Influence of Calcium Nitrate and Ammonium Sulfate on Phytophthora Root Rot of *Persea indica*

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ABSTRACT

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In greenhouse experiments with soil naturally infested with *Phytophthora cinnamomi*, $Ca(NO_3)_2$ at 100, 200, and 300 μg nitrogen and $(NH_4)_2SO_4$ at 300 μg nitrogen per gram of air-dried soil significantly reduced root rot of *Persea indica*, a close relative of avocado. Nitrapyrin at 1 μg/g of air-dried soil also resulted in higher NH_4 levels and significantly reduced root rot and completely prevented stem canker development, indicating direct toxicity of this chemical to *Phytophthora*. Seedlings grown in Hoagland's solution containing Ca^{++} at 160 μg/ml were more resistant to infection by *P. cinnamomi* than those grown in lower Ca^{++} levels. Reduction in inoculum density following application of $Ca(NO_3)_2$ and high levels of $(NH_4)_2SO_4$ also contributed to the reduction of root rot. Sporangium production in mycelial mats incubated in extracts of these soils was also inhibited. Approximately 2.5 to 3 times more bacteria and actinomycetes were present in these soils compared to the nontreated soil or soil treated with a lower level of $(NH_4)_2SO_4$. In separate experiments, addition of $(NH_4)_2SO_4$ to nonsterile and sterile soil extracts suppressed sporangium production and release of zoospores. Addition of $Ca(NO_3)_2$ stimulated sporangium production, but release of zoospores was poor. In nonsterile soil extracts supplemented with $Ca(NO_3)_2$, large numbers of bacteria were attached to the walls of sporangia and hyphae, and these were associated with breakdown of sporangia and lysis of hyphae.

The type of nitrogen applied to soil often influences the severity of plant diseases (17). With diseases induced by species of *Phytophthora*, Apple (2) showed that both ammonium nitrogen (NH_4-N) and nitrate nitrogen (NO_3-N) predisposed a previously resistant tobacco cultivar (Coker 139) to *Phytophthora parasitica* Dastur. Ammonium-N enhanced the severity of citrus root rot caused by *P. citrophthora* R. E. Sm. & E. H. Sm. and *P. parasitica* while NO_3-N decreased it (18). Pal and Grewal (23) similarly reported that high levels of NH_4-N increased the incidence of pigeon pea blight caused by *P. drechsleri* Tucker var. *cajani* Pal, Grewal & Sarbhoy. On the other hand, soils suppressive to root rot caused in avocado (*Persea americana* Mill.) by *P. cinnamomi* Rands had high levels of NO_3-N and NH_4-N, in addition to high organic matter contents and high levels of exchangeable Ca and Mg (11). More recently, Broadbent (10) reported that maintenance of high levels of NH_4-N with nitrapyrin (a nitrification inhibitor) in pot cultures reduced avocado root rot.

In view of the differences in the effects of the form of N on Phytophthora root rots, laboratory and greenhouse experiments were conducted to examine the influence of $Ca(NO_3)_2$ and $(NH_4)_2SO_4$ on root rot caused in *Persea indica* (L.) Spreng (a close relative of avocado) by *P. cinnamomi*.

MATERIALS AND METHODS

Soil, naturally infested with *P. cinnamomi*, was collected from an avocado grove located in Fallbrook, San Diego County, CA, with the following texture and chemical properties: sand, 76.4%; silt, 9.5%; clay, 14.1%; pH (paste), 7.26; organic matter, 1.89%; organic carbon, 1.10%; total N, 0.01%; exchangeable NH_4^+, 1.04 μg/g; NO_3^-, 10.38 μg/g; C/N ratio, 11.0; cation-exchange capacity, 10.67 me/100 g soil; exchangeable Ca and Mg (in me/100 g soil), 7.24 and 3.61, respectively. Total soil N was determined by a macro-Kjeldahl method (9). Exchangeable NH_4^+ and NO_3^- (in micrograms per gram of soil) were evaluated from wet soil by a modified Kjeldahl method (9) using 1 N KCl as the extracting reagent. Quantitative determination of organic carbon and organic matter was carried out according to the procedures of Walkley and Black (29). The methods of Yaalon et al (30) were used to evaluate the cation-exchange characteristics of the soil. Cation-exchange capacity and exchangeable Ca and Mg were determined with the Perkin-Elmer absorption spectrophotometer (model 370A, Perkin-Elmer Corp., Norwalk, CT 06852). *P. cinnamomi* was isolated from the soil by baiting with 6-wk-old *P. indica* seedlings (33). The fungus (mating type A_2) was designated as Pc 354 in the Phytophthora collection, Department of Plant Pathology, University of California, Riverside.

Influence of nitrogen on root rot and stem canker development. Analytical grades of $Ca(NO_3)_2 \cdot 4H_2O$ (CN) and $(NH_4)_2SO_4$ (AS), with and without nitrapyrin (2-chloro-6-trichloromethyl pyridine) (supplied as N-Serve®, Dow Chemical Co., Midland, MI 48640), were added to the soil at 100, 200, and 300 μg N/g of air-dried soil. Concentration of nitrapyrin used was 1 μg/g air-dried soil. Approximately 700 g of the mixed soil was placed in each 10-cm-diameter fiber pot. Nonsupplemented soil and soil treated with nitrapyrin alone acted as controls. Soil pH was measured in 0.01 M $CaCl_2$ according to the method of Schofield and Taylor (26).

