## Effect of Mineral Salts on Aphanomyces euteiches and Aphanomyces Root Rot of Peas

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## ABSTRACT

Of the salts of 11 cations tested, only  $CuSO_4$  and  $FeCl_3$  at concentrations of 50  $\mu g$  cation/ml or less almost completely inhibited growth, zoospore formation, and zoospore germination in *Aphanomyces euteiches* in vitro. The effects of the other cations (Al, B, Ba, Ca, Co, Mg, Mn, Mo, and Zn) on these processes were varied. The addition of Al, Ca, Cu, and Zn at concentrations of 50 and 100  $\mu g/g$  of soil significantly reduced root rot of peas without apparent adverse effects on the plants. The cations were effective in combination with various anions. Most of the salts, which included the chlorides, nitrates, phosphates, sulfates, and carbonates of Al, Ca, Cu, and

Zn, significantly reduced pea root rot. Addition of a complete fertilizer to amended soil did not alter the effectiveness of the cations in disease reduction. The effectiveness of Al, Cu, and Zn was sustained throughout three pea plantings, but that of Ca was lost after the second planting. No additional benefit was derived from using Al, Cu, or Zn in combination with each other. Copper at 50  $\mu$ g/g, or Cu at 25  $\mu$ g/g in combination with either Al or Zn at 25  $\mu$ g/g, almost completely suppressed disease. Possible use of these materials in agriculture to reduce Aphanomyces root rot of peas is discussed.

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For the past 50 years, root rot of peas, caused by Aphanomyces euteiches Drechs., has caused serious losses in the pea-growing areas of the USA. Control or suppression of this disease by economical methods is still very difficult, and resistant cultivars have not been developed.

Chemical fertilizers are known to alleviate the disease under some conditions. Nitrate and ammonium salts, for example, reduced Aphanomyces root rot of peas both in field and greenhouse (7, 12), and Na and K reduced the disease in the field (7, 19) but not in the greenhouse (12). Calcium in the form of gypsum has been reported to suppress the disease in the field (16). Although surveys have shown that trace-element deficiencies occur in agricultural soils of almost all states (1, 2), little work has been done on the effect of the various microelements such as B, Cu, Mg, or Mn on Aphanomyces root rot. There is a deficiency, for example, of B in 41, Zn in 30, Mn in 25, Cu in 13, Mo in 21, and Fe in 25 states. The large pea-producing states of Wisconsin, Minnesota, Oregon, Washington, and New York have reported deficiencies of B, Mn, Mo, Zn, and/or Cu for several crops. Soils in Washington, Oregon, and Wisconsin have been reported specifically deficient in B, Mn, Mo, or Zn for peas (1, 2).

The present study was initiated to determine the effects of several of these cations on the root rot of peas caused by A. euteiches and on various phases in the life cycle of the causal organism.

MATERIALS AND METHODS.—Isolate A7 of A. euteiches was used in all experiments. Corn broth (9) was used for all in vitro experiments and, with the addition of agar, for maintenance of the fungus.

Reagent-grade salts of the following cations were used to determine the effect of the element on the growth, zoospore formation, and zoospore germination of the fungus: Al (as Al<sub>2</sub>[SO<sub>4</sub>]<sub>3</sub>·18H<sub>2</sub>O); Ba (as BaCl<sub>2</sub>·2H<sub>2</sub>O); B (as

 $Na_2B_4O_7\cdot 10H_2O$ ); Ca (as  $CaCl_2\cdot 2H_2O$ ); Co (as CoCl<sub>2</sub>·6H<sub>2</sub>O); Cu (as CuSO<sub>4</sub>·5H<sub>2</sub>O); Fe (as FeCl<sub>3</sub>); Mg (as MgSO<sub>4</sub> ·7H<sub>2</sub>O); Mn (as MnSO<sub>4</sub> ·H<sub>2</sub>O); Mo (as Na2 MoO4 · 2H2O); Zn (as ZnSO4 · 7H2O). Forty ml of corn broth were placed in 125-ml Erlenmeyer flasks and autocalved for 15 min at 1.05 kg-force/cm<sup>2</sup> (15 psi). Aqueous solutions of the above salts were membrane-filter sterilized, and aliquots were aseptically added to the sterile corn broth. The broth was then made to 50 ml with sterile distilled water, after enough mineral solution had been added to give a final cation concentration of 1.0 to 300 µg/ml. Inoculations were made with 5-mm disks of the fungus cut from the edge of 5-day-old colonies grown in petri dishes. Cultures were incubated 21 days at 25 C, after which mycelial dry weight was determined.

