Factors Related to Control of Clubroot of Crucifers in the Salinas Valley of California

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The authors gratefully acknowledge financial assistance provided by the Grower-Shipper Vegetable Association of Central California; fields provided by the Office of Treasurer of Regents of University of California, Old Stage Farms, and d'Arrigo Brothers of California; and statistical advice from F. J. Hills.

Accepted for publication 10 January 1985 (submitted for electronic processing).

ABSTRACT

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.

Control trials were started after clubroot, caused by *Plasmodiophora brassicae*, was discovered on broccoli in the Salinas Valley in 1978. Single applications of lime ($CaCO_3$) controlled clubroot for up to 3 yr and were more effective than pentachloronitrobenzene or calcium cyanamide. Some parameters affected by lime application were evaluated to better understand the mechanism of action of lime and the variable control of clubroot reported in liming trials in which only pH was measured. Lime applied at 5–33 metric tonnes per ha (t/ha) to severely infested Placentia sandy loam soil gave control for 2–3 yr in two trials. Soil pH increased as a curvilinear response to lime and continued to increase each year so that control was obtained at pH 6.7 in the first year and at pH 7.2 in the third year. Extractable calcium increased as a linear response to lime and control was obtained with 12–14 meq/100 g of soil. In contrast, a single application of 2.7–10.8 t/ha to a lightly infested field of Elder sandy loam soil controlled clubroot in the first crop, but not in the second crop when the pH and extractable calcium fell below 7.1 and 14 meq/100 g, respectively. In one trial, lime was as effective when incorporated into the soil 1 day before planting as when added 6 wk before planting. Lime was effective in another experiment in a commercial field and in numerous infested fields treated by growers. We postulate that successful control by liming depends on an interaction between pH and extractable calcium plus magnesium which must exceed approximately 14 meq/100 g of soil from native minerals plus the lime treatment.

Clubroot, caused by *Plasmodiophora brassicae* Wor., has been spreading slowly in California since the coastal area of San Mateo county was infested in the 1940s (18). The pathogen had spread south to the northern part of Monterey County by 1967 (20). In 1978, it was found in the Salinas Valley where, judging by the size of the infested area and the severity of the disease, it probably had been introduced several years earlier. This outbreak was especially threatening because approximately 26,000 ha of crucifers are grown in the valley. Farming and harvesting equipment is moved frequently from field to field and broccoli (*Brassica oleracea* L. var. *italica* Plenck) is the primary economic crop grown on some clubroot-conducive, acidic soils. All collections of *P. brassicae* in California (two from San Mateo Co. and four from the Salinas Valley) have reacted on the European Clubroot Differential set as 16/0/31 or race 7 (14) and probably represent dissemination from one introduction.

The application of lime to soil to control clubroot has been practiced for well over 100 yr. Although liming is often recommended, the literature contains few reports that permit accurate prediction of the degree of control that will be achieved. The earlier literature reviewed by Colhoun (2) and Karling (11) contains many contradictions because it is based primarily on field trials which had many uncontrolled variables and were done on many different soil types. More recently, lime alone has given good control for some authors (6,20) while others report that it must be used with pentachloronitrobenzene (PCNB) for satisfactory control (1,19,21). Temperature, light intensity, inoculum density, and moisture are a few of the factors that cannot be controlled in field trials and that are given as probable causes when liming fails to control clubroot (2,11).