The effect of N on plant growth was studied by using infested soil treated with aerated steam. For this purpose, infested soil was pretreated with aerated steam for 30 min at 60 C. After cooling, the soil was supplemented with CN and AS at identical rates. The experiment was completely randomized with eight seedlings per treatment. Seedlings of *P. indica*, grown previously for 10 wk in sterilized UC mix (5), were used as test plants. All plants received 100 ml of deionized water per day and were examined daily for signs of wilting and canker development. At the end of 9 wk, the plants were removed from the pots and the roots were washed clean of adhering soil particles. Percentage of the root surface area rotted was visually estimated and presence or absence of stem canker was noted. To confirm that the roots and stems were infected with *P. cinnamomi*, representative pieces of necrotic roots and stems were plated on selective PVP-hymexazol antibiotic medium (28). The pH of the soil adhering to the root mass was measured in 0.01 M $CaCl_2$ (26). Soils from each treatment were pooled and analyzed for total N, exchangeable NH_4^+ and NO_3^+ (9), organic carbon and organic matter (29), cation-exchange capacity, and exchangeable bases (30).

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TABLE 1. Influence of $Ca(NO_3)_2$, $(NH_4)_2SO_4$, and nitrapyrin on the severity of *Persea indica* root rot caused by *Phytophthora cinnamomi*

Treatment	Nitrogen added (μg/g soil)	Root rot (%)[y] Without nitrapyrin	NH_4:NO_3	With nitrapyrin (1 μg/g of soil)	NH_4:NO_3
Nontreated soil	0	94 a[z]	0:1	16 a	1:1
Soil + $Ca(NO_3)_2$	100	49 b	1:1	16 a	2:1
	200	47 b	1:1	19 a	2:1
	300	38 b	3:2	7 a	5:1
Soil + $(NH_4)_2SO_4$	100	89 a	1:2	11 a	2:1
	200	77 a	1:3	9 a	6:1
	300	33 b	3:2	10 a	16:1

[y] Percentage surface area of roots rotted. Average of eight seedlings 9 wk in naturally infested soil.
[z] Means followed by the same letter in a column are not signficantly different, $P = 0.05$, by Duncan's multiple range test.

At the end of the experiment, the soils were also tested for ability to support sporangium production and zoospore release. Pc 354 was used as the test organism. Disks (5 mm in diameter) from 4-day-old V-8 juice agar cultures were grown in clear V-8 juice broth (10% strength, v/v) for 24 hr to obtain mycelial mats. The broth was drained and replaced with 10 ml of 10% (w/v) soil extract. Cultures were incubated at room temperature (25 ± 2 C) under continuous cool white lights (1,200 $\mu W \cdot cm^{-2}$, two General Electric F40D daylight fluorescent lamps, General Electric, New York, NY 11022) for 36 hr. Soil extracts were prepared by suspending soil in glass-distilled water (1:10, w/v oven dry wt) overnight and passing it twice through Whatman No. 42 filter papers. Sporangium counts were made by using a Hawksley eelworm counter (Gelman-Hawksley Ltd., Sussex, England). In addition, two plates per treatment were set aside at the end of 36 hr for zoospore release. For this purpose, mycelial mats bearing the sporangia were washed three times with sterilized glass-distilled water, then chilled at 9 C for 30 min before being returned to room temperature for 6 hr. The percent of sporangia-releasing zoospores was determined with the eelworm counter.

Population densities of soil bacteria and actinomycetes were estimated by using the soil dilution plate technique. Ten grams of air-dried soil was placed in a 300-ml prescription bottle containing 90 ml of twice-autoclaved 1% (w/v) soil extract agar. The bottles were shaken at low speed with a wrist-action mechanical shaker for 15 min. Soil dilution series ranging from 10^2 to 10^6 were then prepared. Martin's modified RB-M2 medium (21) was used for the isolation of soil fungi and a 0.3% tryptic soybroth agar (20) for the isolation of soil bacteria and actinomycetes.

Influence of calcium on resistance of *P. indica* to root infection. In the previous experiments, although N was supplied in equivalent amounts, there were imbalances in other ions. Ca is known to influence infection in some instances (1,14,24,27). This study was carried out to determine the influence of Ca on resistance of *P. indica* to root infection by *P. cinnamomi*. Aseptic *P. indica* seeds were obtained by removing the testa and surface sterilizing the remaining parts of the seeds in 20% (v/v) solution of commercial sodium hypochlorite for 30 min. Seeds were then transferred aseptically to 9-cm-diameter petri plates containing 15 ml sterile deionized-distilled (DD) water and allowed to germinate in the dark at 25 ± 1 C. At the end of 2 wk, seedlings were transferred aseptically to capped sterile test tubes (20 × 2.5 cm) with a constriction approximately 8 cm from the base to hold the cotyledons and allow the roots to grow into the nutrient solutions. Nutrient solutions consisted of a 25% (v/v) Hoagland's solution (16) incorporated with 10, 40, 80, and 160 μg Ca^{++}/ml of solution. Calcium ions were supplied in the form of $Ca(NO_3)_2 \cdot 4H_2O$. Nitrogen discrepancies were made up with appropriate amounts of 1 M NH_4NO_3. Seedlings were grown under continuous cool white light (1,200 $\mu W \cdot cm^{-2}$, two General Electric F40D daylight fluorescent lamps) for 8 wk, after which the roots were detached and artificially inoculated with a suspension of zoospores of Pc 354 (10^4 per ml), 15 ml per petri plate. Each plate held three roots of approximately equal length and thickness. After 8 hr, infected roots (three plates per treatment) were stained with a drop of cotton blue in lactophenol. The number of zoospores attracted to each root was determined by blending roots at high speed for 2 min in a Waring Blendor and counting zoospores on an eelworm counter. In addition, the length of the lesions was measured on another set of roots (three plates per treatment) at the end of 36 hr.

TABLE 2. Influence of $Ca(NO_3)_2$ and $(NH_4)_2SO_4$ on stem canker development on *Persea indica* caused by *Phytophthora cinnamomi*[x]

Treatment	Nitrogen added (μg/g soil)	Stem canker (%)[y]	NH_4:NO_3
Nontreated soil	0	87.5 a[z]	0:1
Soil + $Ca(NO_3)_2$	100	37.5 bc	1:1
	200	62.5 ab	1:1
	300	37.5 bc	3:2
Soil + $(NH_4)_2SO_4$	100	100.0 a	1:2
	200	87.5 a	1:3
	300	37.5 bc	3:2

[x] Addition of nitrapyrin at 1 μg/g soil completely controlled stem canker in all treatments.
[y] Percent of eight seedlings with canker after 9 wk in naturally infested soil.
[z] Means followed by the same letter in a column are not significantly different, $P = 0.05$, by Duncan's multiple range test.

