Effect of Exchangeable Soil Aluminum and Alkaline Calcium Salts on the Pathogenicity and Growth of *Phytophthora capsici* from Green Pepper

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Accepted for publication 23 June 1980.

ABSTRACT

MUCHOVEJ, J. J., MAFFIA, L. A., and MUCHOVEJ, R. M. C. 1980. Effect of exchangeable soil aluminum and alkaline calcium salts on the pathogenicity and growth of *Phytophthora capsici* from green pepper. Phytopathology 70:1212-1214.

In greenhouse studies $CaCO_3$ or $Ca(OH)_2$, but not $CaSO_4$ or $CaCl_2$ significantly increased seedling blight caused in green pepper by *Phytophthora capsici*. This enhancement of disease is attributed to immobilization of exchangeable soil aluminum by $CaCO_3$ and $Ca(OH)_2$, but not by $CaSO_4$ or $CaCl_2$. There was no relationship between tissue calcium and disease. In vitro reduction in mycelial growth occurred in culture media amended with Al^{+3} at levels corresponding to natural levels in soil. *P. capsici* was recovered from all soils indicating that suppression was fungistatic rather than fungitoxic.

Additional key words: soil pH, soil fertility.

Exchangeable soil calcium, soil pH, and exchangeable soil aluminum are soil fertility factors that reduce the development of diseases caused by some soilborne plant pathogens. Lewis (10) showed that calcium reduces root rot of peas caused by *Aphanomyces euteiches* Drechs. and that the control was not necessarily accompanied by changes in soil pH. Broadbent and Baker (3) showed that exchangeable calcium is higher in soils suppressive to *Phytophthora cinnamomi* Rands causing root rot of avocado than in conducive soils. Anderson et al (1) controlled clubroot (caused by *Plasmodiophora brassicae* Wor.) in crucifers by applying lime, and Jones and Woltz (7,8) demonstrated that hydrated lime added to soil reduces the occurrence of Fusarium wilt of tomato. Bateman (2) showed that soils with pH 5.5 or lower suppress root rot of poinsettia caused by *Thielaviopsis basicola* (Berk. & Br.) Ferr. and *Pythium ultimum* Trow.

Natural soil aluminum has been shown to reduce pathogenesis of both *Verticillium albo-atrum* Reinke & Berthold and *Sclerotinia sclerotiorum* (Lib.) de Bary (= *Whetzelina sclerotiorum* (Lib.) Korf & Dumont) to sunflowers (12). Ko and Hora (9) demonstrated that natural soil aluminum is toxic to *Neurospora tetrasperma* Shear & Dodge; however, Jackson (6) found that aluminum added to soil as $Al_2(SO_4)_3$ is only of doubtful significance in the control of damping-off of pines grown in sand cultures.

Phytophthora capsici Leonian is an important blight-causing pathogen of green peppers in Brazil. Many Brazilian soils are fairly acid (pH 4.5–5.0) with high amounts of exchangeable soil aluminum and low levels of other mineral nutrients (11). The purpose of this study was to determine the effect of soil fertility factors (Al^{+3}, Ca^{+2}, and pH) on the development of *Phytophthora* blight of pepper (*Capsicum frutescens*).

0031-949X/80/12121203/$03.00/0

MATERIALS AND METHODS

Greenhouse: The soil used in these studies was an acidic oxisol collected from a cerrado region near Uberlândia, MG, Brazil. The original soil pH was 4.8, exchangeable calcium was 0.2 meq/100 g, and exchangeable aluminum was 0.6 meq/100 g of soil. Soils were amended with either 0, 200, 400, 600, 800, or 1,000 $\mu g/g$ Ca^{+2} as either $CaCl_2$, $CaCO_3$, $Ca(OH)_2$, or $CaSO_4$. Soil was then mixed for 1 hr in a rotary twin-arm soil mixer, brought to field capacity, and incubated for 4 mo in polyethylene bags. The soils were then distributed into 200-ml plastic cups, and one pepper seed was planted per cup. All treatments were replicated four times with each replicate consisting of 10 cups and all experiments were repeated twice.

When the seedlings were about 25 days old, each was inoculated with 1 ml of a suspension containing 10^6 zoospores of *P. capsici* placed on the surface of the soil in each cup. The soil moisture was maintained at field capacity for an additional 15 days and the number of dead plants was recorded.

