Effects of Lime Particle Size and Distribution and Fertilizer Formulation on Clubroot Disease Caused by *Plasmodiophora brassicae*

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ABSTRACT

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Lime has historically been recommended and used for clubroot control, but it has not always been effective. This work was done to evaluate variables that can influence the effectiveness of lime, such as the degree of mixing lime with soil, the fineness of limestone, and the residual basicity or acidity of nitrogen sources in the rhizosphere. Thorough mixing of limed soil resulted in a more uniform pH distribution; the pH from one 0.5-g soil microsample to another varied as much as 2 pH units when not thoroughly mixed to as little as 0.2 pH units when thoroughly mixed. This variation in soil pH was not evident using 15-g soil macrosamples. Control was consistently best using thoroughly mixed limed soils in both greenhouse and field trials. Control also improved with decreased particle size of limestone and with use of calcium nitrate, a fertilizer reported to induce a residual basic reaction in the rhizosphere.

Clubroot is a serious disease of crucifers. A good, predictable, and economical control for this disease is needed. Breeding for resistance is promising, although progress is slow. Many partial soil sterilants and fungicides offer adequate control, but the value of these crops does not usually justify their use (1,14). Consequently, farmers often move to new, "clean" land or turn to the old but unpredictable control measure of liming soil to raise the pH (measured in water) to above 7.2.

In many cases, liming has given good control, yet in others poor or negligible control has been reported (7). Larson and Walker (8) correlated failure of control with varying soil moisture levels. Chupp and Sherf (3) reported that a delay of 6–8 wk after liming was required for good control. Colhoun (4) found that high inoculum levels of *Plasmodiophora brassicae* Wor. allowed infection to occur in soils with a pH as high as 8. More recently, Horiuchi and Hori (6) and Myers et al (9) stated that different soils react differently to lime application in relation to clubroot control.

Thus far, attempts to explain the mode of action of lime or the predictability of this control measure have not yielded clear answers. In this work, we have analyzed some of the physical and chemical factors involved in using lime for clubroot control: thoroughness of mixing, lime particle size, and the influence of acidic and basic fertilizers.

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MATERIALS AND METHODS

Soil and lime. A Sultan silt loam soil (fine silty, mixed nonacid mesic Aquic Xerofluvent) located in western Washington and naturally infested with *P. brassicae* was used in all experiments. Hydrated lime was applied to soil at 4,483 kg/ha (2 tons/acre) or its equivalent and was incorporated in the field by rotovating twice. In some pot culture experiments, field mixing was mimicked by briefly mixing lime and moist soil in a plastic bag. All field plots and pot cultures that were thoroughly mixed were passed through a 2-mm sieve and further mixed by hand for about 1 min. All limed soil treatments were planted within 7 days. Unlimed controls were included for all treatments.

Determination of pH. All pH values were obtained using 0.01 M calcium chloride ($CaCl_2$) to minimize water dilution and salt concentration effects (10). This will be referred to as $CaCl_2$-pH to differentiate it from pH measured in distilled water, henceforth called water-pH. About 15-g and less than 0.5-g samples of air-dry soil were used to measure macro- and micro-pH, respectively. Each set of 10 micro-pH soil samples, including three samples taken from inside soil clods, was collected at random from one macro-pH soil sample.

Lime mixing evaluated in the field. Thorough mixing of the soil was not practical on a normal field-plot scale, and miniplots were thus prepared by removing the bottoms from 30-cm-diameter, 50-cm-deep plastic buckets and sinking these into the ground, leaving 5 cm above the soil surface. The four treatments comprised field soil that was limed and not limed, each being either thoroughly mixed or field mixed. Each miniplot was filled and packed with a treated soil, a macro-pH sample was taken, and 25 Chinese cabbage seeds were planted in each. Water was repeatedly applied 7–10 days after planting to induce infection, and clubroot data were collected after 8 wk. The experiment was completely randomized, with four replicates.

