The Role of Calcium and Microorganisms in Suppression of Cucumber Damping-Off Caused by *Pythium splendens* in a Hawaiian Soil

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ABSTRACT

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Amendment of conducive soil with $CaCO_3$, alfalfa meal, or $CaCO_3$ plus alfalfa meal reduced damping-off caused in cucumber seedlings by *Pythium splendens* from 78 to 30, 44, and 11%, respectively. $CaSO_4$ was as effective as $CaCO_3$ or $Ca(OH)_2$ in reducing disease incidence even though it did not increase the soil pH. Growth of cucumber seedlings and the number of roots were greatly increased by amendment of soil with Ca, alfalfa meal, or Ca plus alfalfa meal. Amendment of soil with Ca plus alfalfa meal was the most effective of these treatments. Sporangial germination of *P. splendens* was most responsive to increased nutrients from cucumber root extract in conducive soil followed by Ca-amended soil and alfalfa meal-amended soil. Germination was least responsive to nutrients in suppressive soil and conducive soil amended with Ca plus alfalfa meal. In the cucumber rhizosphere, sporangia of *P. splendens* germinated 67% on conducive soil and 23% on suppressive soil. Germination on conducive soil was decreased to 50% with amendment of Ca, 44% with alfalfa meal, and 27% with Ca plus alfalfa meal. Infection occurred on 81% of the inoculated cucumber roots grown in conducive soil, 25% in suppressive soil and 21% in conducive soil amended with Ca. Amendment of conducive soil with alfalfa meal did not decrease the percentage of root infection, nor did it decrease the size of necrotic lesions on roots. Amendment of soil with Ca increased the concentration of N and Ca in cucumber tissues, whereas amendment of soil with Ca plus alfalfa meal increased the concentration of N, K, and Ca. Two weeks after planting in unamended field conducive soil, 59% of cucumber seedlings were killed by *P. splendens*, whereas only 11, 15, and 8% of seedlings were killed in soil amended with Ca, alfalfa meal, and Ca plus alfalfa meal, respectively. Leaves of cucumber seedlings grown in unamended soil were small and yellowish, while leaves were large and green in amended soil. Soils amended with alfalfa meal and Ca plus alfalfa meal contained 4.0×10^8 and 6.0×10^8 colony-forming units of microorganisms per gram of soil, respectively. Amendment of soil with Ca also increased the total microbial population from 4.6×10^6 to 6.9×10^7 colony-forming units per gram of soil.

About 37% of 81 soil samples collected from different islands in Hawaii were suppressive to sporangial germination of *Pythium splendens* Braun (19). There was a positive correlation of the degree of suppression and soil pH among these soils. About 33% of the pathogen-suppressive soils were also suppressive to damping-off of cucumber seedlings caused by *P. splendens* (19). A study of the mechanisms of suppression in a pasture soil (from South Kohala on the island of Hawaii) which was highly suppressive to *P. splendens* and to cucumber damping-off caused by this fungus suggested that a combination of an unknown abiotic factor and a high microbial population is responsible for suppression of *P. splendens* in the South Kohala soil (14). Results from a recent study suggest that Ca is the unknown abiotic factor and that a combination of high Ca content and high microbial population is the cause of suppression of *P. splendens* in the South Kohala soil (15).

The objectives of this study were to determine the role of Ca and microorganisms in suppression of damping-off caused in cucumber (*Cucumis sativus* L.) by *P. splendens* in the South Kohala soil and to apply the suppression principle to control this disease in the field.

MATERIALS AND METHODS

Soil amendments. Conducive soil from Hilo and suppressive soil from South Kohala were collected and processed as described previously (15). To increase the Ca content in conducive soil, $CaCO_3$ was used in most of the experiments; however, other Ca-containing compounds such as $Ca(OH)_2$ and $CaSO_4$ also were used for comparison. Since suppressive soil contained about 6 mg Ca/g soil more than the conducive soil, this amount of the different Ca sources was used for amendment. To increase the population of indigenous microorganisms, soil was thoroughly mixed with 1% (w/w) alfalfa meal.

