Effect of Injected Antiviral Compounds on Apple Mosaic, Scar Skin, and Dapple Apple Diseases of Apple Trees

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ABSTRACT

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Several synthetic chemicals with known antiviral or antibiotic activity were injected under pressure into apple trees showing symptoms of apple mosaic, scar skin, or dapple apple. Compounds included ribavirin, 2-thiouracil, formycin B, amantadine HCl, methisazone, and oxytetracycline. Ribavirin completely suppressed symptom expression when injected during autumn into orchard trees and greenhouse-grown grafts infected with apple mosaic virus (AMV). Similar injections of oxytetracycline significantly increased the frequency and enhanced the severity of foliar symptoms. Injections of ribavirin in the spring reduced symptom expression but did not completely control AMV infection in orchard trees, whereas injections of oxytetracycline in the spring did not affect foliar symptom expression. The other compounds had little or no effect on apple mosaic symptoms. Injections of the antiviral compounds into orchard trees showing symptoms of scar skin or dapple apple disease and direct application to the fruit of trees with dapple apple disease did not produce significant changes in fruit symptoms.

The successful use of systemic fungicides in controlling plant-pathogenic fungi (8,9) and the use of antibiotics against bacterial and mycoplasmal diseases in plants (6,11) have led to increased efforts in the search for chemical substances effective against viral diseases in plants (1,3,4,12). Work on virus inhibition in plants by chemical methods so far has involved mainly spraying antiviral compounds on virus-infected hosts (1–4). The development of chemicals that can be systemically distributed through a tree and be effective against plant diseases (6,10,11,15) has made pressure injection a very attractive method of applying these chemicals to diseased trees and makes the possibility of controlling viral diseases in trees with injected antiviral compounds worth investigating.

Virus diseases in orchards are controlled primarily through use of virus-free stock. However, established healthy trees may become infected with viruses via vectors, pollen, etc. Also, not all nursery stock is free from viruses, particularly latent ones. At this point, removing the infected trees is the only control measure available (5). Consequently, there is a need to develop practical and effective alternatives to the present method of controlling virus diseases in deciduous fruit trees.

Apple mosaic is a known virus disease of apple resulting in characteristic mosaic symptoms on the foliage of affected trees and therefore was chosen as a model disease to study the effects of injected antiviral compounds on foliar symptoms. Scar skin and dapple apple are apple diseases, probably related, that are presumed to be of viral (or viroid) etiology because of the viruslike symptoms on apple fruits and because they are graft-transmissible. No virus or other pathogen has been associated with these diseases, however. It was hoped, therefore, that the effect of known antiviral and antibiotic compounds on the development and expression of symptoms in apple fruit infected with scar skin or dapple apple disease would provide information concerning the nature of the causal agent(s).

MATERIALS AND METHODS

Apple mosaic virus (AMV) and the agents causing scar skin and dapple apple diseases were obtained from infected apple trees growing in the University of Massachusetts Fruit Research Orchard in Belchertown. Trees used in the field experiments were growing in the university orchard and consisted of 8- and 4-yr-old McIntosh, Red Delicious, and Hyslop Crab apple trees and 2-yr-old McIntosh trees, all grafted on EM VII rootstocks. For greenhouse experiments, healthy scion wood of Golden Delicious apple was grafted onto seedling rootstocks growing in 15-cm pots.

The synthetic antiviral compounds used were ribavirin (Virazole; ICN Pharmaceuticals, Cleveland, OH), 2-thiouracil and formycin B (Sigma Chemical Co., St. Louis, MO), amantadine HCl (Pfaltz and Bauer, Inc., Stamford, CT), methisazone (Marboran; Burroughs Wellcome Co., Greenville, NC), and oxytetracycline HCl (Terramycin Tree Injection Formula; Pfizer Chemical Division, New York).

The symptom frequency and severity observed after each chemical treatment were compared with those of the untreated control, and statistical significance was determined by applying Student's *t* test.

Field experiments. Treatments were applied to individual branches of virus-infected 8- and 4-yr-old apple trees. Injection holes were drilled with a 3-mm diameter drill bit to a depth of 15 mm. Two holes were drilled per branch. Using the injection apparatus developed by Sterrett and Creager (16), 1 ml of an aqueous chemical solution per injection hole was pressure-injected into each branch. Each branch received two injections for a total of 2 ml of chemical solution in concentrations just below the phytotoxic levels determined in preliminary experiments.