Zoospores were prepared by the method of Cunningham & Hagedorn (5), with sterile aqueous solutions of the minerals used in place of sterile distilled water. Zoospore concentrations were determined with a hemacytometer. To observe the effects of the minerals on zoospore germination, droplets of zoospore suspensions were placed on petri dishes containing the mineral-supplemented growth medium, to which agar had been added. Zoospore germinability was determined after 24 hr of incubation at 25 C.

At the time of infestation, the sandy loam soil used (pH 5.8) contained 1.6% total C, 0.09% total N, 8.8 µg NH<sub>4</sub>-N/g, 16.3 µg NO<sub>3</sub>-N/g, and 110 µg PO<sub>4</sub>-P/g. It had a water-holding capacity of 32%. Batches of the soil in the greenhouse were infested with zoospores of A. euteiches as previously described (11) and cropped with peas (Pisum sativum L. 'Early Alaska') until the disease-severity index (DSI) of the harvested plants was greater than 75. A DSI of 0 to 100 was calculated from an individual plant rating scale of 0 (no visible symptoms) to 4

(severe symptoms) by the method of Sherwood & Hagedorn (17). The soil was air-dried before use. Wherever possible, the minerals were added in aqueous solution to obtain the desired concentration. The moist soils were incubated for 3 weeks, after which they were mixed and placed in 11.4-cm diameter plastic pots. The equivalent of 900 g of air-dried soil was used per pot. Ten pea seeds were planted per pot. After a 5-week growing period, the seedlings were removed, counted, and examined for disease symptoms. In some experiments, more than one planting was made. Soil pH was determined electrometrically in a soil slurry containing CaCl<sub>2</sub>(0.01 M) at a ratio of 1:2. Dry weight was determined by drying plants from each replicate treatment in a tared container to a constant weight at 105 C. Six replicates were used throughout, and the data reported were those of the second set of experiments.

RESULTS.-Effect of minerals on growth, zoospore formation, and zoospore germination of A. euteiches.-Differences in growth, zoospore formation, and zoospore germination of A. euteiches were observed as a result of addition of 11 mineral salts to the assay medium (Table 1). At cation concentrations of 50 µg/ml or less, CuSO<sub>4</sub> and FeCl<sub>3</sub> were the only two salts that inhibited almost completely all three processes. Calcium and Mg were the least inhibitory. The five salts Al2(SO4)3, BaCl2, CoCl2, CuSO4, and FeCl3 reduced growth by 95% at cation concentration of less than 25 µg/ml. Similarly, the six salts containing Al, B, Cu, Fe, Mn, and Zn inhibited zoospore formation by 95%. Zoospore germination was not as adversely affected by these salts as was growth and zoospore formation.

The effect of four cations (Al, Ca, Cu, Zn) on growth, zoospore formation, and zoospore germination is shown in Fig. 1. These cations were chosen because they were subsequently shown to be

TABLE 1. Cation concentration (µg/ml) necessary to inhibit growth, zoospore formation, and zoospore germination in Aphanomyces euteiches by 95% (ED<sub>9 5</sub>) in corn-decoction medium

Cation (mineral)	Growth <sup>a</sup>	Zoospore formation <sup>b</sup>	Zoospore germination <sup>C</sup>
A1 (A1, [SO <sub>4</sub> ] <sub>3</sub> )	<10	<10	200
$B(Na_2B_4O_2)$	64	<10	115
Ba (BaCl <sub>2</sub> )	19	75	>300
Ca (CaCl <sub>2</sub> )	>300	> 300	>300
Co (CoCl,)	16	80	>300
Cu (CuSO <sub>4</sub> )	<10	<10	42
Fe (FeCl <sub>2</sub> )	16	<10	50
Mg (MgSO <sub>4</sub> )	>300	>300	>300
Mn (MnSO <sub>4</sub> )	156	<10	>300
Mo (Na, MoO <sub>4</sub> )	200	90	>300
Zn (ZnŚO <sub>4</sub> )	78	40	>300