Plants were then harvested, dried, ground, digested with $HNO_3 + HClO_4$ and analyzed for tissue calcium by using the EDTA titration method (14). Soils were analyzed for pH, Ca^{+2}, and Al^{+3} by

using standard methods (11).

Survival of *P. capsici* in the soil was determined by taking soil samples from inoculated cups in each treatment, letting the soil become air-dry, and plating 50 mg of the soil on selective medium (50 μg/g streptomycin sulfate, 60 μg/g technical PCNB and 100,000 units of Nystatin in cornmeal agar) (4).

Bioassay: The effect of Al^{+3} ions on mycelial growth of *P. capsici* was determined by incorporating $Al(NO_3)_3 \cdot 8H_2O$ at 0 to 0.6 meq Al^{+3} into filtered cornmeal agar (pH 6.0) and the effect of pH on mycelial growth was determined by using HNO_3-Tris to modify the pH of filtered cornmeal agar from 4.1 to 6.5 at increments of 0.3. Plates were inoculated with a 5-mm-diameter disc of *P. capsici* taken from the edge of a 7-day-old culture. Plates were then incubated at 26 C and the diameter of the zone of growth was recorded every 24 hr for 6 days.

RESULTS

The percentage of plants killed by *P. capsici* was greater in soil amended with $Ca(OH)_2$ or $CaCO_3$ than in nonamended soil or in soil amended with $CaCl_2$ or $CaSO_4$ (Table 1). Increased levels of $CaSO_4$ did not significantly increase the amount of seedling disease. In general, $CaCO_3$ amendments resulted in fewer diseased plants than $Ca(OH)_2$ amendments. *P. capsici* was isolated from lesions from diseased plants as well as from soil taken from all treatments.

$CaCl_2$ inhibited seed germination at the 800 and 1,000 μg/g level of Ca^{+2}. All plants grown in $CaCl_2$- or $CaSO_4$-amended soil or in nonamended soil developed a russeting of the leaves indicating acid soil infertility and/or Al^{+3} toxicity.

Soil pH increased with increasing amendments of $Ca(OH)_2$ or $CaCO_3$, but not with $CaCl_2$ or $CaSO_4$. $CaCO_3$ was more effective than $Ca(OH)_2$ in raising soil pH (Table 2). Exchangeable soil aluminum was completely immobilized by the addition of as little as 200 μg/g (1meq Ca/100 g soil) calcium as either $CaCO_3$ or $Ca(OH)_2$, but no exchangeable soil aluminum was immobilized by the addition of $CaCl_2$ or $CaSO_4$.

Tissue calcium increased significantly over the control when any calcium salt was added to the soil (Table 3), however, there was no relationship between tissue calcium and disease. $CaCO_3$ gave the best overall increase in tissue calcium.

As little as 0.4 meq Al^{+3}/100 g (36 μg/g) in the culture medium significantly reduced mycelial growth of *P. capsici*. At 0.1 to 0.3 meq Al^{+3}, *P. capsici* colonies grew at a rate of 0.45 mm diameter increase per day. At 0.4, 0.5, and 0.6 meq Al^{+3} colony (Fig. 1.) growth rates were 0.39, 0.33, and 0.19 mm/day respectively. There was no reduction in mycelial growth of *P. capsici* at pH of 4.1 or higher.

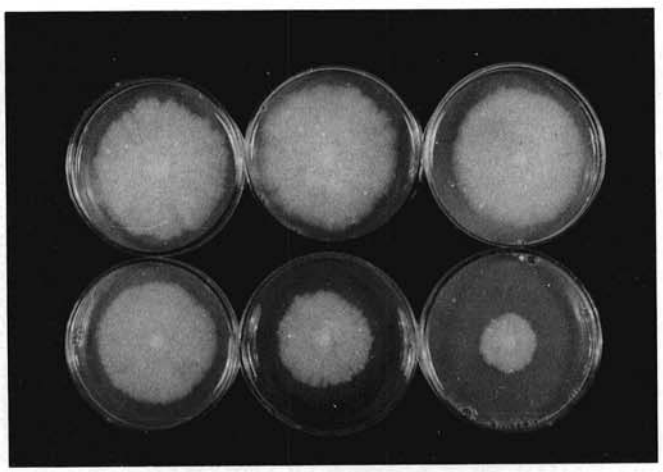

Fig. 1. Al^{+3} inhibition of *Phytophthora capsici* after 6 days of growth on filtered cornmeal agar supplemented with 0.1 to 0.6 meq Al^{+3}. Top row (left to right) 0.1, 0.2, and 0.3 meq Al^{+3}, bottom row (left to right) 0.4, 0.5, and 0.6 meq Al^{+3}.