To determine the effect of Ca on membrane permeability of roots, plants were removed from the test tubes, and the roots were rinsed twice in sterile DD water. The plants were placed in test tubes each holding 25 ml of sterile DD water. Conductivity of water in these tubes was measured with a "Barnstead" Conductivity Bridge 'PM-70 CM' (Sybron Corp., Boston, MA 02132) at 24 C. Sterility of the plants was checked at the beginning and end of the experiment by streaking samples from surfaces of plants or by swabbing 0.5 ml of the nutrient solutions into plates of nutrient agar and PDA.

Influence of N on sporangium production and zoospore release. Calcium nitrate and AS at 10^2, 5×10^2, and 10^3 μg N/ml were added to 10% (w/v) soil extract, with and without the incorporation of nitrapyrin (2 μg/ml). Nonsupplemented soil extract and soil extract supplemented with nitrapyrin alone served as controls. The extracts were kept in the dark at room temperature (25 ± 2 C) for 72 hr. Similar studies were made with sterile soil extracts (double autoclaved at 1.02 kPa [15 psi] for 30 min). Sterility tests on the soil extracts were performed at the beginning and end of the experiment by swabbing 0.5-ml soil extracts on nutrient agar and PDA. The pH of the extracts was determined before mycelial mats were placed in them.

RESULTS

Influence of N on root rot and stem canker development. Application of CN at 100, 200, and 300 μg N/g of air-dried soil resulted in the significant reduction in the percent of the roots infected with *P. cinnamomi* (Table 1). The greatest reduction occurred with the highest levels of N applied. Addition of nitrapyrin (1 μg/g) significantly reduced root rot in all treatments, regardless of the sources of N. Stem canker was also suppressed by the application of the above rates and sources of N (Table 2). No stem cankers were observed on plants in soil amended with

nitrapyrin. Nontreated plants and those that had received 100 and 200 μg N/g soil (in the form of AS) were the first to show symptoms of infection by *P. cinnamomi*. Dark-brown lesions appeared on the stems at the soil line and infected plants wilted approximately 3 wk after inoculation.

Growth response of *P. indica* to N fertilization in soil treated with aerated steam was significantly greater with AS than with CN (Fig. 1). The best growth was obtained with low to moderate levels of AS. Application of CN to soil did not significantly increase plant growth while addition of nitrapyrin to soil adversely affected growth.

Addition of nitrapyrin to the soil effectively reduced the loss of NH_4^+ through nitrification after 9 wk (Figs. 2 and 3). There were two to 100 times more exchangeable NH_4^+ present in nonsteamed soil and two to 50 times more in steamed soil with nitrapyrin than without nitrapyrin. There was no apparent difference in the levels of exchangeable Ca and Mg after 9 wk (Table 3). Presumably, most of the Ca^{++} in the CN-treated soil was lost from the soil, either through leaching or absorption by the plants. There was a slight increase in the pH of soil treated with CN and nitrapyrin. Application of the high level of AS lowered the soil pH from 7.3 to 6.4, probably as a result of nitrification.

There was a significant reduction in the production of sporangia and release of zoospores by Pc 354 in soil extracts prepared from soils treated for 9 wk with CN at 100 to 300 μg N/g of soil and AS at 300 μg N/g of soil (Table 4). The greatest reduction occurred with the highest level of N applied. The highest number of sporangia and zoospores occurred in soil extracts prepared from the nontreated soil and from soil supplemented with 100 μg N/g of soil. Addition of nitrapyrin significantly reduced the production of sporangia and release of zoospores regardless of the N status of the soil.

Enumeration of microbial population densities in soil using the soil dilution plate technique indicated that soils treated for 9 wk with CN at 100 to 300 μg N/g soil and with AS at 300 μg N/g soil had higher population densities of bacteria and actinomycetes (Table 5). Nitrapyrin nullified the stimulatory effects of CN and AS on bacteria and actinomycetes.

Influence of N on sporangium production and zoospore release. Calcium nitrate significantly stimulated sporangium production

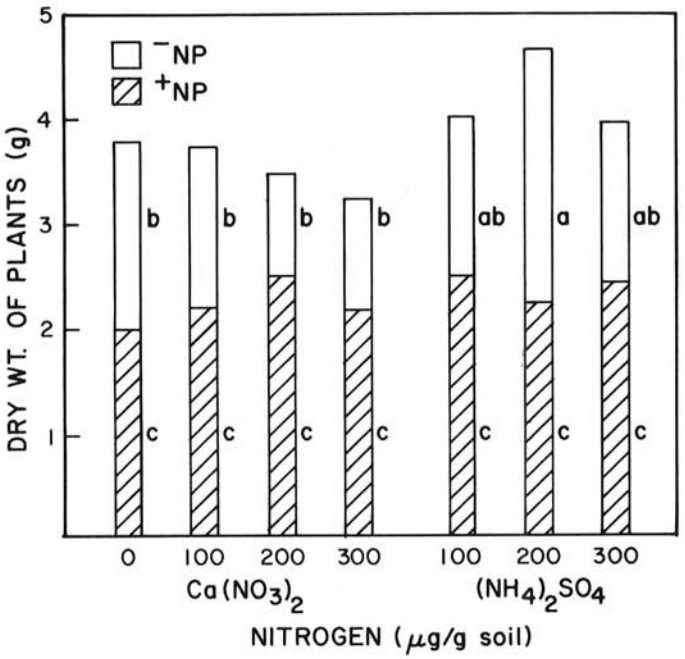


Fig. 1. Dry weight (g) of 4-mo-old *Persea indica* seedlings grown in aerated steam-treated soil supplemented with $Ca(NO_3)_2$ and $(NH_4)_2SO_4$, with and without 1 μg nitrapyrin per gram of air-dried soil. Average of eight plants. Means followed by the same letter are not significantly different from one another, $P = 0.05$, by Duncan's multiple range test.