Assay of disease incidence. Sporangia of *P. splendens* (isolate 106F) were obtained by using the method described by Ko and Ho (17). Spore concentrations were determined by counting the number of spores in 1 μl with a Pipetman digital microliter pipet (P-20D); West Coast Scientific, Inc., Oakland, CA (16). Sporangia (10/g soil) of *P. splendens* were thoroughly mixed with soil in a polyethylene bag. Ten and six cucumber seeds (cultivar Burpee Hybrid), respectively, were planted in 300 and 150 g of soil in a pot. Damping-off of cucumber seedlings was recorded after 10 days. All the experiments were repeated at least once.

Spore germination on soils. Since sporangia of *P. splendens* are sensitive to general soil fungistasis, cucumber root extract was added to overcome that effect. Cucumber root extract was prepared according to the method of Ko and Ho (17). Sporangial suspensions of *P. splendens* were mixed with an equal volume of cucumber root extract at various dilutions before being added to soil blocks. Two drops of spore suspension were added to the smooth surface of a soil block ($50 \times 26 \times 3$ mm) on a glass slide which was then placed in a moist chamber and incubated at 24 C for 6 hr (17). After incubation, spores were stained with rose bengal (1% rose bengal, 5% phenol, and 0.01% $CaCl_2$ in distilled water). Germination of sporangia of *P. splendens* was counted directly on the soil surface with a Zeiss Universal Microscope equipped with a model II C vertical illuminator at $\times$200. Three replicates were used for each treatment and the experiment was done twice.

To determine the response of sporangia to exudates from roots placed on soils with different amendments, cucumber seeds were germinated on moist filter paper in a petri plate in darkness for 3 days; during that time each seed produced a root about 3 cm in length. Sporangia without exogenous nutrients as described above

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were placed on the soil block on which the root of a germinating seed was placed. The soil block on a moist filter paper in a petri plate was kept at 24 C. After 6 hr, sporangia were stained and counted (17). Two replicates per treatment were used and the experiment was done twice.

Assay of host resistance. Conducive soil amended with $Ca(OH)_2$, alfalfa meal, or $Ca(OH)_2$ plus alfalfa meal was incubated for 1–4 wk at 24 C before use. Conducive soil and suppressive soil without amendment were used as controls. Six cucumber seeds were planted in a pot (50 × 70 × 60 mm) containing 150 g of soil. After 5 days, seedlings were gently washed free of soil particles with running water and kept in distilled water in a 300-ml beaker. Two Whatman No. 1 filter papers (70-mm diameter) were placed on each side of a large plastic petri plate (150 × 25 mm) and moistened with distilled water. A cucumber seedling with four long roots separated from other roots in the water was placed in between the two filter papers. These four roots selected for inoculation were placed on filter papers with two roots on each side. All other roots were laid on the middle and covered with the soil used to grow the cucumber seedling. These procedures were carried out carefully to prevent injury to the cucumber roots. Thirty sporangia in 5 μl of sporangial suspension of *P. splendens* were added to a filter paper disk (6-mm diameter) with a Pipetman digital microliter pipet, and the disk was placed upside down immediately on a root tip. After inoculation, the petri plates were covered and placed in a moistened polyethylene bag and incubated at 24 C for 3 days. Infection was observed under a steromicroscope at ×50. Five cucumber seedlings with a total of 20 roots were used for each treatment, and the experiments were repeated twice.

