

Lime and the Control of Clubroot of Crucifers: Effects of pH, Calcium, Magnesium, and Their Interactions

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ABSTRACT

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The effects of pH and nutrients on the development of *Plasmodiophora brassicae* in broccoli were studied in a nutrient solution-sand culture system. At pH >7.2, primary infections were reduced and clubbing prevented, apparently because primary thalli aborted before they released secondary zoospores. As the concentration of calcium or magnesium was increased from 0.5 to 2.5 mM, infection and clubbing were inhibited at a

limiting concentration that varied inversely with the pH. These results may be related to calcium uptake which was less at pH 6.2 than at pH 6.8 or 7.2 with intermediate or low amounts of calcium in the nutrient solution. Calcium and hydrogen ions have low toxicity to resting spores in that incubation of resting spores in 1.0 M CaCl₂ at pH 6.2-7.2 for 1 wk reduced infectivity only slightly from that of NaCl or buffer controls.

Liming the soil is one of the oldest and most widely used control measures for clubroot caused in crucifers by *Plasmodiophora brassicae* Wor. It is commonly believed that liming should raise the soil pH to 7.2 or above for adequate control (12). Many workers, however, have failed to get satisfactory control by increasing soil pH above 7.2. The reasons for these failures have been difficult to determine because many factors may influence the effectiveness of lime treatment. Palm (20) used a nutrient solution-sand culture system to show that some nutrients, including calcium, could inhibit the development of clubroot. The interaction of pH and nutrients has not been determined. Furthermore, field and greenhouse trials have shown that calcium or magnesium in lime may affect disease development independent of pH (1,7,8). The effects of these factors cannot be studied adequately in soils; therefore, we have used a nutrient solution-sand culture technique to permit precise, independent control of each factor. The results of

these studies are reported in this paper. A preliminary report (18) and studies of related edaphic factors (17) have been made.

MATERIALS AND METHODS

Plant culture and sampling system. Acid-washed, flint-shot, quartz sand (100 g) was added to 100-cm³ plastic pots. The sand was saturated with an appropriate nutrient solution containing resting spores of *P. brassicae* to establish a final concentration of 10⁷ spores per gram of sand. The spore preparation and race have been described (1,19). Each pot was seeded with 15 seeds of the susceptible broccoli, *Brassica oleracea* L. var. *italica* Plenck 'Topper,' and incubated at room temperature in clear plastic boxes under fluorescent lights. Starting 4 days after planting and on alternate days thereafter, old nutrient solution was removed by drenching each pot twice with a void volume (20 ml) of fresh solution.

The roots of four plants (two plants per pot, two replicate pots per treatment) were fixed in acetocarmine (10) 12 days after sowing. Developmental types of the pathogen were identified and counted in 100 root hairs in the midsection of each root to determine the

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effects of pH and selected nutrients on infection. Eight developmental stages in the primary phase of the fungus were recognized with phase-contrast microscopy in time-course studies and corresponded to the stages described by others (9). For this paper, the number of thalli in stages 1–3, which consisted of the development of the primary plasmodia, plus stages 4–8, which detailed the development of the primary zoosporangia, were summed to provide a quantitative measurement of infectivity. The number of empty zoosporangia (stage 8), from which secondary zoospores had been released, was used as an estimate of normal maturation of primary thalli.

The remaining plants were maintained until 30 days after sowing when the percentage with clubroot symptoms on the taproot was determined. All experiments were repeated once, but the results of only one experiment are shown.

Nutrient solutions. A nutrient solution was developed to meet the following criteria: to yield >50 root hair infections per 100 root hairs at 12 days and >75% of the plants with clubroot symptoms at 30 days, and to permit plant growth without symptoms of nutrient deficiencies. This solution, which has approximately 0.5 × the macronutrients and 2× the micronutrients of complete Hoagland's solution, contained: macronutrients—MgSO₄ (1 mM), KNO₃ (2.5 mM), Ca(NO₃)₂ (2.5 mM), and KH₂PO₄ (0.5 mM); and micronutrients—H₃BO₃ (92 μM), MnCl₂ (18 μM), CuSO₄ (0.64 μM), H₂MoO₄ (0.14 μM), ZnSO₄ (1.5 μM), and Fe-EDTA (1.3 μM). When calcium was varied, concentrations of magnesium, potassium, nitrogen, sulfur, and phosphorus were kept constant. Calcium concentrations above 2.5 mM were obtained with CaCl₂. When the concentration of calcium was <2.5 mM, NaNO₃ was added to keep the nitrogen level constant. Two controls were included. The chloride control had 50 mM chloride from NaCl to test the effect of the highest concentration of chloride (45 mM) from CaCl₂. The sodium control had 5 mM sodium from Na₂SO₄ to balance the sodium from NaNO₃ at 0 mM calcium. When magnesium concentrations were varied, concentrations of calcium, potassium, nitrogen, sulfur, and phosphorus were kept constant. Sulfur was supplied by Na₂SO₄ at magnesium concentrations below the normal. Higher concentrations of magnesium were obtained with MgCl₂. The chloride control contained 50 mM chloride from NaCl. A sodium control with 50 mM sodium from Na₂SO₄ was included to test higher sodium concentrations than found in the series.