Lime mixing evaluated in the greenhouse. Unlimed and field-limed soils were collected in plastic bags and brought into the greenhouse. Three soil treatments were prepared: unlimed, greenhouse-limed, and field-limed soil. Half of each treatment was sieved and thoroughly mixed, the other was not. The six treatments were replicated three times in plastic pots, 15 cm diameter. Soil macro- and micro-pH samples were collected and 15 seeds of Chinese cabbage planted in each pot. All pots received equal quantities of water as needed except for repeated watering 7–10 days after seeding to induce infection. After 6 wk, clubroot symptoms were visible and percentage of infected plants recorded.

Lime particle size and mixing. Unlimed field soil was brought into the greenhouse and limed with six lime treatments: commercial hydrated lime; laboratory limestone ($CaCO_3$) powder; flour limestone; and a fine (<0.5 mm), medium (0.5–1 mm), and coarse (1–2 mm) limestone obtained by sieving a commercial limestone mix. Half of the soil from each lime treatment was then sieved and thoroughly mixed. The 12 treatments were replicated three times in a completely randomized design. A macro-pH sample was taken from each 15-cm pot, 10–15 seeds of Chinese cabbage were planted, and induction watering was given as in previous experiments. Plants were examined for clubroot symptoms after 6 wk.

Nitrogen fertilizer and liming. A randomized, complete block, split-plot design with 12 treatments and four replicates was established in the field. Each block was divided into a field-limed

and unlimed plot, each containing six rows of Chinese cabbage given a different form of nitrogen fertilizer. All fertilizers were mixed to provide the equivalent of a 10-20-20 ratio at 1,120 kg/ha (1,000 lb/acre) using treble superphosphate and muriate of potash. The N sources shown in Table 1 were mixed with the phosphorus and potassium sources prior to application. Nitrapyrin (N-Serve) was added as recommended by the manufacturer to the $(NH_4)_2SO_4$ and urea to reduce nitrification. All fertilizers were broadcast and incorporated by rototilling to a depth of about 15 cm prior to planting. Plants were repeatedly irrigated 7–10 days after planting, and clubroot data were collected after 8 wk. Disease index was calculated by classifying the plants on a symptom scale of 0–3 as given by Buczacki et al (2).

All experiments were repeated at least twice, except for the lime particle size experiment.

RESULTS

Both in the field and in the greenhouse, thorough mixing significantly reduced disease incidence in all limed soils while having no effect on unlimed soils (Tables 2 and 3). On the limed soils, reduced disease was related to low micro-pH ranges with a higher minimum micro-pH and not to macro-pH (Table 3).

As lime ($CaCO_3$) particle size increased and as thorough mixing decreased, disease incidence increased (Table 4). The least disease followed thorough mixing of the powder form of hydrated lime or $CaCO_3$, whereas greater disease followed the use of coarser limestone and poor mixing. Thus, as the lime became less well distributed in the soil, disease increased.

Nitrogen-fertilizer source also influenced disease incidence (Table 1). There was significantly less disease in both the limed and unlimed plots using $Ca(NO_3)_2$. When about half of the $Ca(NO_3)_2$-N was replaced with KNO_3, the disease control was decreased. The greatest infection occurred on $(NH_4)_2SO_4$ and on urea plots with or without the nitrapyrin treatments.

DISCUSSION

The control of clubroot by raising the water-pH of soil above 7.2 has been a source of controversy for many years (7). Smiley and Cook (10) emphasized that water-pH measurements are highly variable depending on the technique followed and soluble salt levels, whereas $CaCl_2$-pH measurements are not. The variability in water-pH may in part be responsible for the controversy over this control measure. $CaCl_2$-pH measurements were used in this study. Water-pH values have been reported to vary from 0 to 0.9 pH units higher than their equivalent $CaCl_2$-pH values, and this variability precludes the easy comparison of one with the other (5,10). Smiley and Cook (10) reported that, on the average, there is a 0.5 pH unit difference and that this average difference was found to hold true for the soils used in this study. Therefore, a water-pH of 7.2 would be about equivalent to a $CaCl_2$-pH of 6.7.