Field trials. Field trials were carried out in Hilo, Hawaii from August to October of 1984 at the conducive soil location. Since *P. splendens* affects cucumber at the seedling stage, only planting holes were amended and infested shortly after seedbed preparation. Hydrated lime (85% $Ca(OH)_2$), alfalfa meal, and hydrated lime plus alfalfa meal were used as soil amendments. Each treatment consisted of seven planting holes spaced 1 × 1 m and replicated three times. Approximately 3 kg of soil from each of the seven planting holes (20-cm diameter × 18-cm depth) was removed and put into a container. Amendments and infestations were done with a portable power cement mixer, and the soils were put back into the planting holes. Infested, but unamended, soils were used as controls. Ten cucumber seeds were planted in each hole 7 days later. Planting holes were watered once a day when it did not rain. Damping-off of cucumber seedlings was recorded after 14 days and seedlings in each planting hole were subsequently thinned to two plants. Growth of cucumber vines was measured after 45 days.

TABLE 1. Damping-off of cucumber seedlings caused by *Pythium splendens* in conducive soil from Hilo, HI, amended with different calcium and magnesium sources and in a suppressive soil from South Kohala, HI[x]

Soil amendment	Damping-off (%)[y]	Soil pH
Conducive soil		
None	86 b[z]	5.1
$CaCO_3$	28 d	6.8
$Ca(OH)_2$	38 d	6.9
$CaSO_4$	31 d	5.1
$(MgCO_3)_4 \cdot Mg(OH)_2$	100 a	6.8
Suppressive soil		
None	6 e	6.8

[x] Ten cucumber seeds were planted in each pot containing 300 g of soil after soil amendment and infestation with 10 sporangia of *P. splendens* per gram of soil. Four pots were used for each treatment.
[y] Damping-off was recorded after 10 days of planting.
[z] Values followed by the same letter are not significantly different at $P = 0.05$ by Duncan's multiple range test.

TABLE 2. Growth of cucumber seedlings in conducive soil from Hilo amended with $Ca(OH)_2$ and/or 1% alfalfa meal and inoculated with sporangia of *Pythium splendens*[y]

Soil amendment	Height (cm)	Fresh weight (mg/seedling)	Dried weight (mg/seedling)
None	3.4 a[z]	75 a	8.7 a
$Ca(OH)_2$	5.4 b	163 b	14.3 b
Alfalfa meal	5.3 b	171 b	15.0 b
$Ca(OH)_2$ + alfalfa meal	7.3 c	235 c	18.7 c

[y] Hydrated lime containing 85% $Ca(OH)_2$ was used for amendment. Growth of cucumber seedlings was measured 10 days after planting and 30 seedlings were measured for each treatment.
[z] Values followed by the same letter in each column are not significantly different at $P = 0.05$ according to Duncan's multiple range test.

RESULTS

Disease suppression by soil amendments. Amendments of conducive soil with $CaCO_3$, alfalfa meal, or $CaCO_3$ plus alfalfa meal reduced damping-off of cucumber seedlings caused by *P. splendens* from 78 to 30, 44 and 11%, respectively. Since amendment of soil with $CaCO_3$ which reduces disease incidence also involved an increase in soil pH, another alkali, $(MgCO_3)_4 \cdot Mg(OH)_2$, which increases soil pH, and another Ca source, $CaSO_4$, which does not change soil pH, also were tested. Results showed that $CaSO_4$ was as effective as $CaCO_3$ or $Ca(OH)_2$ in reducing disease incidence even though it did not change the soil pH (Table 1). Adjusting the soil pH from 5.1 to 6.8 with 0.4% $(MgCO_3)_4 \cdot Mg(OH)_2$ increased disease incidence.

In a separate experiment, 10 cucumber seeds were planted 7 days after soil amendment and infestation in each 3.7-L pot containing 2,500 *g* of soil. Damping-off of cucumber seedlings was reduced from 75% in unamended soil to 3 and 5% in soils amended with $Ca(OH)_2$ and $Ca(OH)_2$ plus alfalfa meal, respectively. Alfalfa meal amendment alone reduced disease to 15%. Cucumber seedling growth was increased greatly by all amendments. Amendment of soil with $Ca(OH)_2$ plus alfalfa meal was most effective and increased seedling dry weight more than 100% 10 days after seeds were planted (Table 2). Soil amendments increased the number and growth of roots with $Ca(OH)_2$ plus alfalfa meal being more effective than $Ca(OH)_2$ or alfalfa meal alone in enhancing root growth. Root growth in $Ca(OH)_2$ amended soil was about the same as that in alfalfa meal-amended soil.