Among the factors that can be varied experimentally are the form and amount of lime, application of other chemicals, and the timing of application. The forms of lime may be $CaCO_3$ (alone or mixed with $MgCO_3$), CaO, or $Ca(OH)_2$. Sherf (16) emphasizes that at least 1,680 kg of $Ca(OH)_2$/ha must be applied annually at least 6 wk before planting. Fletcher et al (6) reported good control with high rates of either $CaCO_3$ or Na_2CO_3. The minimum desired soil pH has generally been given as pH 7.2 (11,16), but severe clubroot has developed at higher pH values (2,5,8,11). Comparisons are difficult because methods of determining pH have changed. Measurements with the water-saturated paste and 0.01 M $CaCl_2$-saturated paste methods differ by about 0.5 pH unit (15,17). Furthermore, a composite sample from a limed soil may indicate a pH of 7.2, but microsamples of the same soil may vary 1–2 pH units (5,8). Although the lime treatments have been evaluated by measuring their effect on pH and disease control, no attempts have been made to correlate clubroot control with the available magnesium and calcium supplied from native sources plus the lime treatment.

The present trials were established to evaluate control measures for the soils of the Salinas Valley. Because liming was effective, studies were done to test rates and timing of the treatment for practical control, as well as to evaluate the influence of pH and available cations, to better understand the mode of action of lime and to permit prediction of its efficacy. A preliminary report has been presented (7). Related laboratory and greenhouse experiments on the mode of action of lime are presented in a companion paper (12) and abstract (13). Experiments on solarization have been reported previously (14).

MATERIALS AND METHODS

Four major liming experiments were done in the Salinas Valley. Trials I and II were on small plots in a severely-infested field devoted to this experimentation from 1980 to 1982. Five levels of lime application were evaluated for effect on disease severity, plant yield, soil pH, and cation levels for 2–3 yr. These trials were supplemented with trials III and IV in which commercial-scale applications were tested in grower's fields initially having scattered infestations. These trials included only four levels of lime and were similarly evaluated except that plant yield was omitted.

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Lime. The lime used for these plots was spent lime ($CaCO_3$) from a local sugar beet processing factory. The lime was in the carbonate form; 70% passed a 2-mm (10-mesh) soil sieve and 50% passed a 0.425-mm (40-mesh) sieve. All applications were on the basis of 100% $CaCO_3$ after correcting for 40% impurities and moisture content. The lime was broadcast by hand in Trials I and II and by a truck-mounted rotary spreader in Trials III and IV. Lime applications were always made on dry soil that was disked to a depth of 15 cm (usually three times) after lime application. Later, standard beds (15 cm high, 50 cm wide at the top, and on 1 m centers) were formed and the crop was sown.

Small plot trials. A research area was established near Chualar, CA, in a field of Placentia sandy loam soil (3) where a severe clubroot infestation was discovered in broccoli in 1979. There were up to 10^6 resting spores per gram of soil after harvest of this crop (14). A portion of this area was sown with susceptible Chinese cabbage (*B. campestris* L. var. *pekinensis* Rupr. 'Michihli' in April 1980 to locate the most severely infested area and to increase the inoculum. After 7 wk, the planted area was divided into 56 units, each 11 × 15 m. In each unit, six plants were dug at each of nine sites. The research area (45 × 213 m) comprised contiguous units in each of which >45 plants were diseased. Each summer, from 1980 to 1982, the entire research area was sown to broccoli (two rows per bed) that was thinned to approximately 30 cm spacing in the row. Cultivar Topper was sown in 1980 and 1981 and cultivar Green Duke in 1982. The planting and harvest dates for each year were: 4 August and 1 October 1980, 4 June and 25 August 1981, and 7 June and 30 August 1982. A sprinkler system was operated to apply 2.5–3.0 cm of irrigation water approximately once a week while the crop was growing. A fertilizer mixture of ammonium phosphate and potassium sulfate (560 kg/ha) was broadcast before planting and ammonium nitrate (336 kg/ha, also broadcast) was applied at the mid-growth stage.

Lime was applied to Trial I on 25 June 1980 in a randomized complete block design. There were five lime treatments (0, 4.3, 8.5, 17, and 34 metric tonnes/hectare [t/ha]) each applied to four replicate plots (7.6 × 7.6 m). Broccoli was sown annually from 1980 through 1982 with no further applications of lime.