To determine the effects of pH, the nutrient solutions were made in 0.01 M buffers having a pKa in the desired pH range. These buffers met the following criteria: low or negligible metal-binding constant for cations including magnesium, calcium, manganese, and copper, inert or not known to participate in or to stimulate chemical reactions in plants, and not toxic to host or pathogen at 0.01 M. The buffers were MES [2-(*N*-morpholino)ethanesulfonic acid, sodium salt], pKa 6.15; PIPES [piperazine-*N,N'*-bis-(2-ethanesulfonic acid) monosodium salt, monohydrate], pKa 6.8; HEPES [*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid], pKa 7.55; and TRIS [tris(hydroxymethyl)aminomethane], heavy metal-free, pKa 8.3 (Calbiochem-Behring Corp., La Jolla, CA 92039).

Calcium uptake. The roots from all the seedlings in a pot grown for 15 or 30 days with or without *P. brassicae* were harvested, freed of sand, oven-dried at 100 C, digested with perchloric acid, and analyzed for calcium by atomic absorption analysis (11). There were four replicate pots per treatment and approximately 20 plants per replicate. This experiment was done once.

Calcium and resting spore viability. Samples of resting spores were added to tubes with 10 ml of 1 M CaCl₂ in 0.01 M PIPES at pH 6.2, 6.8, or 7.2 and incubated 1 wk at room temperature. Other samples were placed in 1 M NaCl or buffer alone at each pH as checks. The resting spores were sedimented at 2,987 g for 20 min, resuspended in 30 ml of distilled water, and sprayed into 800 g of greenhouse soil mix being tumbled in a laboratory seed-treating drum. The soil was distributed into four replicate pots that were randomly placed in saucers on a greenhouse bench, sown with broccoli, and carefully watered to prevent splash contamination. The samples of resting spores were added to the tubes at two

concentrations which yielded either 10² or 10⁶ resting spores per gram of soil mix. A spore concentration of 10²/g of soil mix is the threshold for 100% clubbing of bait seedlings in this system (19). The plants were grown for 42 days and examined for clubroot. The experiment was repeated once with comparable results. Results of the second experiment are reported here.

RESULTS

pH. The total number of infections, the number of infections that matured and released secondary zoospores, and the percentage of clubbing were high between pH 5.4 and 7.1 with any of three buffers (Table 1). The total number of infections was reduced, but not eliminated, at pH 7.3–8.0; however, neither discharged sporangia nor clubroot symptoms were observed. Because infection was affected by pH, but not by the buffer, PIPES was used exclusively in the remaining trials.

Calcium. The calcium content of the nutrient solution influenced infection and clubroot development, and this effect was influenced by pH (Table 2). Although infections occurred in the absence of added calcium, the continued growth of both the pathogen and the host were affected adversely and no clubroot developed. With 0.5 mM or more of calcium, host and pathogen grew well and clubroot developed until a limiting concentration of calcium that reduced infection and clubbing was reached. This concentration varied inversely with pH.

Magnesium. The effects of magnesium on clubroot development were similar to those of calcium (Table 3). Infection, as well as host, pathogen, and disease development, were poor at pH 6.2 and 6.8 without added magnesium. Host and pathogen development occurred over a span of concentrations, but pathogen development, especially the liberation of zoospores and induction of clubbing, decreased at higher concentrations of magnesium. All stages from infection to clubbing were inhibited by pH 7.2 in this trial.

Calcium uptake. Incorporation of calcium into uninoculated roots was depressed at pH 6.2 in solutions with 1.5 or 7.5 mM calcium compared to pH 6.8 or 7.2 (Fig. 1). No significant differences in incorporation were observed at 25 mM calcium. Similar results were obtained with plants grown for 15 days in infested sand and with plants grown for 30 days in either infested or noninfested sand.