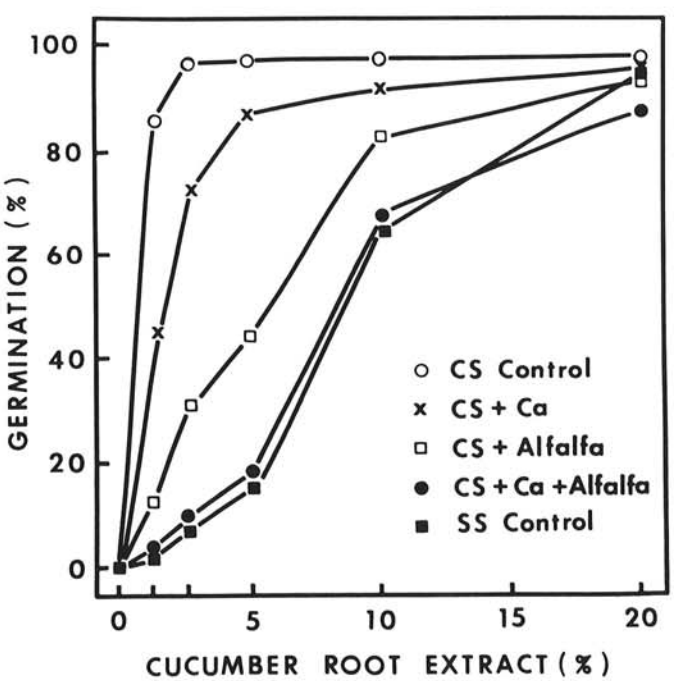

Fig. 1. Germination of sporangia of *Pythium splendens* amended with different concentrations of cucumber root extract on conducive soil (CS) amended with Ca, alfalfa meal, or Ca plus alfalfa meal, or on suppressive soil (SS).

Effect of Ca and microorganisms on spore germination in soil. When different concentrations of cucumber root extract were used to amend a sporangial suspension of *P. splendens*, germination of sporangia increased as the concentration of cucumber root extract increased on all the soils tested. However, soils with different amendments showed different dosage response curves (Fig. 1). Spore germination in conducive soil was most responsive to increased nutrient concentration followed by Ca-amended soil and alfalfa meal-amended soil. Germination was least affected by nutrients in suppressive soil and conducive soil amended with Ca plus alfalfa meal. The dosage response curves of these two soils were similar. About 8.3 and 8.0% of the cucumber root extract were needed for 50% germination of sporangia of *P. splendens* on suppressive soil and conducive soil amended with $CaCO_3$ plus alfalfa meal, respectively. Only 0.6% root extract was needed for 50% germination on conducive soil. Conducive soil amended with $CaCO_3$ or alfalfa meal required 1.7 and 5.6% cucumber root extract, respectively, to induce 50% germination.

When roots from cucumber seeds germinated in moist chambers were placed on suppressive soil or conducive soil amended with $Ca(OH)_2$, alfalfa meal, or $Ca(OH)_2$ plus alfalfa meal to stimulate sporangial germination of *P. splendens*, there were significant differences in percentage germination (Table 3). Within 2 mm from roots, 67% germinated on conducive soil and 23% germinated on suppressive soil. Germination was 50% on $Ca(OH)_2$ amended soil, 44% on alfalfa meal amended soil and 27% on soil amended with $Ca(OH)_2$ plus alfalfa meal. Germination on suppressive soil and conducive soil amended with $Ca(OH)_2$, alfalfa meal, or $Ca(OH)_2$ plus alfalfa meal occurred mainly within 2 mm from roots, whereas on unamended conducive soil some germination occurred even at 5–6 mm from roots.

