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PHYTOPHTHORA ROOT ROT OF AVOCADO IN RELATION TO NITRITE

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The research reported in this paper is a continuation of investigations into the relations of nutrition and aeration to diseases of citrus and avocado initiated by Dr. H. D. Chapman and his coworkers of the Department of Soils and Plant Nutrition and carried on cooperatively with the Department of Plant Pathology. The authors are indebted to both Dr. H. D. Chapman and Dr. L. J. Klotz for their suggestions and continued interest in this problem.

Avocado root rot caused by the fungus *Phytophthora cinnamomi* Rands is in particular associated with excess soil moisture. Under waterlogged conditions soil aeration may be restricted such that the supply of oxygen in the root zone is reduced, possibly to the extent that the avocado is damaged. Root damage under waterlogged conditions can result from various factors. Reduced soil oxygen favors the accumulation of certain products such as carbon dioxide, methane, nitrites, sulfides, et cetera, which maybe harmful to the plant if they persist. In addition to the direct effect, such conditions associated with impeded drainage and excess moisture may indirectly damage the avocado by making the plant more sensitive to saprophytic fungi present in the soil. Klotz and coworkers (2, 3, 4) suggested such a possibility.

Curtis and Zentmyer reported the effect of oxygen supply on Phytophthora root rot of avocado in nutrient solution cultures. Their study, which relates to poor aeration and drainage of soils, showed that the rate of root invasion by *P. cinnamomi* is greatly reduced when the oxygen supply is restricted. In soils, however, toxic materials associated with anaerobic conditions could make the root more susceptible to attack by NO_2-N prevented the development of the root rot disease. This experiment was repeated since the initial results were unexpected. However, identical results were obtained. A maintained concentration of 2 ppm NO_2-N in nutrient solution adjusted to pH 4.5 prevented the development of avocado root rot caused by *P. cinnamomi.*

Table 1. Progress of avocado root rot in solution culture adjusted to pH 4.5 and containing 2 ppm NO_2-N.

Treatment No.	Treatment	Percent root rot or damage on: 4th day	6th day	9th day	30th day
1	N.S.*	0	0	0	0
1a	N.S.* + *P. cinnamomi*	33	85	99	100
2	N.S.*, 2 ppm NO_2	0	0	3	4
2a	N.S.*, 2 ppm + *P. cinnamomi*	3	3	4	4
3	2 ppm NO_2 pretreated, N.S.*	0	0	0	0
3a	2 ppm NO_2 pretreated, N.S.* + *P. cinnamomi*	42	87	100	100

*N.S. = Complete nutrient solution, pH 4.5.

The acidity of nutrient solution has a marked effect on the toxicity of nitrite. The more acid the solution (lower pH value), the more toxic a fixed concentration of nitrite becomes. Therefore, a second series of experiments was carried out wherein the influence of neutral pH reactions of nitrite on Phytophthora root rot was observed. The acidity of the nutrient solution was decreased to a reaction value of pH 6.5. Nitrite concentrations extending up to 50 ppm NO_2-N were maintained in complete nutrient solutions. Avocado seedlings which were growing in these cultures were inoculated with *P. cinnamomi* and the resulting disease development observed. The data are presented in figure 2. Along the vertical axis is plotted the development of root rot, expressed as percent of roots damaged. The horizontal axis is calibrated in days after inoculating the solution cultures. Results of each of the nitrite treatments, 0, 5, 10, 15, 20, 30, 40, and 50 ppm NO_2-N are plotted as individual curves. This family of curves illustrates the retarding effect of nitrite on avocado root rot. For example, avocado seedlings receiving only the nutrient solution (no nitrite) manifested typical root rot symptoms within 4 days after being inoculated with *P. cinnamomi.* Eight to 10 days lapsed before the 20 ppm NO_2-N treated plants developed similar disease symptoms. The two higher levels of nitrite, 40 and 50 ppm NO_2-N, completely arrested disease development.

With regard to the injurious action of these nitrite concentrations on the *root* rot fungus.

These observations suggested the advisability of investigating in more detail the action of nitrite on avocado root rot. Solution culture techniques were employed to evaluate the nitrite-root rot interrelation. In addition, laboratory experiments were performed to assess the specific effect of nitrite on the fungus *P. cinnamomi.*

RESULTS AND DISCUSSION

The first phase of the greenhouse work was the determination of the extent to which nitrites alter the resistance of avocado to root rot. The experimental procedure consisted of growing avocado seedlings in nutrient solution adjusted to pH 4.5 for several weeks before making the nitrite treatments and inoculating with the fungus P. *cinnamomi.* Three sets of seedlings were grown. One set received a conventional nutrient solution; another received, in addition to the nutrient solution, a 5-day pre-treatment of 2 ppm NO_2-N immediately before being inoculated with the root rot fungus; and the last set received, continuously, a nutrient solution plus 2 ppm NO_2-N. One-half of the seedlings of each set was inoculated (see fig. 1, a schematic sketch of the experimental setup). Lesions appeared on roots in all inoculated treatments except where a continuous concentration of 2 ppm NO_2-N was maintained. A summary of the experiment is presented in table 1. Compared to the control plants (Treatment No. 1), the nitrite pretreatment had no effect on predisposing the avocado to a more rapid attack by the fungus. Treatment 2, the maintained nitrite concentration, is especially interesting because rather than weakening the avocado's resistance to Phytophthora root rot, the presence of 2 ppm the avocado at pH 6.5, 50 ppm NO_2-N caused little or no injury to the plant. In the pH 4.5 series, the roots of the plants growing in the presence of a 2 ppm concentration appeared to be slightly more stubby than those of the control plants.