Calcium and resting spore viability. Incubation of resting spores with 1 M calcium chloride significantly reduced spore viability but

TABLE 1. Development of *Plasmodiophora brassicae* and clubroot in broccoli in nutrient solution-sand culture at different pH levels

pH	Buffer ^a	Infections ^b		Plants clubbed ^c
		Mature	Total	
5.4	MES	27 ± 17	76 ± 22	100
5.8	MES	16 ± 14	70 ± 14	100
6.1	PIPES	27 ± 18	64 ± 21	100
6.2	MES	20 ± 21	117 ± 32	100
6.6	HEPES	53 ± 5	109 ± 6	100
6.7	PIPES	26 ± 16	84 ± 23	100
7.0	HEPES	14 ± 16	56 ± 42	100
7.1	PIPES	15 ± 7	90 ± 9	75
7.3	HEPES	0	11 ± 3	0
7.5	HEPES	0	14 ± 16	0
7.6	TRIS	0	10 ± 9	0
8.0	TRIS	0	3 ± 4	0
8.2	TRIS	0	0	0

^a MES = 2-(*N*-morpholino)ethanesulfonic acid, monosodium salt; PIPES = piperazine-*N,N'*-bis-(2-ethanesulfonic acid), monosodium salt, monohydrate; HEPES = *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; and TRIS = tris-(hydroxymethyl)aminomethane.

^b Average number of infections per 100 root hairs ± standard deviation in roots of two seedlings from each of two replicate pots 12 days after sowing. Mature = empty zoosporangia that had released secondary zoospores. Total = all primary thalli at any stage of development, including mature.

^c Percentage of plants with clubroot in duplicate pots 30 days after sowing.

TABLE 2. Development of *Plasmodiophora brassicae* and clubroot of broccoli at different calcium and pH levels^a

Ca ^d (mM)	Infection ^b at pH:						Plants clubbed ^c (%) at pH:		
	6.2		6.8		7.2		6.2	6.8	7.2
	Mature	Total	Mature	Total	Mature	Total			
0	3 ± 2	42 ± 2	3 ± 1	30 ± 11	3 ± 1	10 ± 4	0 ^e	0 ^e	0 ^e
0.5	17 ± 16	62 ± 22	13 ± 17	86 ± 7	10 ± 8	64 ± 9	100	100	20
1.0	34 ± 17	92 ± 9	15 ± 8	77 ± 26	0 ± 0	53 ± 12	100	100	25
1.5	54 ± 1	83 ± 3	19 ± 6	89 ± 1	3 ± 3	69 ± 15	100	86	67
2.5	20 ± 11	88 ± 12	10 ± 4	79 ± 19	0 ± 0	12 ± 8	100	100	17
3.5	14 ± 13	60 ± 16	4 ± 1	80 ± 8	0 ± 0	5 ± 2	100	50	0
5.0	41 ± 22	76 ± 21	10 ± 11	95 ± 22	0 ± 0	6 ± 3	100	71	0
7.5	29 ± 16	78 ± 22	3 ± 4	36 ± 19	0 ± 0	3 ± 2	67	50	0
25.0	11 ± 7	41 ± 20	0 ± 1	10 ± 9	0 ± 0	2 ± 1	0	0	0
Cl Ck ^f	32 ± 18	93 ± 11	1 ± 2	53 ± 32	0 ± 0	2 ± 3	100	100	25
Na Ck ^g	14 ± 9	85 ± 11	9 ± 5	82 ± 12	0 ± 0	11 ± 5	100	100	17

^aThe pH of the nutrient solutions that contained 1 mM magnesium was maintained with 0.01 M PIPES buffer.

^bAverage number of infections per 100 root hairs ± standard deviation in roots of two seedlings from each of two replicate pots 12 days after sowing. Mature = empty zoosporangia that had released secondary zoospores. Total = all primary thalli at any stage of development, including mature.

^cAverage percentage of plants with clubroot in two replicate pots 30 days after sowing.

^dCalcium provided by Ca(NO₃)₂ balanced with NaNO₃ to maintain N up to 2.5 mM Ca and by CaCl₂ above that.

^ePlant development abnormal, and growth severely arrested.

^fChloride check with 2.5 mM Ca plus 50 mM Cl from NaCl.