DISCUSSION

Disease enhancement by the addition of $CaCO_3$ or $Ca(OH)_2$, but not by the addition of $CaCl_2$ or $CaSO_4$ indicates that Al^{+3} ions may retard the development of *P. capsici* in soil. According to Tisdale and Nelson (13) the addition of lime ($Ca[OH]_2$ or $CaCO_3$) neutralizes soluble soil aluminum by forming the insoluble aluminum salt, $Al(OH)_3$. The addition of $CaCl_2$ or $CaSO_4$ would form the salts $AlCL_3$ and $Al_2(SO_4)_3$ which have solubilities of 69.9 g and 31.3 g/100 ml H_2O, respectively (15). This substantiates our results that $CaCO_3$ or $Ca(OH)_2$ limits the solubility and availability of soil aluminum. The lowest amount of $Ca(OH)_2$ we added in our experiments was sufficient to immobilize the aluminum present in our soil.

The results from the bioassay also confirmed that Al^{+3} ions, and not pH, supresses mycelial growth of *P. capsici*. This, and the fact that *P. capsici* was recovered from all infested soils, indicates that the suppression is fungistatic rather than fungitoxic.

The lack of correlation between tissue calcium and disease occurrence indicates that disease suppression is not due to inhibition of pathogen-produced pectinases that have been implicated in the suppression of other diseases (5).

The use of $CaCl_2$ and $CaSO_4$ in quantities as low as 1 meq Ca/100 g soil (200 μg/g) provided sufficient calcium for plant growth; however, there was an apparent sensitivity of green pepper seedlings to the exchangeable aluminum with these salts. It is not known if this sensitivity will limit field production, but manipulation of exchangeable soil aluminum appears to be useful in the control of *P. capsici* blight.

TABLE 1: Effect of calcium salts on death of green peppers (expressed as percent dead seedlings) inoculated when 25 days old with *Phytophthora capsici* in the greenhouse

Ca salt added	Percent dead seedlings[a] in soil amended with indicated μg Ca per gram of soil					
	0	200	400	600	800	1,000
$CaCl_2$	0	27	8	7	...	...
$Ca(OH)_2$	0	45	84	90	50	65
$CaCO_3$	0	20	47	50	63	40
$CaSO_4$	0	0	10	10	9	10
LSD ($P = 0.05$) = 22						

[a] Seedlings were evaluated 15 days after inoculation.

TABLE 2. Effect of calcium salts on the pH of amended soil sampled after harvesting[a]

Ca salt added	pH of soil amended with indicated μg Ca per gram of soil					
	0	200	400	600	800	1,000
$CaCl_2$	4.8	4.3	4.3	4.3	...	...
$Ca(OH)_2$	4.8	6.1	6.2	6.7	7.1	7.5
$CaCO_3$	4.8	6.0	6.4	7.0	7.5	8.0
$CaSO_4$	4.8	5.1	5.1	5.0	4.8	4.8

[a] Pepper plants were inoculated with *Phytophthora capsici* at 25 days and were harvested 15 days later.

TABLE 3. Effect of soil amendments of calcium on the calcium content[a] of tissue of green pepper seedlings

Ca salt added	Percent Ca in seedlings grown in soil amended with indicated μg Ca per gram of soil					
	0	200	400	600	800	1,000
$CaCl_2$	0.19	0.88	0.89	0.91	...	...
$Ca(OH)_2$	0.19	0.77	0.91	0.92	1.16	0.97
$CaCO_3$	0.19	0.80	0.86	0.94	1.01	1.38
$CaSO_4$	0.19	0.67	0.84	0.99	0.92	1.06
LSD ($P = 0.05$) = 0.12						

[a] Expressed as percent calcium in tissues.

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