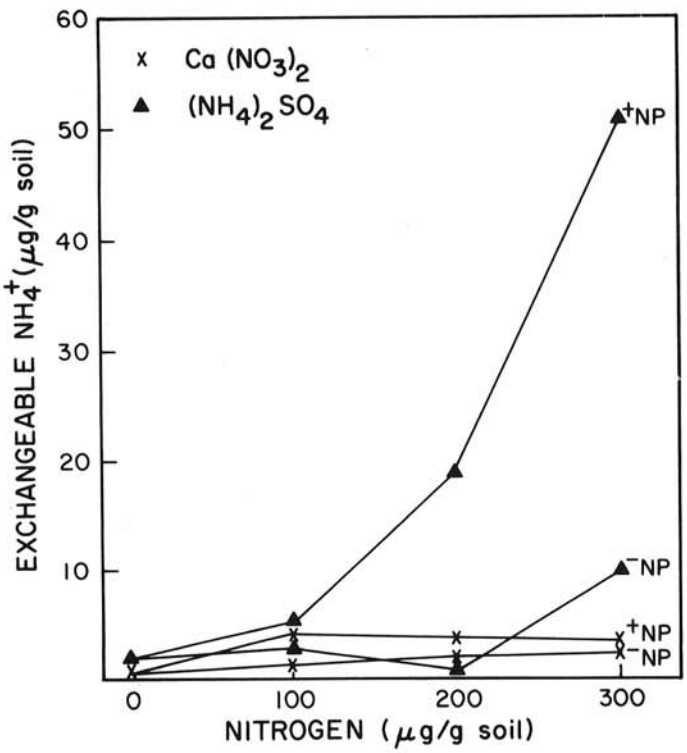


Fig. 2. Influence of nitrapyrin (NP) at 1 μg/g soil on exchangeable NH_4^+ content of soil planted with *Persea indica* seedlings. Soil previously treated with $Ca(NO_3)_2$ and $(NH_4)_2SO_4$ at 100, 200, and 300 μg N/g soil for 9 wk.

TABLE 3. Initial hydrogen-ion concentration (pH) of soil following amendment with $(NH_4)_2SO_4$, $Ca(NO_3)_2$, and nitrapyrin (NP), and the pH and levels of exchangeable Ca and Mg of the soil after 9 wk of growth of *Persea indica* seedlings

Treatment	Nitrogen added (μg/g soil)	Soil pH[y]		Exchangeable cation in soil (me/100 g air-dried soil)	
		Initial	After 9 wk	Ca	Mg
Nontreated soil		7.3	7.1	8.7	3.5
Soil + NP[z]		7.4	7.5	6.3	2.9
Soil + $Ca(NO_3)_2$	100	7.4	7.6	8.1	2.8
	300	7.3	7.2	5.8	2.7
	100 + NP	7.3	7.6	9.9	2.7
	300 + NP	7.2	7.5	5.5	2.7
Soil + $(NH_4)_2SO_4$	100	7.4	7.0	6.8	3.0
	300	7.3	6.4	2.3	2.2
	100 + NP	7.3	7.3	5.3	2.4
	300 + NP	7.3	7.4	7.9	2.8

[y] Measured in 0.01 M $CaCl_2$ (26).
[z] Nitrapyrin at 1 μg/g air-dried soil.

while AS was inhibitory (Figs. 4 and 5). Although CN stimulated sporangium production, the majority of these sporangia did not release zoospores. In the nonsterile soil extracts supplemented with CN many of the sporangia were abortive and the hyphae became lysed and disintegrated after 36 hr. Bacteria and protozoa were associated with the fungal structures. Sporangia produced in sterile soil extract supplemented with CN frequently proliferated externally and the percentage of sporangia that released zoospores was low. Application of AS at 10^2 μg N/g of soil had little or no influence on zoospore release. Drastic reduction in the percentage of sporangia that released zoospores occurred when higher levels of AS were applied. Differentiation of zoospores occurred in sporangia produced in high AS supplemented soil extracts; however, these sporangia did not produce zoospores.

Influence of Ca on resistance of *P. indica* to root infection. The ion efflux (electrical conductivity) data suggest that high Ca levels decreased permeability of the root membrane slightly (Fig. 6). Application of high Ca levels increased resistance of roots to infection by *P. cinnamomi* zoospores even though the differences in the number of zoospores attracted to each root was not significant (Table 6). With high levels of Ca (160 μg/ml), the average length of root lesion was 4.4 mm. With the low Ca treatments (10 to 80 μg/ml), the average length of lesion ranged from 9.3 to 13.0 mm. The average number of zoospores attracted to each 3-cm-long root piece varied from 1,300 to 1,500 with most of them attracted to the zone of elongation.

DISCUSSION

Control of soilborne plant pathogens through quantitative and qualitative manipulation of nutrients may be attributed to reduced inoculum density or increased host resistance or both. In the present studies, severity of *P. indica* root rot was significantly