^gSodium check with 2.5 mM Ca plus 5 mM Na from Na₂SO₄.

TABLE 3. Development of *Plasmodiophora brassicae* and clubroot of broccoli at different magnesium and pH levels^a

Mg ^b (mM)	Infections ^c at pH:						Plants clubbed ^d (%) at pH:		
	6.2		6.8		7.2		6.2	6.8	7.2
	Mature	Total	Mature	Total	Mature	Total			
0	2 ± 2	87 ± 5	9 ± 3	35 ± 6	0 ± 0	5 ± 3	0	9	0
0.5	11 ± 5	82 ± 16	16 ± 6	67 ± 10	0 ± 0	9 ± 9	0	27	18
1.5	31 ± 14	88 ± 1	22 ± 6	64 ± 20	1 ± 1	1 ± 2	11	27	0
2.5	24 ± 17	76 ± 13	9 ± 3	64 ± 7	0 ± 0	0 ± 0	100	18	0
3.5	18 ± 3	106 ± 6	8 ± 2	57 ± 8	3 ± 3	14 ± 17	86	10	0
5.0	26 ± 13	91 ± 3	22 ± 10	72 ± 10	0 ± 0	0 ± 0	75	0	0
12.5	27 ± 6	87 ± 4	2 ± 1	13 ± 9	0 ± 0	0 ± 0	44	0	0
25.0	10 ± 2	62 ± 16	0 ± 0	2 ± 1	0 ± 0	0 ± 0	0	0	0
Cl Ck ^e	20 ± 8	78 ± 5	12 ± 5	57 ± 9	0 ± 0	0 ± 0	100	25	0
Na Ck ^f	18 ± 11	70 ± 15	14 ± 4	60 ± 1	2 ± 1	2 ± 1	100	10	0

^aThe pH of the nutrient solutions that contained 2.5 mM calcium was maintained with 0.01 M PIPES buffer.

^bMagnesium provided by MgCl₂ above 1 mM Mg.

^cAverage number of infections per 100 root hairs ± standard deviation in roots of two seedlings from each of two replicate pots 12 days after sowing. Mature = empty zoosporangia that had released secondary zoospores. Total = all primary thalli at any stage of development, including mature.

^dAverage percentage of plants with clubroot in two replicate pots 30 days after sowing.

^eChloride check with 50 mM Cl from NaCl.

^fSodium check with 50 mM Na from Na₂SO₄.

TABLE 4. Viability of resting spores of *Plasmodiophora brassicae* as determined by infection of broccoli bait plants after exposure to calcium or sodium at three pH levels for 1 wk^a

Chemical	pH	Clubroot ^b (%)	
		10 ² spores/g	10 ⁶ spores/g
CaCl ₂	6.2	21 ^c	100
	6.8	7 ^c	99
	7.2	0 ^c	99
NaCl	6.2	85	90
	6.8	85	99
	7.2	69	96
Nil	6.2	99	100
	6.8	87	99
	7.2	87	99

^aResting spores were incubated 1 wk at room temperature in 0.01 M PIPES buffer at three pH levels with 1 M CaCl₂ or NaCl, removed by centrifugation, and added to soil mix in which broccoli bait plants were grown for 6 wk.

^bAverage percentage of plants with clubroot in four replicate pots 30 days after sowing.

^cThese averages differed significantly from all others, *P* = 0.01.

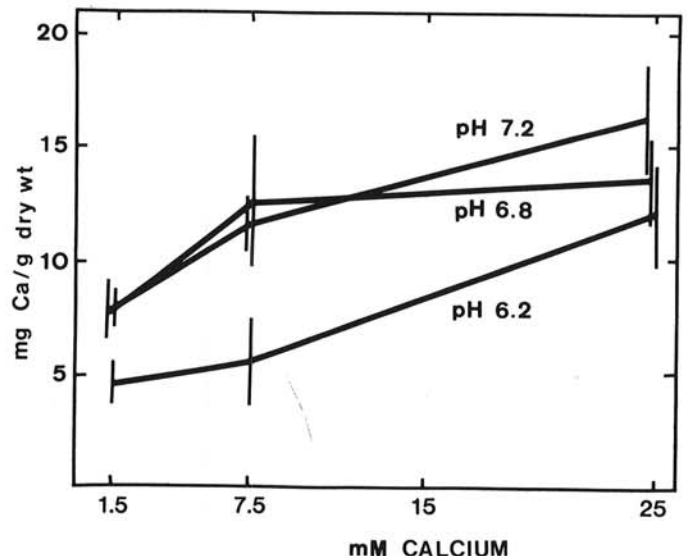


Fig. 1. Incorporation of calcium into roots of broccoli grown for 15 days in nutrient solutions at three pH values and with three amounts calcium. Vertical bars are the standard deviations for four replicates.

only at the low spore concentration (Table 4). The pH at which the spores were incubated had no significant effect. None of the plants in 16 noninfested pots distributed among the experimental pots were infected by *P. brassicae*; this confirmed the absence of splash contamination.