<sup>&</sup>lt;sup>a</sup> Dry weight of mycelium in control flasks = 29.8 mg/50 ml medium.

effective in the reduction of Aphanomyces root rot in the greenhouse. Calcium, in concentrations of 50 µg Ca/ml in corn broth, significantly increased growth of the fungus over the control. Higher concentrations caused a decrease in growth. Toxicity of Zn to growth occurred with concentrations greater than 10 μg Zn/ml, and Al and Cu were toxic to growth even at 1 µg/ml (Fig. 1-A). Calcium did not stimulate zoospore formation as it did growth, but it caused a gradual decline with increasing concentration. The toxicity of Al, Cu, and Zn to zoospore formation was evident with low amounts of cation; Cu prevented formation at 5 μg/ml, Zn and Al at 10 μg/ml (Fig. 1-B). The four cations were more toxic to growth and zoospore formation than to zoospore germination (Fig. 1-C). Both Ca and Zn significantly stimulated zoospore germination at 5 and 10 µg/ml. Both elements decreased germination at higher concentrations. Copper inhibited zoospore germination completely at 50 µg/ml. Aluminum, on the other hand, even at 250 µg/ml, did not prevent germination completely.

TABLE 2. The effect of mineral amendments on Aphanomyces root rot of peas in greenhouse trials

Cation (mineral)	Added cation concentration (µg/g)	Disease- severity index <sup>a</sup>	Dry weight (mg/plant)
Cu (CuSO <sub>4</sub> )	100	7 ab	253 a
A1 $(A1, [SO_4]_3)$	100	19 ab	168 b
Ca (CaCl <sub>2</sub> )	100	27 ab	165 b
$Zn (ZnSO_A)$	100	33 b	122 cd
Ba (BaCl <sub>2</sub> )	100	57 c	118 cd
Mn (MnSO <sub>4</sub> )	100	60 cd	89 f
Mg (MgSO <sub>4</sub> )	100	64 cd	113 cde
Fe (FeCl <sub>3</sub> )	100	71 cd	170 b
$B(Na_2B_4O_2)$	25	75 cde	94 ef
Mo (Na2 MoO4)	100	77 cde	132 c
Control	100	80 de	106 def
Co (CoCl <sub>2</sub> )	50	92 e	114 cde

<sup>&</sup>lt;sup>a</sup> Based on scale in which 0 indicates all roots apparently healthy, and 100 indicates all roots and epicotyls rotted.

Effect of minerals on pea root rot.-Salts of Al, Ca, Cu, Ba, and Zn added to soil at a rate of 100 µg cation/g significantly reduced pea root rot (Table 2). Calcium, Cu, and Al were particularly effective. No statistical differences were observed among treatments in percent stand, but there were differences in dry weights of plants. Plants grown in nonamended soil had an average weight of 106 mg/plant, compared to plants from soil amended with Cu (253 mg/plant), Fe (170 mg/plant), Al (168 mg/plant), Ca (165 mg/plant), or Mo (132 mg/plant). Although Zn and Ba decreased DSI, they did not increase plant weight. Molybdenum, on the other hand, significantly increased plant weight, but did not reduce the DSI. The greatest amount of growth occurred in Cu-amended soil. In addition to reducing

b Zoospores formed under control conditions =  $1.39 \times 10^{5}$ /ml.

c Zoospore germination under control conditions = 66%.

b Numbers followed by same letter are not significantly different at the 5% level.

