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SAPONINS IN RELATION TO THE CONTROL OF AVOCADO ROOT ROT WITH ALFALFA MEAL

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Alfalfa meal, when mixed with soil infested with the fungus *Phytophthora cinnamomi,* gives excellent control of root rot of avocado seedlings under greenhouse conditions (4). In field trials, involving several hundred avocado trees, however, the material has given inconsistent results. Apparently this relatively poor performance under field conditions is related to the fact that in treating an established tree it is impossible to mix the alfalfa meal with the soil mass in which the roots are growing, as can be done when the treatments are applied to seedlings growing in containers in the greenhouse.

In the original paper on alfalfa meal published in 1963 it was suggested that this was a form of "biological control." based on the large increase in saprophytic fungi and bacteria in the soil treated with alfalfa meal in comparison with untreated soil. Other possible mechanisms suggested included: changes in the water-holding capacity of the soil, inhibition or reduction of spore formation by the pathogen, and production of chemicals during decomposition of the organic matter which were toxic to the pathogen.

Because of the beneficial effects of alfalfa meal we have continued research along the lines of biological control, arid have studied further the reason for the action of alfalfa meal.

Alfalfa meal is known to contain appreciable amounts of saponins; the concentration ranges from 1.5 to 3.5 per cent. Thus, it seemed possible that, saponins might be involved in the control of avocado root rot with alfalfa meal. This paper concerns our research on the toxicity of saponins to Phytophthora cinnamomi.

The organic chemicals known as sapogenins are widely distributed in higher plants (2). Saponins are glycosides of the sapogenins, and are equally widespread in higher plants, occurring in such diverse types of plants as alfalfa, and species of *Agave, Yucca, Primula,* and *Digitalis.* In a survey of plants in Malaya, saponins were found in 77 of the 542 species of plants analyzed (1). Saponins have been reported by Zimmer et al. (6) to be very toxic to the fungus *Trichoderma viride* and in other reports (2) to have various effects on higher plants.

The saponins used in our experiments were supplied by Dr. E. D. Walter of the Western Utilization Research and Development Division. U. S. Department of Agriculture, Albany, California, Two different types were sent to us by Dr. Walter: a water-soluble fraction and a water-insoluble fraction; both of these were used in our tests.

The toxicity of the two saponin fractions was tested in the laboratory on growth of mycelium, an production of sporangia, and on germination of zoospores of *Phytophthora cinnamomi.*

To test the effect on mycelial growth of the fungus the chemicals were mixed with potato-dextrose agar at dosages of 100, 500, 1,000, and 10,000 ppm. Results presented in the following table showed that the saponins showed only slight toxicity to the mycelial or vegetative growth of the avocado root rot fungus.

Concentration of saponin (ppm)	*Reduction of growth of mycelium in agar test*	
	WS*	WI
	Per Cent	Per Cent
100	14	15
500	14	15
1,000	15	15
10,000	30	16

*WS = water-soluble fraction
WI = water-insoluble fraction

The toxicity of the saponins to the mycelium of *P. cinnamomi* in soil was tested by the soil vial method developed here (3). This method involves either applying the materials dissolved or suspended in water, as a drench, or mixing the materials with the soil. In the drench test the chemicals must penetrate 1 inch of soil; in the mix test penetration is not a factor but chemicals may be directly inactivated by soil contact.

In the soil test, water-soluble saponin, when mixed with the soil at 100 ppm, reduced growth from the mycelial disc when plated on cornmeal agar by 20-30 per cent as compared with growth from discs in untreated soil. Reduction was similar at 1,000 ppm and was similar with the water-insoluble fraction. Neither material was effective in penetrating the soil when applied as a drench. In this respect, the saponins act similarly to alfalfa meal which is effective only when mixed with the soil, not when applied to the surface and watered in. The similarity of action is additional evidence that saponins may be involved in the effectiveness of alfalfa meal in reducing avocado root rot.

The effect of the saponins was also tested on production of sporangia, and on germination of zoospores produced in the sporangia. To produce sporangia, soil extract was prepared from a non-sterile soil as previously described (5). This extract was diluted with equal parts of solutions or suspensions of saponins in sterile demineralized water to test the effect on production of sporangia. Zoospores were produced from sporangia by reducing the temperature of the solution in which sporangia had been produced, then the suspensions of zoospores were pipetted into depression slides containing measured amounts of saponins. Germination percentages of zoospores were determined in the presence of 0, 10, 50, 100, and 1,000 ppm of the saponins.

As shown in the next table, formation of sporangia was prevented by water-soluble saponin at 2,000 ppm, and was greatly reduced by the water-insoluble form at 1,000 and 2,000 ppm. Both types of saponin greatly reduced germination of zoospores, the primary infective spore of *P. cinnamomi,* at the highest concentration tested. Water-

soluble fraction inhibited zoospore germination by 75 per cent at a concentration of 2,000 ppm, while germination was completely prevented by water-insoluble fraction at 2,000 ppm.

Concentration of Saponin	*Sporangia Produced*		*Inhibition of Zoospore Germination*	
	WS	WI	WS	WI
ppm	No.[1]	No.	Per Cent	Per Cent
0	8.8	8.8		
10	11.3	7.6	0	0
100	11.5	6.2	27	19
500	8.5	6.8	30	33
1,000	5.7	2.1	68	36
2,000	0	0.5	75	100

[1]Mean number of sporangia in 24 microscope fields 1 mm. in diameter.
WS = water-soluble fraction
WI = water-insoluble fraction

In various laboratory and greenhouse tests alfalfa meal has been effective in preventing avocado root rot when mixed with the soil at rates of from 3.2 to 5 per cent. Assuming a saponin content of 3 per cent, alfalfa meal applications at the 5 per cent rate would add 1,500 ppm of saponin to the soil. This amount of saponin, as seen from the above tables, would prevent or greatly reduce formation of sporangia, reduce zoospore germination to a very low level, and retard mycelial growth to some extent.

The results of these tests indicate that saponins could be partly responsible for the control of Phytophthora root rot of avocado seedlings by adding alfalfa meal to infested soil.

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