DISCUSSION

Despite the long-standing emphasis on pH to evaluate clubroot control by liming, there have been only two prior studies attempting to separate the effects of pH on *P. brassicae* from those of calcium and magnesium without the complications introduced by using soil, especially soil treated with lime. MacFarlane (16) showed a reduction in primary infections in solution culture at pH 8 compared to pH 5 or 6, but his use of phosphate buffers made it impossible to alter pH without varying other nutrients and he could not demonstrate any effect of calcium. Presumably calcium was precipitated from solution as calcium phosphate. Palm (20) used a sand-nutrient culture system and phosphate buffers. Although our sand-nutrient culture system was similar to his, our results contrast markedly. For example, he reported a peak of infection at pH 4.5 to 5.2 with a 77% decline in infection at pH 6.2. Although we did not test pH < 5.4, infection was high from pH 5.4 to 7.1 with no decline at pH 6.2. Increasing the pH above 7.1 reduced the number of primary infections and affected *P. brassicae* in a manner not previously reported. The few thalli that began to develop did not produce secondary zoospores; thalli that developed at pH 8.0 were misshapen and aborted. No clubs developed on these plants. These observations, like those obtained by manipulating soil water matric potentials (5), support the hypothesis that reinfection by secondary zoospores is necessary for the clubbing phase of the disease (9).

Calcium or magnesium affected *P. brassicae* but their effects showed a pH-dependent interaction. Increasing the calcium or magnesium content of the nutrient solution decreased the number of infections and the amount of clubbing. The amount of these cations needed to prevent clubbing was smaller as the pH was increased from 6.2 to 7.2. This interaction seems to be related to the pH-dependent incorporation of calcium by the host. When there was a low concentration of calcium in the external solution, calcium incorporation into the roots was less efficient at pH 6.2 than at 6.8 or 7.2. Soybeans likewise required more calcium for normal growth rates at lower pH levels (14). Results for the magnesium-pH interaction were similar although we did not study the incorporation of magnesium by the plant. Palm (20) varied calcium from 0 to 4 mM at pH 5.9 and reported a sharp peak of infection at 1 mM calcium, whereas we found high infection from 0.5 to 7.5 mM calcium at pH 6.2. The discrepancies between our results and those of Palm (20) in pH, calcium, and magnesium effects may have resulted from his measurement of infection at 6 days compared to ours at 12 days, the different composition of the nutrient solutions used, or his use of phosphate buffers compared to our use of PIPES. We have not attempted to resolve the discrepancies.

The present results improve our understanding of the mechanism of the action of lime and may help to explain the erratic behavior of lime in controlling clubroot (1,3,6-8,12). We hypothesize that the degree to which clubroot is controlled may depend on the balance between pH and the concentration of the cations, calcium and magnesium (1). The pH of the soil is probably the most important factor influencing disease development, but high concentrations of calcium and magnesium may give control at pH < 7.2. Similarly, low concentrations may permit disease development at pH > 7.2. This hypothesis also is consistent with our observations on the different responses of soils to liming (17). The amount of exchangeable calcium and magnesium in different types of soils correlated positively with increased clubroot control when the soils were limed (17). Our results are consistent with the theory that a balance of nutrients influences host-parasite development (13). Furthermore, pH can play a significant role in controlling this balance, at least in clubroot of crucifers. These conclusions must be extrapolated to field conditions with caution because there are other important factors that vary in field soils or in other climates

and that also may contribute to the amount of clubroot that develops (2,3,12).