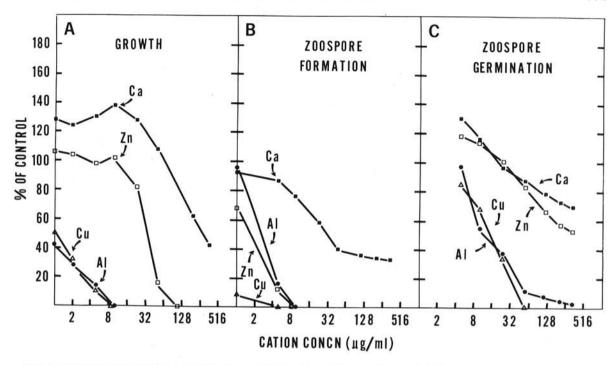


Fig. 1. Influence of increasing concentrations of Al, Ca, Cu, and Zn on A) growth, B) zoospore formation, C) zoospore germination in Aphanomyces euteiches. Cations added as the sulfates of Al, Cu, and Zn, and the chloride of Ca. Dry weight of mycelium in control flasks = 29.8 mg/50 ml medium; zoospores formed under control conditions = 1.39 × 10<sup>5</sup>/ml; zoospore germination under control conditions = 66%.

DSI, Al, Ca, and Zn caused an increase in plant weight over the control. In most cases, the salts could be added to soil in amounts up to 100 µg cation/g of soil without a visible adverse effect on the pea seedlings. The exceptions are noted in Table 2.

determine the minimal effective concentrations of selected cations in the reduction of disease, salts were added to infested soil at rates of 10, 50, and 100  $\mu$ g of Al, Ca, Cu, and Zn/g of soil. Although Aphanomyces root rot was significantly reduced by the addition of 50 and 100 µg of cation/g of soil, the greatest reduction occurred with the cations Al, Cu, and Zn. The DSI for plants grown in Ca-, Al-, Cu-, and Zn-amended soils at 50 µg/g were 40, 11, 10, and 10, respectively. The DSI for plants from nonamended soil was 78. None of the salts was very effective at 10 µg of cation/g of soil in disease reduction.

In addition to using the sulfates of Al, Cu, and Zn, and the chloride of Ca as vehicles for cation addition, an experiment was performed to determine the effectiveness of salts containing other anions in disease suppression. The salts, which included the chlorides, nitrates, sulfates, phosphates, and carbonates of Al, Ca, Cu, and Zn, were added to soil at a rate of 100 µg cation/g. All combinations except CaSO<sub>4</sub>, AlPO<sub>4</sub>, CaCO<sub>3</sub>, Al<sub>2</sub>O<sub>3</sub>·CO<sub>2</sub>, CaHPO<sub>4</sub>, and Zn<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> significantly reduced pea root rot (Table 3). The ineffective salts are insoluble, and this may account for their lack of effectiveness. The two other insoluble salts, CuCO<sub>3</sub>·Cu(OH)<sub>2</sub> and 5ZnO·2CO<sub>3</sub>,

however, were effective to various degrees. The most effective materials were the water-soluble chlorides of Al, Cu, Zn, the nitrate of Al, and the sulfates of Al, Cu, and Zn. In general, Ca salts induced the least disease suppression. Amended soil pH values varied from 4.9 to 5.7.

TABLE 3. Aphanomyces root rot of peas as affected by added A1, Ca, Cu, and Zn (100 µg/g of soil) in combination with various anions

Anion		Disease-sever	ity indexa			
	Cationb					
	<u>A1</u>	Ca	Cu	Zn		
Carbonate	93 dc,d	97 d	29 b	63 c		
Chloride	6 a	38 b	5 a	6 a		
Nitrate	4 a	72 c	33 b	31 b		
Phosphate	83 d	92 d	66 c	75 cd		
Sulfate	6 a	79 cd	10 a	12 a		

a Based on a scale in which 0 indicates all roots apparently healthy, and 100 indicates all roots and epicotyls

b Cations added as the following salts: A12O3 ·CO2, 

<sup>C</sup> Numbers followed by same letter are not significantly

different at 5% level.