Rototilling lime into a silt loam soil under our moist field conditions did not give an adequate distribution of the lime. Consequently, although the average soil $CaCl_2$-pH was 6.7 or above, disease control was not obtained and the soil pH varied as much as two pH units from one microsite to another. In terms of clubroot control, the critical component of the pH range would seem to be how frequently and how low the micro-pH values fall. The more frequently low pH microsites occur, as in poorly mixed soils, the greater the chances for infection. It follows that a growing root in field-limed soil will pass through these low pH microsites where the pathogen can be active and infection can occur. Thus, a macro-pH of a limed soil based on a large soil sample could be misleading and inaccurate in predicting the degree of control. In thoroughly mixed soils, on the other hand, the lime was better distributed and the pH variation between microsites was far less, with markedly fewer low pH microsites. The result was that growing roots encountered far fewer, if any, low pH microsites and thus showed much less infection.

Lime particle size is another important parameter affecting lime distribution (Table 4). Furthermore, smaller lime particle size, which would aid in better lime distribution, was correlated with better control. It appeared that the lime source was less important than the surface area of the liming material, which is related to the rate of neutralization of acidic exchangeable cations and ease of obtaining homogeneous mixtures of soil and lime (Table 4).

Of the fertilizer sources tested, only $Ca(NO_3)_2$ significantly reduced clubroot disease. Smiley and Cook (11) reported that differential uptake of cations and anions influenced take-all of wheat by changing the acidity in the rhizosphere. With $Ca(NO_3)_2$, a greater equivalent uptake of NO_3^- than Ca^{++} reduced acidity; with $(NH_4)_2SO_4$, the greater equivalent uptake of NH_4^+ than SO_4^{--} increased acidity. Similar reasoning could explain the results given in Table 1. Moreover, where about half of the $Ca(NO_3)_2$-N was replaced by KNO_3-N, the control of clubroot was less, probably because crucifers take up K^+ more rapidly than Ca^{++}, reducing the difference in uptake between NO_3^- and its associated cations, K^+ and Ca^{++}. Thus, we might expect the rhizosphere pH not to rise as much as with $Ca(NO_3)_2$ alone. The use of nitrapyrin with $(NH_4)_2SO_4$ and urea was to prevent nitrification and force the plant to absorb NH_4^+, creating a more acidic rhizosphere and increased clubroot infection. However, no significant increase in disease was observed (Table 1). A possible explanation is that without nitrapyrin, nitrification increased acidity by conversion of the basic NH_4^+ cation to the acidic NO_3^- anion, and this increased the acidity as much as the effect of nitrapyrin. Thus, of the fertilizers tested, only $Ca(NO_3)_2$ would have the effect of increasing the soil and rhizosphere pH, and this may explain its effect in reducing disease.

In this study lime did control clubroot,

Table 1. Clubroot of Chinese cabbage in the field in limed and unlimed soils following the use of acidic and basic nitrogen fertilizer sources[w]

Nitrogen source	Limed soils[x]		Unlimed soils	
	Infected plants (%)	Disease index[y]	Infected plants (%)	Disease index
$Ca(NO_3)_2$	0 a[z]	0 a	86 a	1.4 a
$KNO_3 + Ca(NO_3)_2$	24 ab	0.3 a	99 b	2.5 b
$(NH_4)_2SO_4$	32 b	0.4 a	100 b	2.8 b
$(NH_4)_2SO_4$ + nitrapyrin	35 b	0.5 a	100 b	2.7 b
Urea	32 b	0.4 a	100 b	2.9 b
Urea + nitrapyrin	38 b	0.4 a	100 b	2.6 b

[w] All fertilizers were mixed to provide the equivalent of 1,120 kg of a 10-20-20 fertilizer per hectare.
[x] Hydrated lime applied at 4,483 kg/ha and rototilled twice prior to planting.
[y] Disease index based on a scale of 0–3 (2).
[z] All numbers within columns followed by the same letter are not significantly different based on Duncan's multiple range test ($P = 0.05$). Means of representative data from experiments with four replicates, 20 plants per replicate.