Injections with all chemicals were made in the autumn of 1979 and spring of 1980. Amantadine HCl and methisazone were found in preliminary experiments to be either ineffective or too difficult to put into solution and were therefore omitted from subsequent experiments. During the last week of October 1980, branches of apple trees with apple mosaic or scar skin disease were injected with 2 ml of aqueous solutions of ribavirin at 8 and 12 mg/ml, formycin B at 0.5 and 1 mg/ml, or oxytetracycline at 12 and 26 mg/ml. A similar series of injections were made between 5 May and 10 May 1981 on another set of branches.

In July 1979, 2-yr-old McIntosh trees growing in the orchard were each injected with 2 ml of aqueous solution of ribavirin (4 mg/ml), 2-thiouracil (4 mg/ml), or oxytetracycline (5 mg/ml). One week later each tree was inoculated by grafting with two AMV-infected buds. The trees were observed for development of virus symptoms and phytotoxicity during the next two growing seasons.

In June and again in July 1982, all (100–130) fruit on two or three branches

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of Hyslop Crab trees with dapple apple disease were rubbed with a cotton swab containing one of four antiviral compounds: ribavirin, 2-thiouracil, amantadine HCl, and oxytetracycline. The first three compounds were applied at 0.1 or 0.5 mg/ml in June and 1 or 5 mg/ml, respectively, in July and the fourth, at 0.3 or 1 mg/ml in June and 3 or 10 mg/ml, respectively, in July.

Control treatments consisted of inoculated but uninjected branches or trees. In August, leaves from AMV-infected branches were collected and counted, and the degree of symptom severity was visually rated. Fruit from branches with scar skin or dapple apple disease were collected in September and evaluated similarly. Virus symptoms covering less than 25% of the leaf or fruit surface were rated light and given a value of 1, symptoms covering 25–50% of the surface were rated moderate and given a value of 2, and symptoms covering more than 50% of the surface were rated severe and given a value of 3. For each treatment, a disease severity index was calculated by multiplying the percentage of leaves or fruit in each severity class by its respective numerical value, adding the values for all classes, and dividing the sum by 100.

Greenhouse experiments. Young Golden Delicious grafts were inoculated at bud break with two buds infected with AMV. After virus symptoms began to appear on the leaves, the new twigs were cut back to about half their length and the remaining leaves removed. One week later, the rootstocks were injected with an antiviral compound. One hole per tree was drilled with a 1-mm diameter drill bit to a depth of 6 mm at 15 cm above the soil line. The trees were pressure-injected with 0.5 ml of aqueous solution of the antiviral compounds, using the pressure injection apparatus mentioned previously. Chemical treatments at concentrations determined from preliminary experiments consisted of aqueous solutions of ribavirin at 0.1, 0.3, and 0.5 mg/tree, oxytetracycline at 0.3, 0.5, and 1 mg/tree, and formycin B at 0.005, 0.01, and 0.05 mg/tree. Preliminary injections included amantadine HCl and methisazone treatments, but neither compound had any apparent effect on virus symptoms and both were omitted in subsequent experiments. Control treatments consisted of inoculated but uninjected grafts.

AMV-infected orchard branches and Golden Delicious grafts injected with ribavirin and showing no virus symptoms in summer after treatment were indexed onto healthy Golden Delicious grafts in the greenhouse. Six buds from each branch or tree that remained symptomless after ribavirin treatment were placed on three Golden Delicious grafts (two buds per graft) to determine the presence or absence of AMV in the symptomless branch or tree.

RESULTS

Preliminary orchard injections. The results from injections of antiviral compounds during autumn 1979 and spring 1980 into tree branches with apple mosaic, scar skin, or dapple apple disease were inconclusive because disease symptoms were distributed poorly on the foliage and fruit of inoculated trees during the summer of 1980. The results of these experiments tended to support subsequent data obtained from similar experiments the next year, however. Branches injected with ribavirin showed a lower frequency (6–25% vs. 30–68%) and severity (0.07–0.29 vs. 0.12–0.91) of apple mosaic symptoms than any of the other treated branches. Symptoms on branches injected with amantadine HCl, 2-thiouracil, methisazone, and formycin B were similar to those on control branches. None of the injected compounds appeared to have a significant effect on the fruit symptoms of trees with scar skin or dapple apple disease.

Autumn injections of branches. The results of autumn injections of antiviral compounds into AMV-infected McIntosh branches are shown in Table 1. Four of six branches injected with 16 or 24 mg of ribavirin in October 1980 were completely free from viral symptoms during the 1981 growing season. Indexing showed that AMV was present in the symptomless branches. Furthermore, the ribavirin-injected branches that did show symptoms had many fewer leaves with symptoms and symptoms were much milder than in any of the other injected or control branches (Table 1). Formycin B injections at 1 and 2 mg per branch resulted in foliar symptoms that were not significantly different from those on control branches. Oxytetracycline injections of 40 mg per branch, however, resulted in significantly higher percentages of foliar symptoms per branch than control branches. Moreover, the symptoms on the oxytetracycline-treated branches at both 24 and 40 mg were significantly more severe than symptoms on control branches (Table 1).