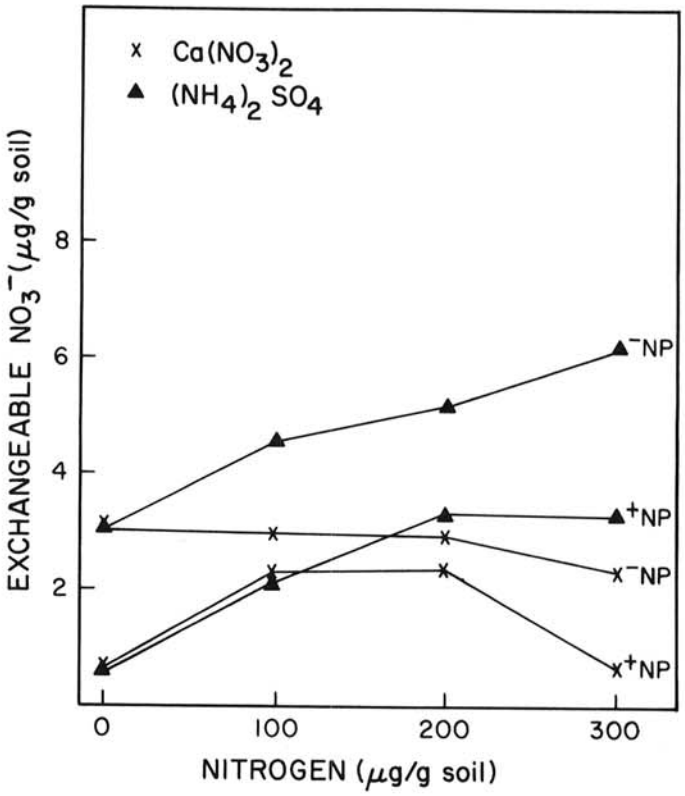


Fig. 3. Influence of nitrapyrin (NP) at 1 μg/g soil on exchangeable NO_3^- content of soil planted with *Persea indica* seedlings. Soil previously treated with $Ca(NO_3)_2$ and $(NH_4)SO_4$ at 100, 200, and 300 μg N/g soil for 9 wk.

TABLE 4. Influence of $Ca(NO_3)_2SO_4$, and nitrapyrin on production of sporangia and release of zoospores by *Phytophthora cinnamomi* in nonsterile soil extracts prepared from amended soils planted with *Persea indica* seedlings

Source of soil extract	Nitrogen added (μg/g soil)	Without nitrapyrin		With nitrapyrin (1 g/g of soil)	
		Sporangia ($\times 10^2$)[y]	Zoospore release (%)	Sporangia ($\times 10^2$)[y]	Zoospore release (%)
Nontreated soil		30.4 a[z]	62.0 a	6.9 a	20.3 a
Soil + $Ca(NO_3)_2$	100	17.0 b	50.5 a	8.4 a	21.8 a
	300	13.0 b	29.6 b	9.4 a	16.7 a
Soil + $(NH_4)_2SO_4$	100	31.0 a	49.4 a	12.4 a	33.3 b
	300	12.7 b	14.2 c	8.5 a	5.0 c

[y] Number of sporangia produced per mycelial mat incubated in 10% (w/v) soil extract for 36 hr.
[z] Means followed by the same letter in a column are not significantly different, $P = 0.05$, by Duncan's multiple range test.

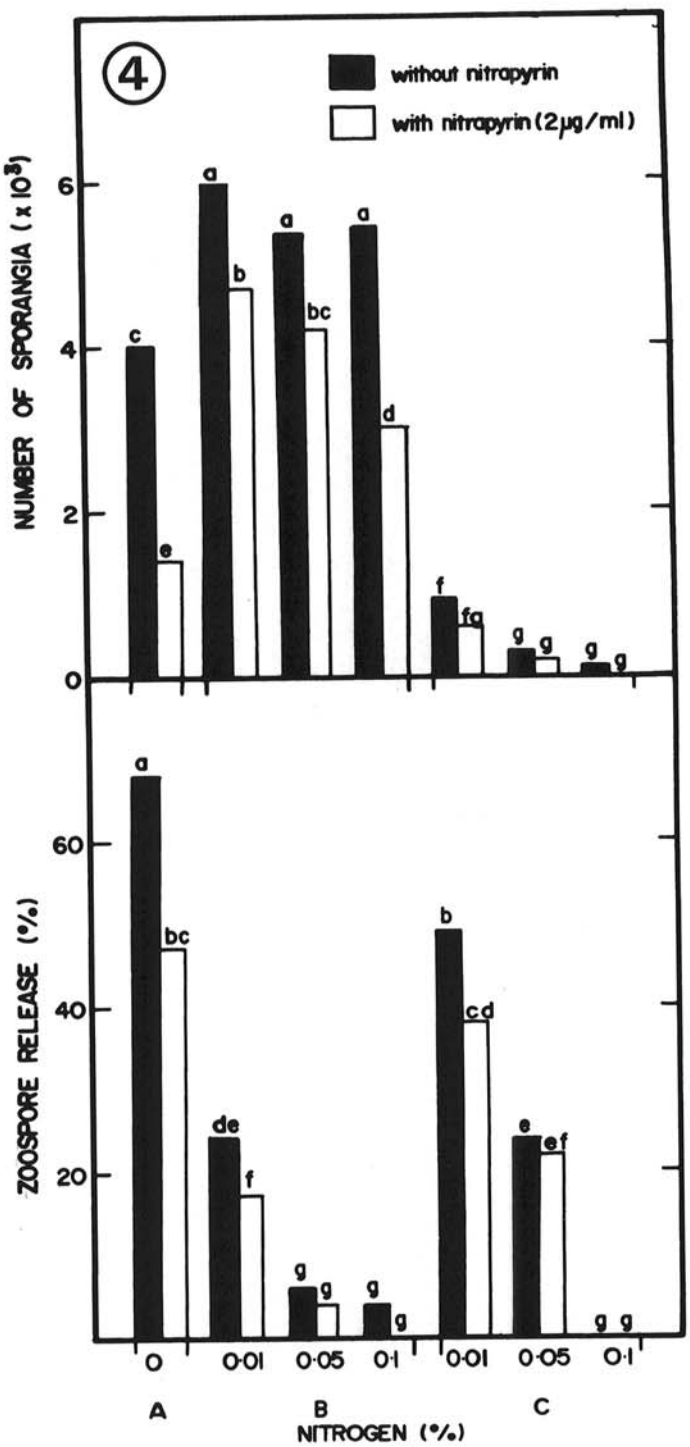
TABLE 5. Influence of $Ca(NO_3)_2$, $(NH_4)_2SO_4$, and nitrapyrin (NP) on the number of bacteria, actinomycetes, and fungi in soil planted with *Persea indica* seedlings