TABLE 3. Germination of sporangia of *Pythium splendens* on a suppressive soil from South Kohala, HI and a conducive soil from Hilo, HI, with different amendments at various distances from roots of cucumber seedlings[y]

Soil amendment	Germination (%) at distances from root of: 0–2 mm	3–4 mm	5–6 mm
Conducive soil			
None	67 a[z]	18 a	7 a
$Ca(OH)_2$	50 b	4 bc	0 b
Alfalfa meal	44 b	7 b	0 b
$Ca(OH)_2$ + alfalfa meal	27 c	2 c	0 b
Suppressive soil			
None	23 c	1 c	0 b

[y]Cucumber seeds were germinated on moist filter paper in petri plates under darkness for 3 days.
[z]Values followed by the same letter in each column are not significantly different, $P = 0.05$, according to Duncan's multiple range test.

TABLE 4. Susceptibility of cucumber roots grown in a suppressive soil from South Kohala, HI and a conducive soil from Hilo, HI, with different amendments, to infection by *Pythium splendens*

Soil amendment	Infection (%)[x]	Necrotic lesion length (mm)[y]
Conducive soil		
None	81 a[z]	20 a
$Ca(OH)_2$	21 b	8 b
Alfalfa meal	83 a	18 a
$Ca(OH)_2$ + alfalfa meal	20 b	9 b
Suppressive soil		
None	25 b	11 b

[x]Five seedlings with four roots per seedling were each inoculated with 30 sporangia at the root tip. Readings were made 3 days after inoculation. Data were from one of two separated experiments with similar results.
[y]Necrotic lesion length was the average of measurements from all infested roots in each treatment.
[z]Values followed by the same letter in each column are not significantly different, $P = 0.05$, according to Duncan's multiple range test.

Effect of Ca on host resistance. Necrosis occurred on 81% of the inoculated roots grown in conducive soil, whereas only 25% of the roots grown in suppressive soil developed necrotic symptoms (Table 4). The average length of the necrotic lesions on infected roots grown in suppressive soil was only about one-half of that on roots grown in conducive soil. Percentages of infection and size of necrotic lesions on roots grown in conducive soil amended with $Ca(OH)_2$ or $Ca(OH)_2$ plus alfalfa meal were about the same as those on roots grown in suppressive soil. Amendment of conducive soil with alfalfa meal did not decrease the percentage of root infection, nor did it decrease the size of necrotic lesions that developed on roots grown in it.

Tissues of the entire cucumber seedlings grown for 10 days in conducive soils amended with $Ca(OH)_2$, alfalfa meal, or $Ca(OH)_2$ plus alfalfa meal were analyzed by X-ray quantometer by the Cooperative Extension Service, University of Hawaii. Amendment of soil with $Ca(OH)_2$ increased the concentration of N and Ca in the cucumber tissue, whereas amendment of soil with alfalfa meal increased the concentration of N and K (Table 5). Cucumber seedlings grown in conducive soil amended with $Ca(OH)_2$ plus alfalfa meal contained higher concentrations of N, K, and Ca than those grown in unamended conducive soil.

Field trials. In the first experiment 2 wk after planting, 59% of the cucumber seedlings were killed by *P. splendens* in the unamended conducive soil, whereas only 11, 15, and 8% of seedlings were killed in conducive soil amended with $Ca(OH)_2$, alfalfa meal, and $Ca(OH)_2$ plus alfalfa meal, respectively (Table 6). Leaves of cucumber seedlings grown in unamended soil were small and yellowish, while leaves were large and green on seedlings grown in amended soils. Amendment of soil with $Ca(OH)_2$, alfalfa meal, or both also increased the height of cucumber plants (Table 6).