Spring injections of branches. The results of spring injections are shown in Table 2. Branches injected with 16 or 24 mg of ribavirin had a significantly lower percentage of leaves with symptoms than control branches. Severity of the symptoms was also significantly lower than in control branches. Branches injected with 1 mg of formycin B or 24 mg of oxytetracycline developed foliar symptoms similar to those on untreated branches. Branches injected with 2 mg of formycin B or 40 mg of oxytetracycline had lower frequency and severity of leaf

Table 1. Effect of autumn injections of antiviral compounds into branches of McIntosh apple trees with apple mosaic disease on foliar symptom frequency and severity

Treatment		Number of branches			
Chemical	Amount (mg/branch)	Treated	With symptoms	Percent of leaves with symptoms[a,b]	Disease severity index[a,c]
Ribavirin	16	6	2[d]	4**[e]	0.05**
	24	6	2[d]	8**	0.10*
Formycin B	1	6	6	50	0.72
	2	6	6	47	0.64
Oxytetracycline	24	6	6	61	1.07*
	40	6	6	70*	1.08*
Control	···	6	6	53	0.72

[a] Each number is the average of six replicates.
[b] Per total number of branch leaves.
[c] Disease severity index = (% leaves with light symptoms × 1) + (% leaves with moderate symptoms × 2) + (% leaves with severe symptoms × 3) divided by 100.
[d] Indexing results showed apple mosaic virus present in the four symptomless branches 10 mo after treatment.
[e] Treatment is significantly different from respective control at $P = 0.05$ (*) and $P = 0.01$ (**).

Table 2. Effect of spring injections of antiviral compounds into branches of McIntosh apple trees with apple mosaic disease on foliar symptom frequency and severity

Treatment		Number of branches			
Chemical	Amount (mg/branch)	Treated	With symptoms	Percent of leaves with symptoms[a,b]	Disease severity index[a,c]
Ribavirin	16	4	4	11**[d]	0.13**
	24	4	4	27*	0.34
Formycin B	1	4	4	51	0.62
	2	4	4	31	0.36
Oxytetracycline	24	4	4	46	0.51
	40	4	4	32	0.34
Control	···	4	4	49	0.55

[a] Each number is the average of four replicates.
[b] Per total number of branch leaves.
[c] Disease severity index = (% leaves with light symptoms × 1) + (% leaves with moderate symptoms × 2) + (% leaves with severe symptoms × 3) divided by 100.
[d] Treatment is significantly different from respective control at $P = 0.05$ (*) and $P = 0.01$ (**).

Table 3. Effect of injections of antiviral compounds into 2-yr-old McIntosh apple trees on apple mosaic virus infectivity and foliar symptom development

Treatment		Number of trees			Percent of leaves with symptoms[a,b]		Disease severity index[a,c]	
Chemical	Amount (mg/tree)	Treated 1979	With symptoms 1980	With symptoms 1981	1980	1981	1980	1981
Ribavirin	4	6	0[d]	2[d]	0**[e]	13**	0**	0.21*
2-Thiouracil	4	6	6	6	47	54	0.66*	0.78
Oxytetracycline	5	6	6	6	27*	37*	0.39	0.51
Control	···	6	6	6	40	67	0.42	0.69

[a] Each number is the average of six replicates.
[b] Per total number of tree leaves.
[c] Disease severity index = (% leaves with light symptoms × 1) + (% leaves with moderate symptoms × 2) + (% leaves with severe symptoms × 3) divided by 100.
[d] Indexing results showed apple mosaic virus present in the symptomless trees 2 yr after treatment.
[e] Treatment is significantly different from respective control at $P = 0.05$ (*) and $P = 0.01$ (**).

Table 4. Effect of injected antiviral compounds on symptoms in Golden Delicious grafts infected with apple mosaic virus in the greenhouse

Treatment		Number of plants		Percent of plants with symptoms
Chemical	Amount (mg/graft)	Treated	With symptoms	
Ribavirin	0.1	43	10	23
	0.3	39	0[a]	0
	0.5	43	0[a]	0
Oxytetracycline	0.3	30	29	97
	0.5	29	29	100
	1.0	30	30	100
Formycin B	0.005	30	18	60
	0.01	30	16	53
	0.05	30	17[b]	100
Control	···	12	12	100

[a] Indexing results showed apple mosaic virus present in the symptomless trees 5 mo after treatment.
[b] Other 13 trees were dead.

symptoms than any other treatment group except ribavirin at 16 mg per branch.