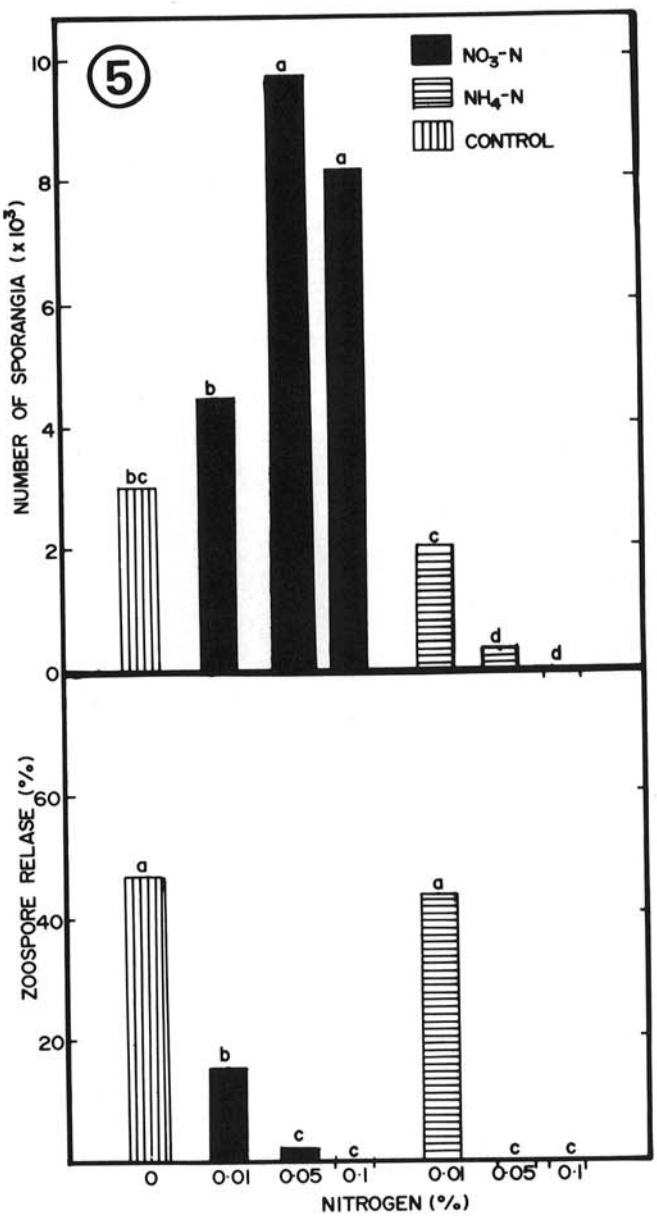
Treatment	Nitrogen added (μg/g soil)	Colony number/g air dried soil[y]	
		Bacteria and air-dried soil ($\times 10^6$)	Fungi ($\times 10^4$)
Nonsupplemented soil		31 def	29 a
Soil × NP[z]		56 cde	18 c
Soil + $Ca(NO_3)_2$	100	73 b	45 ab
	300	100 a	49 a
	100 + NP	27 def	31 bc
	300 + NP	55 bcd	33 abc
Soil + $(NH_4)_2SO_4$	100	20 f	18 c
	300	79 ab	21 c
	100 + NP	70 bc	30 bc
	300 + NP	30 def	18 c

[y] Means followed by the same letter are not significantly different, $P = 0.05$, by Duncan's multiple range test.
[z] Nitrapyrin at 1 μg/g air-dried soil.

reduced through the application of CN at 100–300 μg N and AS at 300 μg N/g soil. Application of AS at lower rates did not control root rot, although growth of host plants was significantly increased. This may result from the relatively high residual NO_3 levels present in this soil.

Decrease in inoculum density following application of CN and high levels of AS in soil appears to be a major factor contributing to the decrease of root rot. Mycelial mats of *P. cinnamomi* grown in these soil extracts produced significantly fewer sporangia and these released a lower percentage of zoospores as compared to mats grown in soil extracts derived from soil treated with lower rates of AS or nontreated soil. The reduction in inoculum density following application of CN and the high level of AS could be the result of antagonistic effects of bacteria and actinomycetes since their numbers in these treated soils were greatly increased. Forms of antagonism generally recognized to explain the activity of soil microflora against various soilborne pathogens include antibiosis and various forms of competition (6). Additional studies showed that AS added to nonsterile soil extracts significantly suppressed sporangium production and zoospore release. The suppressive effect was proportional to the amount of AS added. Reduction in root rot severity appears to be related to high NH_4/NO_3 ratio in soil (Table 1). Ammonia can be released when NH_4 salts are applied to soils and soil solutions, with the greatest release from calcareous soils at high soil pH (13,25). Control of avocado root rot by amending soil with alfalfa meal was attributed in part to the fungicidal action of NH_3 towards *P. cinnamomi* (15). It is possible that NH_3 is involved in the suppression of sporangium production and release of zoospores in soil extracts supplemented with high levels of AS. Addition of CN to nonsterile soil extracts stimulated the production of sporangia. The percentage of these sporangia producing zoospores was poor. Similar results were obtained with sterile soil extracts supplemented with CN, suggesting that CN





Figs. 4 and **5.** Sporangium production and release of zoospores of Pc 354 in **4,** nonsterile and **5,** sterile soil extracts supplemented with $Ca(NO_3)_2$ and $(NH_4)_2SO_4$ at various rates for 72 hr. **A,** 10% (w/v) nonsterile soil extract alone; **B,** with $Ca(NO_3)_2$; **C,** with $(NH_4)_2SO_4$. Bars with the same letters are not significantly different, $P = 0.05$ by Duncan's multiple range test.

could directly increase sporangium production, independent of microbial activity.

