptoms	Back-index	
4 May 1981	n n n <sup>a</sup>	...	n n	...	E E n	...	E n	...	All n
10 June	n n n	---	n n	--	E E E	+	E E	...	All n
24 June	n n n	--+	n n	-	E E E	+	E E	...	All n
23 July									
Tips <sup>b</sup>	n n n	...	n n	...	E E E	+	E n	-	All n
Base <sup>c</sup>	n n n	...	n n	...	E E E	+	E E	+	All n
28 August									
Tips	n n n	--(-)	n n	--	E E E	+++	n n	--(-)	All n
Base	n n n	---	n n	--	E E E	-++	E E	--	All n
12 May 1982	n n n	...	n n	...	E E E	...	n n	...	All n

<sup>a</sup>Each symbol represents one tree or back-check, n = no symptoms, E = epinasty typical of GRM, - = back-index negative, + = back-index positive, and (-) = buds died within 10 days.

<sup>b</sup>Leaves and bark patches near tips of 1981 shoots.

<sup>c</sup>Leaves and bark patches near base of 1981 shoots.

shoots was 60 days. In our tests, however, treatment was continued for several months, and even under the shortest treatment regime (group 1B), back-inoculations remained negative and symptoms had not reappeared 2 yr after the end of the treatment. The treated trees are therefore considered free of GRM but will be kept under observation for another 3 yr.

The inability of ribavirin applications to eliminate NRSV from peach confirms previous results that showed that NRSV infection of *C. quinoa* could not be prevented or influenced by foliar applications of 500 ppm ribavirin (6). Cheplick and Agrios (1), on the other hand, observed symptom remission when they injected McIntosh apple trees infected with apple mosaic with ribavirin. Although apple mosaic virus is considered a strain of NRSV, these two results are not necessarily incompatible because their injection application provides a continuous supply of the chemotherapeutic material, presumably for several days, whereas our weekly foliar application provided the material intermittently but for several weeks.

From a practical point of view, foliar application of ribavirin offers the advantage that it is considerably cheaper, faster, and simpler than thermotherapy, meristem culture, micrografting, or selection by indexing of naturally virus-free material. It should be extremely useful in the treatment of stone fruit trees,

which are especially sensitive to thermotherapy (2).

The main factor limiting the usefulness of ribavirin chemotherapy is the apparently narrow range of plant viruses against which it appears to be active (6). However, new antiviral compounds are constantly being developed, and combinations of several of these may eventually permit elimination of all viruses and VLAs from infected plant tissue. In principle, this method should be feasible and economically useful for elimination of viruses and VLAs from tree fruits and from other vegetatively propagated crops, such as small fruits, potatoes, ornamentals, and many plantation crops, which rely on a continuous supply of healthy propagation material.

This is the first report of an apparently successful chemotherapeutic elimination of a VLA from a woody host.

#### ACKNOWLEDGMENTS

I wish to thank G. Mink for the serological identification of the rugose mosaic strain of the NRSV isolate. I also thank Linda Green, Moira Timmons, and Mardi Dolfo for their competent technical assistance and helpful comments.

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