d Disease-severity index of plants from nonamended soil

To determine the influence of a commercial fertilizer on the effectiveness of 100  $\mu$ g/g of soil of Al, Ca, Cu, and Zn, a water-soluble preparation (NPK, 20:20:20) was added to soil at the time of mineral amendment to provide an additional 75 µg N/g. The soils were planted to three consecutive crops of peas. The added fertilizer had no appreciable influence on the effectiveness of Al, Ca, Cu, or Zn. Only in the third planting did the fertilizer reduce the effectiveness of Al and Zn. In the Cu-amended soil, DSI values were less than 10 in all three plantings, regardless of fertilizer addition. In this experiment, as in previous ones, Ca was the least effective of the four cations. Nevertheless, in the first planting, the DSI values of 35 and 29 for Ca, with and without fertilizer, compared favorably with those for the corresponding control treatments, 81 and 92. The initial effectiveness of Ca, with and without fertilizer, gradually declined with consecutive plantings, so that after the second planting the beneficial effect of the cation was lost entirely.

TABLE 4. Effect of cation combination with A1, Cu, and Zn on Aphanomyces root rot of peas

Cation <sup>a</sup> (µg/g of soil)	Disease-severity indexb	Dry weight (mg/plant)		
Cu (25) + Zn (25)	1 a <sup>c</sup>	194 b		
Cu (50)	3 a	209 a		
Cu(25) + A1(25)	7 a	200 ab		
Cu (25)	32 b	210 a		
Zn (50)	64 c	147 c		
A1 (50)	70 cd	106 d		
A1(25) + Zn(25)	80 cde	138 c		
A1 (25)	90 de	97 d		
Zn (25)	93 de	103 d		
Control	97 e	97 d		

a Cations added as the sulfates of A1, Cu, and Zn.

b Based on a scale in which 0 indicates all roots apparently healthy, and 100 indicates all roots and epicotyls rotted.

<sup>c</sup> Numbers followed by the same letter are not significantly different at 5% level.

To determine whether the most effective cations were synergistic at low rates, the sulfates of Al, Cu, and Zn were added to Aphanomyces-infested soil alone or in combination, so that each cation was added at either 25 or 50 µg/g of soil. Copper at 50  $\mu g/g$ , or Cu at 25  $\mu g/g$  in combination with either Al or Zn at 25 μg/g, suppressed disease almost completely in this heavily infested soil (Table 4). Copper at 25 µg/g also significantly reduced pea root rot, whereas Al or Zn at 25 μg/g did not. Although both Al and Zn at 50 µg/g significantly reduced diseases, the effect in neither case was as striking as that of Cu. The data showed that, in every instance, Cu was necessary to give the greatest disease reduction. The treatments that resulted in the lowest DSI values also resulted in the greatest amounts of pea growth. All the treatments in which Cu was included, gave pea tissue dry weight greater than 190 mg/plant, compared to the 97 mg/plant of peas from nonamended soil.

Effect of minerals on pea development.-An experiment was performed to see the effect of Al, Ca, Cu, and Zn on pea development in noninfested soil after 6 weeks of growth. The salts of these cations, added at a rate of 100 µg cation/g of soil, reduced neither stand nor plant dry weight over that of seedlings grown in nonamended soil. The percent stand varied between 90 and 100. Dry weight varied between 130 and 170 mg/plant. The dry weight of plants from amended soil was not higher than that of plants from nonamended soil in this experiment, as they were in experiments that involved infested soil (Tables 2, 4). This was because the control plants from noninfested soils were initially healthy and vigorous and displayed none of the deterioration and decay of control plants from infested soil. Assayed electrometrically at the time of planting, the pH values of soils to which salts were added were not appreciably different from the pH of the nonamended soil.

DISCUSSION.-The data indicate that the inhibitory cations were just as effective against A. euteiches as they are reported to be against other fungi (3, 18). Aluminum, Cu, and Zn at a concentration of less than 25 µg/ml inhibited zoospore formation (ED95); and Al and Cu prevented growth (ED95). In addition, Al, Cu, and Zn also suppressed Aphanomyces root rot. These three cations may have reduced disease by a direct toxic effect on the pathogen in soil. The toxic effect, which might involve inhibition of zoospore formation, would be evident especially in the rhizosphere, where the cations could be solubilized from clay colloids as a result of the action of the acidic exudates. Calcium could reduce disease by affecting zoospore activity. Mitchell & Yang (10) indicated that CaCl2 at 0.05 M 1,800 µg Ca/ml) caused zoospores to differentiate but remain in the hyphae. At half this concentration, spores were exuded but remained at the orifice of the sporangium.