Microscopic examination of the sporangia produced in nonsterile soil extract incorporated with CN suggested that bacteria were associated with sporangial breakdown and hyphal lysis. Bacteria were attracted to the walls of sporangia and mycelia and internal proliferation of the new sporangia occurred under these conditions. However, the new sporangia did not generally release zoospores. The production of sporangia by *P. cinnamomi* is dependent upon the production of metabolites and/or the depletion of nutrients by soil bacteria (32). Among the bacteria implicated are *Pseudomonas* spp. (3,4,19,22) and *Chromobacterium violaceum* (31). *Bacillus subtilis* var. *niger* and certain species of *Pseudomonas* and *Flavobacterium* were associated with sporangial breakdown and hyphal lysis (12).

Seedlings grown aseptically in nutrient solutions containing high levels of Ca were more resistant to infection by *P. cinnamomi* than those grown in lower levels of Ca. In one of the experiments, roots of seedlings grown in nutrient solution containing Ca^{++} at 160 μg/ml were significantly more resistant to infection by zoospores than those that had received less Ca. Whether this was due to reduced penetration or to reduced colonization within the host tissues was not determined. Divalent cations such as Ca^{++} and Mg^{++} contribute to the structural integrity of plant tissues by forming salt bridges between uronic acid carboxyls of adjacent uronide polymers and possibly between these polymers and proteins (7). Changes in the electrical conductivity of sterilized deionized distilled water in which intact roots were immersed indicated that roots of seedlings grown in lower Ca concentrations were more permeable than those that had received higher levels of Ca. Decreased permeability of roots is probably not the mechanism for increased resistance since there were no significant differences in the number of zoospores attracted to these roots. Bingham et al (8) observed some reduction of root infection when avocado seedlings were grown in nutrient solutions containing high levels of NO_3-N (1,000 μg/ml) or K (1,500 μg/ml). They further reported that alteration of ratios of exchangeable Ca, Mg, K, Na, and H in soil in which avocado seedlings were grown had no subsequent effect on the infection of the root by *P. cinnamomi*.

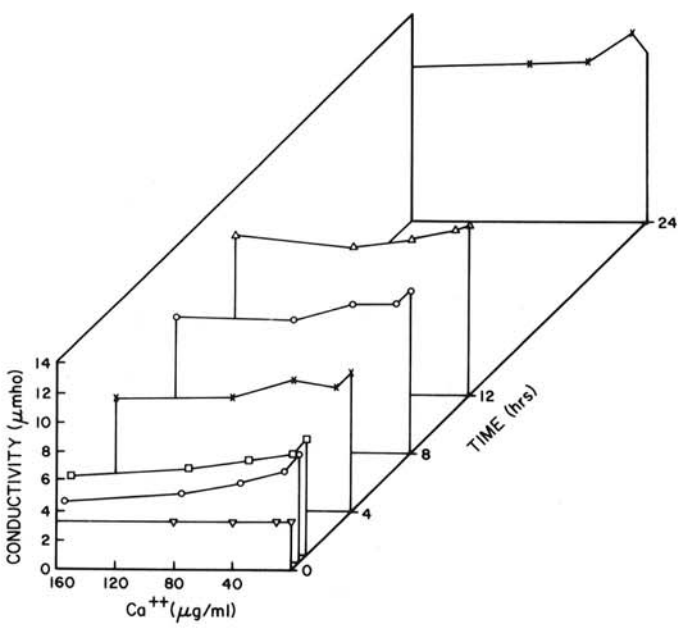


Fig. 6. Efflux of ions from roots, as measured by electrical conductivity changes, of 2-mo-old *Persea indica* seedlings grown in 25% (v/v) Hoagland's solution (16) with different levels of Ca^{++}.

TABLE 6. Attraction of zoospores of *Phytophthora cinnamomi* to roots of *Persea indica* seedlings grown in 25% (v/v) Hoagland's solution (16) supplemented with $Ca(NO_3)_2$ at various concentrations, and the subsequent root lesion size after 36 hr

Ca^{++} (μg/ml)	Number of zoospores[y]	Root lesion (mm) after 36 hr[y]
10	1,367 a[z]	9.3 a
40	1,310 a	13.3 a
80	1,500 a	12.7 a
160	1,300 a	4.4 b

[y] Average of nine roots per treatment.
[z] Means followed by the same letter are not significantly different, $P = 0.05$, by Duncan's multiple range test.

There was no canker development in treatments incorporated with nitrapyrin, and the percent of root rot in all nitrapyrin-treated soils, regardless of N source, was significantly lower than in the control. This strongly suggests that control of root rot with nitrapyrin is the result of direct toxicity of the compound to the fungus. Broadbent (10) reported that maintenance of a high level of NH_4-N with nitrapyrin in pot cultures reduced avocado root rot. In view of the present findings on the toxicity of nitrapyrin to *P. cinnamomi*, past reports on the influence of NH_4-N plus nitrapyrin as the nitrification inhibitor on disease control probably need to be reevaluated using varying sources of NH_4-N and several different nitrification inhibitors.

The soil used in the present studies was collected from an avocado grove heavily infested with *P. cinnamomi*. This soil was characterized by low organic matter content (1.89×10^4 μg/g soil), low total N (10^3 μg/g), and a relatively low exchangable Ca status (7.24 me/100 g soil). The soil thus had certain properties similar to the soils in Australia reported as conducive to Phytophthora root rot (11). Due to its low cation-exchange capacity and low organic matter content, most of the Ca added to the soil remained free in the soil solution, resulting in the depletion of most of the calcium ions by 9 wk. Among the factors to be considered when nutrients are used to reduce diseases is the ability of the nutrients to be retained in the soil within the root zones for a maximum period of time.