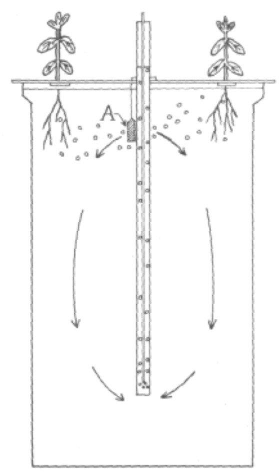

Fig. 1. Sketch of solution culture apparatus showing position of plant, aerator, and inoculum bag (A).

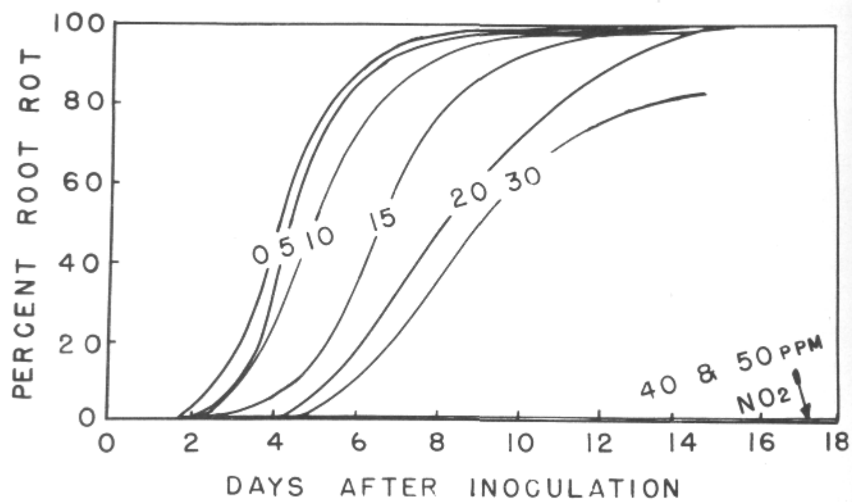

Fig. 2. Progress of avocado root rot in relation to concentration of nitrite maintained.

This study indicates that nitrite does not lower the resistance of avocado roots to invasion by *P. cinnamomi.* Actually it appears that the fungus is more sensitive than the avocado plant to nitrite.

Table 2. Mycelial growth of *P. cinnamomi* colonies on agar at pH 4.5 and 6.5 with increasing amounts of nitrite.

Treatment		Diameter of colonies in:		
pH	ppm NO_2-N	3 days	5 days	7 days
		mm.	mm.	mm.
4.5	0	47	69	89
4.5	2	43	66	88
4.5	4	24	47	75
4.5	6	0	0	0
6.5	0	41	59	90
6.5	15	21	35	79
6.5	30	14	27	56
6.5	45	0	10	14
6.5	60	0	7	12

Laboratory experiments with *P. cinnamomi* and nitrite provided additional evidence that the fungus is sensitive to nitrite. Working with an agar plate technique, the mycelial growth of fungal colonies under various nitrite treatment was observed. Results of a typical experiment are given in table 2.

The fungitoxic concentration of nitrite appears to be approximately 6 ppm NO_2-N according to results of the agar plate experiment conducted at pH 4.5. A significant growth inhibition occurred in the 4 ppm NO_2-N treated set. Agar plate tests at pH 6.5 indicate that 45 ppm NC^-N is approximately the concentration lethal to Phytophthora. Growth restriction was brought about by a 30 ppm NO_2-N concentration. Some extremely weak and sparse growth was observed in the two highest nitrite levels (45 and 60 ppm NO_2-N).

The possibility of nitrite inhibiting zoospore germination was examined also. However, in order to have experimental material, zoospores of a species related to *P. cinnamomi* were used since no method has been developed to produce a pure zoospore suspension of *P. cinnamomi.* Using a method outlined by Dr. L. J. Klotz of the Citrus Experiment Station, zoospore suspensions of *P. citrophthora* were prepared; results obtained with *P. citrophthora* should pertain to *P. cinnamomi.*

Germination of the *P. citrophthora* zoospores in solutions containing nitrite was observed with a microscope. At pH 4.5, germination was sharply reduced by the addition of 1 ppm NO_2-N; nitrite concentrations greater than 4 ppm reduced germination to about 10 percent.

Germination of zoospores in solutions adjusted to pH 6.5 was reduced significantly by as little as 5 ppm NO_2-N. Nitrite concentrations of 40 ppm or more reduced the germination percentage to about 25 percent.

It is evident from the results of the above-mentioned experiments, that the fungus responds similarly to the avocado with respect to the nitrite-pH relation. This relation applies to mycelial growth and as well to germination of zoospores (zoospores of another species, *P. citrophthora).* Accordingly, it seems unlikely that nitrites, under field conditions, lower the resistance of the avocado to Phytophthora root rot. Actually, the data suggest the opposite effect; a maintained nitrite concentration, if high enough, would be fungistatic, thus retarding the activity of the disease.

It appears that a waterlogged condition, so often associated with Phytophthora root rot of avocado, favors the dissemination of the fungus throughout the soil by zoospores moving through the soil-water in between the soil particles. According to the investigations relating a reduced disease activity to reduced oxygen tensions, and the nitrite relation reported herein, the excess soil moisture does not appear to favor the fungus in preference to the host avocado except by providing a more favorable medium for the zoospore to move in.

LITERATURE CITED

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