Studies of the process or processes affected by calcium and pH between resting spore germination and establishment of the secondary thallus in the host protoplasm need to be done. For example we have not resolved whether high pH and calcium were fungistatic or fungicidal to resting spores. The infectivity of resting spores was not affected by high pH in the absence of calcium but was diminished by 1 M calcium. This decrease was an indication of loss of viability not greater than 10% based on the threshold for 100% clubbing of bait seedlings at 10^2 spores per gram of soil in our bioassay system (19). Part of this reduction, however, could have been caused by a fungistatic effect of residual calcium on resting spore germination or zoospore infection. Even if high calcium was fungicidal, only a small proportion of resting spores would be exposed to 1 M calcium at microsites in the field. In addition, lime was effective when applied 1 day before planting (1). Thus, calcium and high pH may have little fungicidal effect on resting spores (4,6,7,12,15) but may affect pathogen penetration or development in the host.

LITERATURE CITED

1. Campbell, R. N., Greathead, A. S., Myers, D. F. and De Boer, G. J. 1985. Factors related to control of clubroot of crucifers in the Salinas Valley of California. *Phytopathology* 75:665-670.
2. Colhoun, J. 1953. Observations on the incidence of club-root disease of *Brassicae* in limed soils in relation to temperature. *Ann. Appl. Biol.* 40:639-644.
3. Colhoun, J. 1953. A study of the epidemiology of the clubroot disease of *Brassicae*. *Ann. Appl. Biol.* 40:262-283.
4. Colhoun, J. 1973. Effects of environmental factors on plant disease. *Annu. Rev. Phytopathol.* 11:343-364.
5. Dobson, R. L. and Gabrielson, R. L. 1983. Role of primary and secondary zoospores of *Plasmodiophora brassicae* in the development of clubroot in Chinese cabbage. *Phytopathology* 73:559-561.
6. Dobson, R. L., Gabrielson, R. L., Baker, A. S., and Bennett, L. 1983. Effects of lime particle size and distribution and fertilizer formulation on clubroot disease caused by *Plasmodiophora brassicae*. *Plant Dis.* 67:50-52.
7. Fletcher, J. T., Hims, M. J., Archer, F. C., and Brown, A. 1982. Effects of adding calcium and sodium salts to field soils on the incidence of clubroot. *Ann. Appl. Biol.* 100:245-251.
8. Hamilton, H. A., and Crête, R. 1978. Influence of soil moisture, soil pH, and liming sources on the incidence of clubroot, the germination and growth of cabbage produced in mineral and organic soils under controlled conditions. *Can. J. Plant Sci.* 58:45-53.
9. Ingram, D. S., and Tommerup, I. C. 1972. The life history of *Plasmodiophora brassicae* Woron. *Proc. R. Soc. Lond.; B, Biol. Sci.* 180:103-112.
10. Jensen, W. A. 1962. *Botanical Histochemistry*. W. H. Freeman and Co., San Francisco and London. 408 pp.
11. Johnson, C. M., and Ulrich, A. 1959. Analytical methods for use in plant analysis. *Calif. Agric. Exp. Stn. Bull.* 766. 78 pp.
12. Karling, J. S. 1968. *The Plasmodiophorales*. 2nd ed. Hafner Publ. Co., New York and London. 256 pp.
13. Lewis, R. W. 1953. An outline of the balance hypothesis of parasitism. *Am. Nat.* 87:273-281.
14. Lund, Z. F. 1970. The effect of calcium and its relation to several cations in soybean root growth. *Soil Sci. Soc. Am. Proc.* 34:456-459.
15. MacFarlane, I. 1952. Factors affecting the survival of *Plasmodiophora brassicae* Wor. in the soil and its assessment by a host test. *Ann. Appl. Biol.* 39:239-256.
16. MacFarlane, I. 1958. A solution culture technique for obtaining root hair, or primary, infection by *Plasmodiophora brassicae*. *J. Gen. Microbiol.* 18:720-732.
17. Myers, D. F., and Campbell, R. N. 1981. Clubroot of crucifers in California: Soils respond differentially to lime for clubroot control. (Abstr.) *Phytopathology* 71:1005-1006.
18. Myers, D. F., and Campbell, R. N. 1982. Clubroot of crucifers: Interaction of pH and nutrition in disease control. (Abstr.) *Phytopathology* 2:998.
19. Myers, D. F., Campbell, R. N., and Greathead, A. S. 1983. Thermal inactivation of *Plasmodiophora brassicae* Woron. and its attempted control by solarization in the Salinas Valley of California. *Crop. Prot.* 2:325-333.
20. Palm, E. T. 1963. Effect of mineral nutrition on the invasion and response of turnip tissue to *Plasmodiophora brassicae* Wor. *Contrib. Boyce Thompson Inst.* 22:91-112.