Results of the second experiment were similar to those of the first experiment. Damping-off of cucumber seedlings was reduced from 53% in unamended soil to 6% in $Ca(OH)_2$ amended soil, 17% in alfalfa meal amended soil and 5% in soil amended with $Ca(OH)_2$ plus alfalfa meal. Growth of cucumber seedlings was not measured in this experiment. The total microbial populations determined 7 days after soil amendment by using the same methods as those described previously (15) were much higher than those obtained from the laboratory test. Soils amended with alfalfa meal and

TABLE 5. Elemental analyses[z] of cucumber seedlings grown in conducive soil from Hilo, HI, amended with $Ca(OH)_2$ and/or alfalfa meal

Soil amendment	Elemental concentration (%) N	P	K	Ca	Mg	S	Si
None	1.73	0.27	1.61	0.91	1.27	0.57	0.27
$Ca(OH)_2$	4.72	0.21	1.72	4.12	0.67	0.39	0.10
Alfalfa meal	4.92	0.18	4.92	1.11	1.11	0.39	0.20
$Ca(OH)_2$ + alfalfa meal	4.82	0.17	5.22	3.07	0.49	0.26	0.08

[z]Tissue analyses was done by X-ray quantometer by the Cooperative Extension Service, University of Hawaii. Elemental concentrations were based on oven dried entire cucumber seedling tissues.

TABLE 6. Damping-off caused in cucumber seedlings in field plots by *Pythium splendens* and growth of cucumber in conducive soil from Hilo, Hi, variously amended with $Ca(OH)_2$ and/or alfalfa meal in the field (Experiment I)

Soil amendment	Damping-off (%)[x]	Growth in height (cm)[y]
None	59 a[z]	31 a
$Ca(OH)_2$	11 bc	67 c
Alfalfa meal	15 b	50 b
$Ca(OH)_2$ + alfalfa meal	8 c	85 d

[x]Damping-off of cucumber seedlings was recorded 14 days after planting.
[y]Height of main vine was measured after 45 days of planting. Values were averages of 30 cucumber plants.
[z]Values followed by the same letter in each column are not significantly different at $P = 0.05$ according to Duncan's multiple range test.

$Ca(OH)_2$ plus alfalfa meal contained 4.0×10^8 and 6.0×10^8 cfu/g soil, respectively. Amendment of soil with $Ca(OH)_2$ also increased the total microbial population from 4.6×10^6 to 6.9×10^7 cfu/g of soil.

DISCUSSION

Ko and Lockwood (18) provided evidence suggesting that nutrient deprivation imposed by microbial activity is responsible for the inability of spores of most fungi to germinate on natural soil, a phenomenon commonly called soil fungistasis. Amendment of sporangia of *P. splendens* with 5% cucumber root extract overcame the general fungistasis in the conducive soil. Under such conditions, germination of *P. splendens* was still inhibited on the soil from South Kohala (14). This apparently is due to the strong nutrient deprivation environment imposed by the high microbial activity because sporangia of *P. splendens* germinated freely on suppressive soil when the root extract used to amend sporangia suspension was increased from 5 to 20% (Fig. 1). The concentration of root extract required for 50% germination increased when conducive soil was amended with either Ca or alfalfa meal. This suggests that both high Ca content and high microbial population contribute to the creation of a strong nutrient deprivation environment in the suppressive soil. The role of Ca in enhancing the nutrient deprivation condition of soil is not known. It is possible that soil microorganisms become more active because they are more effective in obtaining nutrients from the environment in the presence of high Ca. The fact that the total microbial population in conducive soil amended with alfalfa meal plus Ca was higher than that amended with alfalfa meal only (15), and that the total microbial population in the reinfested sterilized soil was higher when it was amended with Ca (13) are consistent with such an explanation.