The sample portion of each plot was the central 4.6 × 4.6-m area. Soil samples taken near planting and harvest dates were composites from the bed tops at nine sites within the sample area. Soil pH was determined by the water-saturated paste method (15). Extractable calcium (XCa) and magnesium (XMg) were measured by the Versene titration method after extraction with ammonium acetate (10). Total calcium was determined by atomic absorption analysis of perchloric acid digests. At harvest time, the plants (approximately 100) in the sample area were dug and rated individually for clubroot by using the 1-4 scale in which 1 = no clubroot and 4 = >50% of the primary root clubbed (14). A disease severity index from 1 to 4 was calculated for each plot (14). The results were tested by analysis of variance with orthogonal partitioning of treatments into linear and quadratic components. If only linear trends were significant ($P = 0.05$), the linear line of best fit was obtained by the least squares method. If there was a significant quadratic trend ($P = 0.05$), an asymptotic line was approximated by trial and error. In 1981 and 1982, the average fresh weight of plants, including roots, was determined for each disease severity class. In the 1982 harvest the plants in each class were also rated for marketability, i.e., whether they had formed a head large enough to be harvested for processing. The percentage of marketable heads and plant biomass were calculated for each disease severity class in each plot only if there were >10 plants in each class.

Trial II was placed adjacent to trial I in an area planted to broccoli in 1980. The design was the same as in trial I, but the lime treatments were reduced to 0, 2.9, 5.6, 11.4, and 22.9 t/ha to test the minimum effective dose. Lime was applied once on 24 April 1981. The cultural procedures, harvesting, and data collection for the 1981 and 1982 crops were the same as in Trial I.

Large-scale lime trials. Additional infested fields were discovered in 1980 and parts of two such fields were made available for trials. The fields were managed by the growers except for the application of lime in the experimental area and the collection of data. Both fields were sprinkler irrigated.

Trial III was located on Chualar loam soil (3). Stunted, severely clubbed plants were observed in a spring crop of cauliflower (*B. oleracea* L. var. *botrytis* L.) in one area of about 200 m^2 in May 1980. There were many small, scattered infection centers in the next cauliflower crop in this field in the fall of 1980. Trial III was established in 1981. It was designed with one nontreated control adjacent to each treated plot. The lime treatments were distributed in a randomized complete block with four replications. Each plot was 12 m (12 beds) wide × 122 m long. Three lime application rates were used: 3.4, 7.0, and 13.9 t/ha. The lime was spread on 9 March 1981 and cauliflower was sown (one row per bed) in May. The sampling area was the central 8 × 53 m of each plot and contained about 600 plants. Composite soil samples for pH were collected from the sampling area of each plot about 2 mo after the crop was sown. Because of the scattered distribution of diseased plants, two different types of disease incidence data were collected. First, the number of plants in disease severity class 4 were counted 2 mo after planting. They were recognized by their small size, wilted leaves, and the club that usually protruded from the ground, but they were not dug so they could be included in the evaluation at harvest. Second, at harvest time, 100 randomly selected plants were dug in each sampling area and rated for clubroot severity.

Trial IV was located on Elder sandy loam soil (3). Clubroot was first observed on broccoli in this field in March 1981 as small, widely dispersed infection centers. Trial IV was designed as a randomized complete block with three replications. Lime was spread on 20 May 1981 at 2.7, 5.4, and 10.8 t/ha. Each plot was 15.3 m × 107 m long. The field was planted to broccoli twice; the planting and harvest dates were 3 August and 9 November 1981, and 20 March and 24 June 1982. Soil samples were taken for pH determinations before the lime was applied (average pH 5.5 for eight samples) at planting and harvest time in 1981, but only at harvest in 1982. At maturity of each crop, randomly selected plants (200 in 1981, 100 in 1982) were dug and rated as above.