Aluminum, Ca, Cu, or Zn could be added to soil on a practical basis for the control of Aphanomyces root rot. These four cations, added at a rate of 100 μg/g of soil, did not adversely affect morphology or appearance of the pea plant, nor did this amount cause an appreciable alteration in soil pH. This suggests that relatively large amounts could be used without causing plant damage. Many heavy metal ions can be added to soil without any appreciable deleterious effect on the environment as a result of leaching. Copper, for example, may be held by the colloidal fraction of the soil with such a degree of security that it cannot be displaced readily by other soil cations in the ordinary range of soil acidity. Copper has also been fixed in soil by chelate linkages with lignoproteins and other humic compounds (15). Zinc, added as ZnSO4, could remain in the top 2.5 cm of soil even after leaching with 1 meter of water

In practice, salts of Al are not generally added to

soils to control deficiencies (13). Calcium, Cu, and Zn, however, are commonly added to control deficiencies. Copper sulfate may be required in amounts of 55.7 to 222.9 kg/ha (50 to 200 lb/acre) for normal yields of vegetables and field crops (15). Other soluble salts, such as CuCl<sub>2</sub> or Cu(NO<sub>3</sub>)<sub>2</sub>, as well as the insoluble oxides of Cu, have been used with equally good results as Cu fertilizers (15). Zinc sulfate has been added to field soils in amounts up to 89.2 kg/ha (80 lb/acre) (4). Calcium is frequently added to soils in many kg/ha as lime, gypsum, Ca(NO<sub>3</sub>)<sub>2</sub>, or CaCl<sub>2</sub> to control Ca deficiency or to adjust soil acidity (4). Calcium in the form of lime has been found to reduce Fusarium wilt of tomato (8), and in the form of gypsum to reduce Aphanomyces root rot of peas (16). Neither gypsum nor lime reduced Aphanomyces root rot in the greenhouse in this investigation. Results of the present study indicated that in many instances the anionic portion of the soluble salt did not influence the effectiveness of the cation. The nitrate, sulfate, and chloride of Al, for example, were equally effective in disease reduction. The insoluble phosphate and carbonate, on the contrary, were ineffective. All the salts of Cu were effective in disease suppression, but the soluble ones were more effective than the insoluble ones. This suggests that a variety of commercially available minerals and ores might be used effectively.

Broadcast application of the amount of salts used in these experiments (100  $\mu$ g/g) would involve, at most, about 557 kg salt/ha. Except for Ca, clearly this is neither feasible nor desirable. But, by "in-row" application (14), only a fraction of this amount would be used. For example, when applied at a rate of 6.7 kg/ha (6 lb/acre), a material present in a 5.1 X 5.1 cm (2 X 2 inch)-treated zone of soil has a concentration of 100  $\mu$ g/g of soil for a 50.8-cm (20-inch) row spacing. Since the data indicate that 25 to 50 µg of Al, Cu, or Zn/g of soil could be effectively used to suppress pea root rot, it is possible that relatively small amounts of Al, Cu, or Zn salts could be applied to advantage in agricultural practice. The observation that Al, Cu, and Zn were effective during several consecutive plantings, suggests that these materials would not have to be added every year. The addition of small increments might be sufficient to prolong the beneficial effects of the materials.

Since the addition of a complete fertilizer did not influence the effectiveness of the cations, there appeared to be no alteration in cation availability as a result of addition of cations and fertilizers in combinations. Antagonism or synergism in cation effectiveness also was not observed. Thus, the use of only a single cation would be necessary. Similar results were obtained by Evans et al. (6), who showed that Cu compounds alone significantly reduced late blight of potatoes caused by *Phytophthora infestans*, but the addition of Al as an adjuvant was not successful.

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