Injections of young trees. The results of injecting 2-yr-old McIntosh trees with antiviral compounds in July 1979, followed by inoculation with AMV 1 wk later, are shown in Table 3. By August 1980, the six trees injected with ribavirin showed no virus symptoms, whereas all other treated and control trees showed typical mosaic symptoms. Two years after the injections, two of the six ribavirin-treated trees were showing typical mosaic symptoms; the remaining four trees were still symptomless. Indexing results showed that apple mosaic virus was present in all six trees at this time. The percentage of leaves with symptoms in oxytetracycline-treated trees was lower than that of control trees, but the severity of symptoms was similar in both. In trees treated with 2-thiouracil, on the other hand, the percentage of leaves with symptoms was similar to that of control trees but the symptoms were more severe the first year after treatment (Table 3).

Injections of grafts in the greenhouse. Results in greenhouse-grown Golden Delicious grafts inoculated with AMV and injected with antiviral compounds are shown in Table 4. Ribavirin at 0.3 and 0.5 mg per graft completely suppressed virus symptoms in foliage produced after injection. Indexing results 5 mo after treatment showed that apple mosaic virus was present in the symptomless trees. Oxytetracycline did not suppress foliar symptom development in the virus-infected plants. Grafts injected with the antibiotic had more severe symptoms than did plants in any of the other treatment groups, including the control. Leaves of oxytetracycline-treated plants that showed mosaic patterns had larger and brighter yellow or white areas and smaller green areas than leaves of the other grafts. Formycin injections at 0.005 and 0.01 mg resulted in moderate suppression of virus symptoms, with 60 and 53% of the grafts, respectively, showing foliar symptoms after treatment.

Effects on scar skin and dapple apple diseases. No noticeable change in fruit symptoms on Red Delicious apple trees with scar skin disease or Hyslop Crab apple trees with dapple apple disease occurred after branches were injected with antiviral compounds during either autumn or spring. Hyslop Crab fruit swabbed with 2-thiouracil or ribavirin had a lower, but not significantly different, dapple apple disease severity index than untreated fruit.

Phytotoxicity of antiviral compounds. Many branches injected with an antiviral compound during either autumn or spring had circular, depressed necrotic areas of bark around the injection sites. On branches injected with ribavirin, newly emerging leaves occasionally appeared narrower than normal and slightly chlorotic. Formycin B injections of 2 mg per branch appeared to be the most toxic to trees. By mid-July, much of the foliage of branches treated with formycin B was smaller than normal and chlorotic. On branches injected with oxytetracycline during autumn, leaf expansion was about 1 wk behind that of the rest of the tree and of the other trees and the expanding leaves were chlorotic.

DISCUSSION

The results indicate that ribavirin can completely suppress the expression of AMV symptoms when injected into young apple trees or branches during autumn. Indexing showed that virus was present in all trees remaining symptomless as a result of treatment with the compound, suggesting that ribavirin suppresses symptom expression but does not completely and permanently inactivate the virus. This conclusion is in agreement with earlier reports (1,4,7,13). On the other hand, when ribavirin was injected into AMV-infected orchard trees in the spring, the percentage and severity of foliar symptoms were significantly reduced but symptom expression was not suppressed entirely. The reasons for this are not known but possibly ribavirin is distributed slower or less effectively when injected in the spring than in autumn. Similar results have been reported with spring and autumn injections of other compounds into trees (14).

Oxytetracycline injections of orchard trees in the autumn or of grafts in the greenhouse significantly increased the frequency and enhanced the severity of foliar symptoms. Tetracyclines are known inhibitors of protein synthesis but apparently have no antiviral activity. How oxytetracycline increased severity of symptoms on AMV-infected plants is not known. A reasonable speculation may be that chlorosis is enhanced by the combined effects of virus infection and oxytetracycline-inhibited plant protein synthesis on chloroplast development.

Formycin B appeared to have no significant effect on apple mosaic symptoms and was the most phytotoxic

compound at the concentrations administered.

Injections of antiviral compounds into branches with scar skin or dapple apple disease had no apparent effect on fruit symptom expression. The low transpiration rate of the fruit relative to that of the foliage may have prevented the accumulation of significant levels of the chemicals in the fruit. The slight reduction in symptom severity of dapple apple disease in Hyslop Crab fruit swabbed with 2-thiouracil and ribavirin while still growing on the tree may indicate some uptake of these compounds by the fruit.

Much of the phytotoxicity observed after chemical injection might be eliminated by using larger volumes of more dilute antiviral solutions. This approach might also improve final distribution of the compounds throughout the plant. Ribavirin injections of this nature might result in greater suppression of apple mosaic symptoms than the small, concentrated injections reported in this paper.

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