Other chemicals. In 1980, a trial in which PCNB and lime were incorporated into the beds was established adjacent to Trials I and II. There were four replicates in a randomized complete block design and each plot extended 7.6 m along a single bed. Three rates of PCNB (75% wettable powder) and lime were applied to the surface of preformed beds and incorporated with a rototiller into the upper 8 cm of the beds 1 day before planting. Although the application rates were calculated on a broadcast basis, only the beds were treated. Thus, the amount of chemical applied per hectare was half of that indicated. The plot was planted, maintained, and harvested as described for Trial I except that the sample area was 5.8 m long and contained approximately 40 plants.

A granular form of calcium cyanamide, Perlka (SKW Trostberg Aktiengesellschaft, Trostberg, W. Germany), at 1 t/ha was tested in a trial near Trials I and II in 1982. There were three replications in a randomized complete block design with each plot measuring 7.6 × 7.6 m. Perlka was applied 30 April 1982. Nitrogen equivalent to that contained in the Perlka was added to the check plots as ammonium nitrate, and the soil was disked twice and irrigated. After 14 days, the plots were cultivated, bedded, maintained, and harvested with Trials I and II. Weed counts were made 16 days after planting within a 0.3 × 0.3-m frame randomly placed on the bed surface at six locations in each plot.

RESULTS

Effect of lime on disease, pH, and exchangeable calcium in small plots. The results of trials I and II were similar (Fig. 1). Lime application reduced disease severity and significantly increased the soil pH and XCa. The reduction in disease severity was not as great in Trial II as in Trial I. In both trials, clubroot increased in the second or third crop after liming with 2.9–8.5 t/ha. The pH increased at each successive crop. The pH data obtained at each harvest were consistent with this trend but are not presented. The pH values associated with clubroot control increased from 6.7 in the first year of Trial I to pH 7.3 in the second or third years of both

trials. As expected, lime applications produced a highly significant, linear increase in XCa (Fig.1) as well as in total calcium (*unpublished*). Extractable calcium values of 11–12 meq or more per 100 g were associated with good control in the 1981 and 1982 crops of both plots. The average reduction in XCa of 0.7 meq/100 g from 1981 to 1982 apparently represents leaching during winter rains (44 cm, 30% above normal). The XMg averaged 2.12 ± 0.3 meq/100 g and was not affected by the lime treatments.

Effect of clubroot on plant growth. Only the 1982 crop in Trials I and II was raised to maturity so that marketable yield and plant weight could be rated. Yield data from both trials have been combined (Table 1). The plants in severity classes 1 and 2 did not differ greatly. The smaller size and yield of the plants in class 1 was probably caused by the inclusion of small, doublet plants that germinated late, grew under the older plants, and were not thinned. Plant growth and yield were reduced in disease severity class 3,