LITERATURE CITED

1. Anderson, W. C., Gabrielson, R. I., Haglund, W. A., and Baker, A. S. 1976. Clubroot control in crucifers with hydrated lime and PCNB. Plant Dis. Rep. 60:561-565.
2. Apple, J. L. 1961. The development of black shank in tobacco as influenced by host nutrition. Phytopathology 51:386-389.
3. Ayers, W. A. 1971. Induction of sporangia in *Phytophthora cinnamomi* by a substance from bacteria and soil. Can. J. Microbiol. 17:1517-1523.
4. Ayers, W. A., and Zentmyer, G. A. 1971. Effect of soil solution and two soil pseudomonads on sporangium production by *Phytophthora cinnamomi*. Phytopathology 61:1188-1193.
5. Baker, K. F. (ed.). 1957. The U.C. system for producing healthy container-grown plants. Calif. Agric. Exp. Stn. Man. 23:1-332.
6. Baker, K. F., and Cook, R. J. 1974. Biological Control of Plant Pathogens. W. H Freeman, San Francisco. 433 pp.
7. Bateman, D. F., and Miller, R. L. 1966. Pectic enzymes in tissue degradation. Annu. Rev. Phytopathol. 4:119-146.
8. Bingham, F. T., Zentmyer, G. A., and Martin, J. P. 1958. Host nutrition in relation to *Phytophthora* root rot of avocado seedlings. Phytopathology 48:144-148.
9. Bremmer, J. M. 1965. Inorganic forms of nitrogen. Pages 1179–1237 in: Methods of soil analysis, Part 2. Chemical and microbiological properties. No. 9. Agronomy. C. A. Black, D. D. Evans, J. L. White, L. E. Ensminger, and R. C. Dinauer, eds. Am. Soc. Agronomy, Madison, WI. 1572 pp.
10. Broadbent, P. 1977. Organic amendments for avocados. Aust. Nurserymen's Association Ltd., Annu. Conf., Hobart, Tasmania. 38 pp.
11. Broadbent, P., and Baker, K. F. 1974. Behaviour of *Phytophthora cinnamomi* in soils suppressive and conducive to root rot. Austr. J. Agric. Res. 25:121-137.
12. Broadbent, P., and Baker, K. F. 1974. Association of bacteria with sporangium formation and breakdown of sporangia in *Phytophthora* species. Austr. J. Agric. Res. 25:139-145.
13. Fenn, L. B., and Escarazaga, R. J. 1976. Ammonia volatilization from surface application of ammonium compounds on calcareous soils. V. Soil water content and method of nitrogen application. Soil Sci. Soc.

Am. 40:537-541.
14. Gill, D. L. 1972. Effect of gypsum and dolomite on *Pythium* diseases of seedlings. J. Am. Soc. Hortic. Sci. 97:467-471.
15. Gilpatrick, J. B. 1969. Role of ammonia in the control of avocado root rot with alfalfa meal soil amendment. Phytopathology 59:973-978.
16. Hoagland, D. R., and Arnon, D. I. 1938. The water-culture method for growing plants without soil. Calif. Agric. Exp. Stn. Circ. 347. Berkeley, CA.
17. Huber, D. M., and Watson, R. D. 1974. Nitrogen form and plant disease. Annu. Rev. Phytopathol. 12:139-165.
18. Klotz, L. J., De Wolf, T. A., and Wong, P. 1958. Decay of fibrous roots of citrus. Phytopathology 48:616-622.
19. Manning, W. J., and Crossan, D. F. 1966. Effects of a particular soil bacterium on sporangial production in *Phytophthora cinnamomi* in liquid cultures. Phytopathology 56:235-237.
20. Martin, J. K. 1975. Comparison of agar media for counts of viable soil bacteria. Soil Biol. Biochem. 7:401-402.
21. Martin, J. P. 1950. Use of acid, rose bengal and streptomycin in the plate method for estimating soil fungi. Soil Sci. 69:215-232.
22. Marx, D. H., and Haasis, F. A. 1965. The induction of aseptic sporangial formation in *Phytophthora cinnamomi* by metabolic diffusates of soil microorganisms. Nature (Lond.) 206:673-674.
23. Pal, M., and Grewal, J. S. 1976. Effect of NPK fertilizers on the *Phytophthora drechsleri* blight of pigeon-pea. Indian J. Agric. Sci. 46:32-35.
24. Ranney, C. D., and Bird, L. S. 1958. Influence of fungicides, calcium salts, growth regulators, and antibiotics on cotton seedling diseases when mixed with the covering soil. Plant Dis. Rep. 42:785-791.
25. Rolston, D. E. 1978. Volatile losses of nitrogen from soil. Pages 163–193 in: Management of Nitrogen in Irrigated Agriculture. P. F. Pratt, ed. Univ. of Calif., Riverside. 442 pp.
26. Schofield, R. K., and Taylor, A. W. 1955. The measurement of soil pH. Soil Sci. Soc. Am. Proc. 19:164-167.
27. Sitterly, W. R. 1962. Calcium nitrate for field control of tomato southern blight in South Carolina. Plant Dis. Rep. 46:492-494.
28. Tsao, P. H., and Guy, S. O. 1977. Inhibition of *Mortierella* and *Pythium* in a *Phytophthora* isolation medium containing hymexazol. Phytopathology 67:796-801.
29. Walkley, A., and Black, I. A. 1934. An examination of the Degtareff method for determining soil organic matter and proposed modification of the chromic acid titration method. Soil Sci. 37:29-38.
30. Yaalon, D. H., Schnylenborgh, J. V., and Slager, S. 1962. The determination of cation-exchange characteristics of saline and calcareous soils. Neth. J. Agric. Sci. 10:217-221.
31. Zentmyer, G. A. 1965. Bacterial stimulation of sporangium production in *Phytophthora cinnamomi*. Science 50:1178-1179.
32. Zentmyer, G. A., and Erwin, D. C. 1970. Development and reproduction of *Phytophthora*. Phytopathology 60:1120-1143.
33. Zentmyer, G. A., and Ohr, H. D. 1978. Avocado root rot. Univ. Calif., Div. Agric. Sci., Berkeley Leafl. 2440. 15 pp.