There were some similarities between the nature of pathogen suppression and the disease suppression when comparisons were made using soil from South Kohala as the suppressive soil and soil from Hilo as the conducive soil. Soil from South Kohala suppressed germination of *P. splendens* and *P. aphanidermatum* (Edson) Fitzpatrick, but not *Phytophthora capsici* Leonian and *P. palmivora* (Butler) Butler (14). It also suppressed diseases caused by *P. splendens* and *P. aphanidermatum*, but not diseases caused by *P. capsici* and *P. palmivora* (13). Conducive soil from Hilo became suppressive to *P. splendens* when it was amended with Ca and alfalfa meal. The same treatment also rendered Hilo soil suppressive to the disease caused by the pathogen. Like pathogen suppression, the disease suppression in South Kohala soil was not due to pH because increasing the pH of Hilo soil to 6.8 with $(MgCO_3) \cdot Mg(OH)_2$ did not make it suppressive to damping-off caused in cucumber seedlings by *P. splendens*. Moreover, amendment of Hilo soil with $CaSO_4$ which did not change soil pH also decreased the disease caused by *P. splendens*.

Pathogen suppression appears to be different from that of disease suppression in that amendment of Hilo soil with Ca decreased the disease by more than 60% (Table 1), whereas it decreased germination of the pathogen by less than 10% (15). This suggests the possibility that, in addition to enhancing the nutrient deprivation potential of soil, Ca may also increase the resistance of the host to the pathogen. Results from this study showed that 81% of cucumber roots grown in conducive soil were infected by *P. splendens* but only 21% were infected when the soil was amended with Ca, and the average length of necrotic lesions on roots was decreased by 60% when the soil was amended with Ca (Table 6). Ca is essential for the conversion in plant tissues of pectin to Ca pectate which is resistant to degradation by polygalacturonase produced by the pathogen (2,5). It also inhibited the activity of polygalacturonase (4). This may account for the restriction of lesion size on roots grown in Ca-amended soil.

Multiple changes are probably induced by high Ca content in hosts which may be attributed to a decrease in disease incidence in suppressive soil. In addition to increase in resistance, Ca amendment also increased root growth which in turn could reduce the damage caused by the pathogen. *Pythium* spp. have been classified by Garrett (7) as unspecialized parasites which are very destructive to juvenile tissues of seedlings but are restricted by mature tissues. Since Ca increased the growth rate of cucumber seedlings, it may also shorten their susceptible period and, therefore, reduce the percentage of roots infected (5,20).

The application of various Ca salts to soil reduces crop losses caused by *Pythium ultimum* Trow (24), *P. myriotylum* Drechsler, and *P. aphanidermatum* (8), *Sclerotium rolfsii* Saccardo (25), *Fusarium oxysporum* f. sp. *lycopersici* (Saccardo) Snyder & Hansen (4,11,12), *Plasmodiophora brassicae* Wor. (6), *Rhizoctonia solani* Kühn (1), *Phytophthora cinnamomi* Rands (3,21), and *Aphanomyces euteiches* Drechsler (22,23). On the other hand, Ca also has been reported to increase incidence of diseases caused by *Streptomyces scabies* (Thaxter) Waksman & Henrici (9), *Phymatotrichum omnivorum* (Shear) Duggar, *Fusarium* spp., *Verticillium albo-atrum* Reinke & Berthold, and *R. solani* (10).

Disease incidences in amended conducive soils in small pot tests were consistently higher than that in large pot tests or field trials. This probably reflected the amount of plant debris that was incorporated into the soil during preparation to serve as a nutrient amendment. The same soil was used for the large-pot tests. Conducive soil used in small-pot tests was sieved to remove large fragments of organic matter and kept in moist conditions for more than 1 mo before use to exhaust the available nutrients (15). Such an explanation was supported by the observations that the total microbial population in the field soil increased by more than 10-fold 1 wk after amendment with Ca only, whereas the same treatment did not increase the microbial population in conducive soil used in small-pot tests (13). This may also be the basis for absence of significant differences in disease incidence between Ca-amended and Ca plus alfalfa meal-amended conducive soils in large-pot tests and field trials.

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