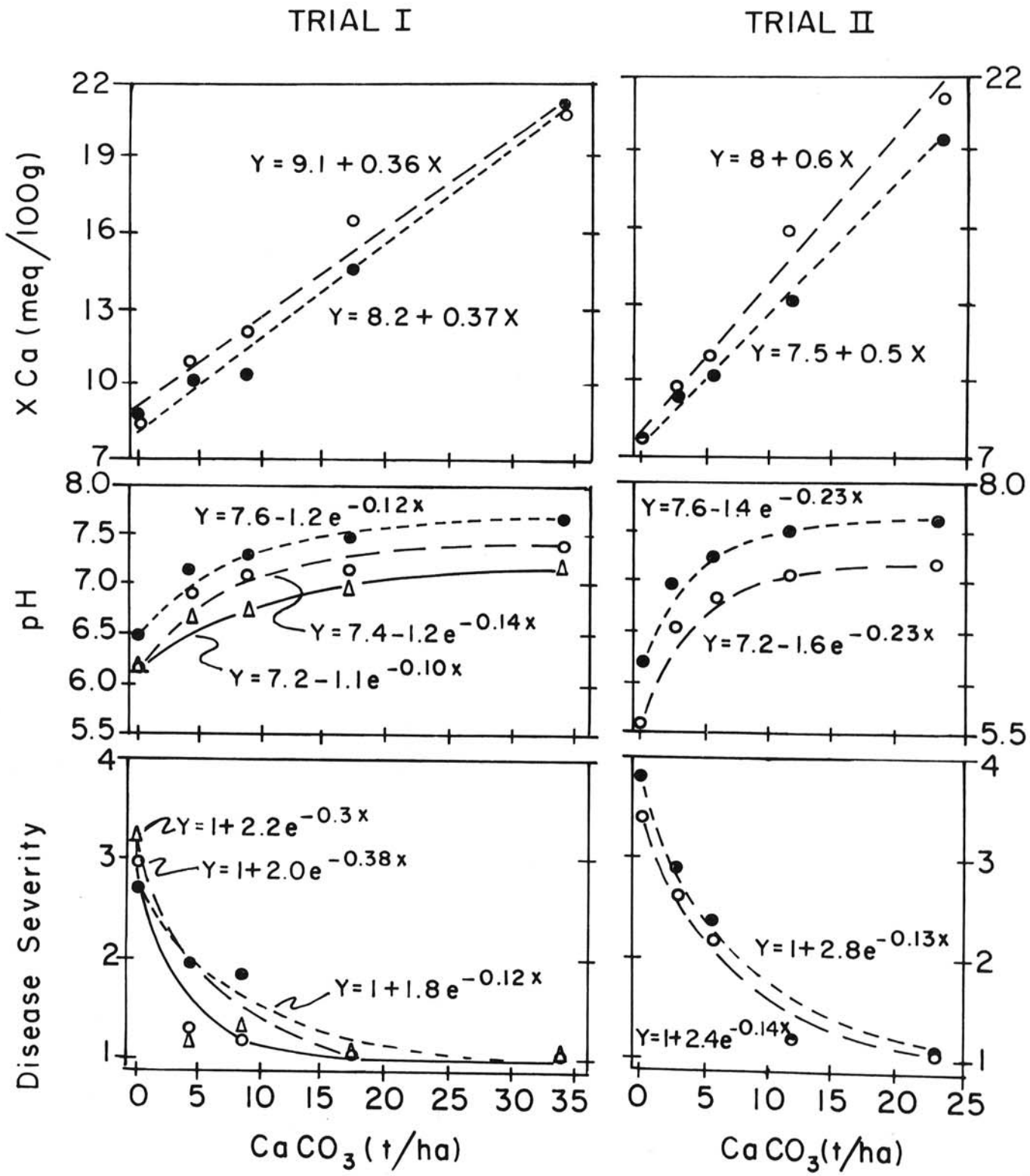

Fig. 1. Relationship of lime ($CaCO_3$) to severity of clubroot of broccoli, soil pH, and extractable calcium (XCa) in Trials I and II on Placentia sandy loam soil: Δ——Δ, 1980 crop; o——o, 1981 crop; •——•, 1982 crop. Disease severity scale ranges from 1 = no clubs to 4 = >50% of taproot clubbed are described in Table 1. $R^2 > 0.98$ for all regression lines.

which did not occur frequently, and they were reduced greatly in class 4. In the latter class, most of the plant weight was the large, galled primary root and the plants were nearly dead. Similar relationships between disease severity classes and fresh weight were observed in the 1981 crop in both trials.

Effect of lime on disease, pH and exchangeable calcium in large scale trials. Lime applications in Trial III increased the soil pH and resulted in a low incidence of clubroot (Fig. 2). Although pH was directly related to the amount of lime added to the soil, it was not increased above 7.0 by any treatment. All applications of lime reduced the number of severely clubbed plants at midseason and increased the number of healthy plants at harvest time.