Table 2. Clubroot of Chinese cabbage in field miniplots following field or thorough mixing of lime

Soil treatment[x]	Infected plants (%)[y]	Average pH[z] (macro)
No lime; field mix	91 a	6.0
No lime; thorough mix	96 a	6.2
Lime; field mix	34 b	7.0
Lime; thorough mix	6 c	7.0

[x] Hydrated lime applied at 4,483 kg/ha in a silt loam soil and rotovated twice for field mix. Thorough mix was the additional mixing of soil and lime after passing through a 2-mm sieve.
[y] Combined means of two experiments with four replicates of 15 plants each. All numbers followed by the same letter are not significantly different based on Duncan's multiple range test ($P = 0.05$).
[z] $CaCl_2$-pH determinations.

Table 3. Incidence of clubroot on Chinese cabbage and soil pH in the greenhouse following field, greenhouse (GH), and thorough mixing of limed soil

Soil treatment[x]	Infected plants (%)[y]	Soil pH[z]	
		Macro	Micro-pH range
No lime; field mix	100 a	5.9	5.6-6.3
No lime; thorough mix	100 a	6.0	5.9-6.1
GH limed; GH mix	48 c	7.3	6.0-8.0
GH limed; thorough mix	18 d	6.9	6.6-6.95
Field limed; field mix	86 b	6.4	5.7-6.8
Field limed; thorough mix	25 d	6.5	6.4-6.65

[x] Hydrated lime applied in the field at 4,483 kg/ha in a silt loam soil and rotovated twice for field mix or applied in the greenhouse (GH) and shaken briefly in plastic bags for GH mix. Thorough mix was the additional mixing of soil and lime after passing through a 2-mm sieve.

[y] Combined means of three experiments with four replicates, 10 plants per replicate. All numbers followed by the same letter are not significantly different based on Duncan's multiple range test ($P = 0.05$).

[z] $CaCl_2$-pH. Micro-pH range based on 10 micro-pH soil samples (<0.5 g each) taken from one macro-pH soil sample (15 g).

Table 4. Clubroot of Chinese cabbage in a silt loam soil limed with different particle sizes following greenhouse (GH) or thorough mixing

Source and size of lime particles[w]	Infected plants (%)[x]		pH[y] (macro)
	GH mix	Thorough mix	
$Ca(OH)_2$ (powder)	8 b[z]	0 a	7.4
Laboratory $CaCO_3$ (powder)	10 b	0 a	7.4
Agricultural flour limestone	12 c	8 b	7.3
Fine limestone (<0.5 mm)	54 e	27 d	7.3
Medium limestone (0.5–1 mm)	70 f	48 e	7.2
Coarse limestone (1–2 mm)	...	80 g	7.1
No lime	97 h	100 h	5.8

[w] All lime was applied at the equivalent of 4,483 kg of hydrated lime per hectare.

[x] Means of three replicates, 10 plants per replicate. GH mix is the incorporation of lime by brief shaking in plastic bags. Thorough mix was the additional mixing of soil and lime after passing through a 2-mm sieve.

[y] Average $CaCl_2$-pH values.

[z] All numbers in both columns followed by the same letter are not significantly different based on Duncan's multiple range test ($P = 0.05$).

but how lime actually functions in inhibiting this disease is as yet unknown. Although pH is stressed in this paper as a possible direct controlling factor, it may only be an assay of how well lime is distributed in the soil. Ca^{++} or secondary pH effects on minor elements such as iron, copper, and others must also be considered. Whatever the controlling factor, an effective distribution of lime was essential to control. This emphasizes the importance of specifying conditions related to lime distribution, including soil preparation, soil moisture, soil texture, lime particle size, quantity of lime, incubation interval between lime application and planting, and fertilizer type used.

Chupp and Sherf (3) recommended an incubation interval of 6–8 wk for control, and this may reflect the time required for lime redistribution under field conditions. Welch et al (13) and Waring and Wisbey (12) have obtained good control using 10–20 tons of lime per acre, which could also greatly improve lime distribution. Myers et al (9) and Horiuchi and Hori (6) have reported that liming of different soil types gives different levels of clubroot control. This may reflect the fact that some soil textures or moisture levels enable lime to be better distributed or redistributed than others. The lack of information on lime distribution may be a major reason for the contradictory results summarized by Karling (7). For practical purposes, methods are needed to achieve economically the more uniform distribution of lime in field soils.

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