Trial IV was planted for 2 yr after lime was applied and provided a demonstration of the explosive increase in disease severity. In the first year, clubroot infection centers were widely scattered in two of three blocks; this reduced the effect of lime applications on clubroot severity to marginal significance ($P = 0.945$) (Fig. 3). By the second year, the inoculum had increased markedly and severe clubroot occurred uniformly in the checks. Lime applications still reduced clubroot severity significantly ($P > 0.99$), but even at the highest rate the control was not as good as in the second years of Trials I and II. Lime treatments significantly increased pH and XCa ($P > 0.99$). The pH, however, decreased from the first to the second year in the intermediate lime treatments but remained at about 7.1–7.2 in the highest lime treatment. The XCa, which was only increased to 14.9 meq/100 g at the highest lime rate, was assayed before planting in 1981 and after harvest in 1982. Thus, the reduction, averaging 1.5 meq/100 g for all treatments, represents the calcium removed by two crops plus leaching by winter rains. In the second crop, the best control of clubroot was associated with pH 7.1 and XCa of 13.9 meq/100 g, but this level of control was not satisfactory. The extractable magnesium averaged 2.8 ± 0.7 meq/100 g and was not affected by lime treatments.

Other chemicals. The control of clubroot was better with lime than with PCNB when each was incorporated in beds 1 day before planting (Fig. 4). The effect of lime was comparable to that achieved in the same year in Trial I in which lime was applied 6 wk before planting.

Perlka reduced clubroot incidence in the 1982 plot. The average disease severity index was 2.43 ± 0.7 compared to 3.67 ± 0.2 in the checks. The pH was slightly higher in the Perlka replicates (pH 6.7 ± 0.5) than in the checks (pH 6.2 ± 0.4), but the XCa was the same in both. By comparing the pH of each plot with the regression line for Trial II (which was the most nearly comparable lime plot), the Perlka plots had an average disease severity index 0.66 ± 0.3 less than expected for their pH values whereas the checks did not differ. Perlka did not reduce the number of weeds 16 days after sowing.

TABLE 1. Relationships between clubroot severity and growth of broccoli in Trials I and II in the Salinas Valley of California in 1982

Disease severity class[a]	Number of plots[b]	Yield and size of broccoli[c]	
		Marketable heads (%)	Biomass[d] (g)
1	35	80.8 ± 9.6	645 ± 174
2	10	90.2 ± 11.7	859 ± 244
3	1	50	422
4	22	1.4 ± 2.4	139 ± 40

[a] 1 = no clubs on roots; 2 = one or two clubs on secondary roots; 3 = up to half of the taproot clubbed or numerous clubs on lateral roots; 4 = >50% of the taproot clubbed.
[b] Number of plots out of 40 with more than 10 plants in a given disease severity class and which were used for yield calculations.
[c] Results given as mean and standard deviation of the averages calculated for each plot in which there were more than 10 plants in a given disease severity class.
[d] Average fresh weight of intact plant including roots.

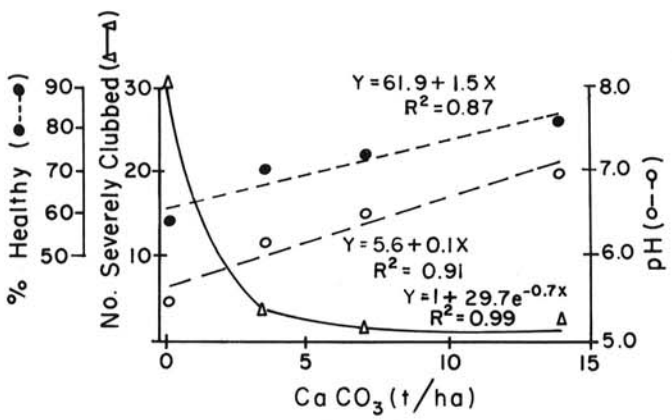

Fig. 2. Relationship of lime ($CaCO_3$) to incidence and severity of clubroot of cauliflower and to soil pH in Trial III on Chualar loam soil, 1981. Clubroot control rated by the number of severely clubbed plants at midseason and by the percentage of plants free of clubbing when plants were dug at harvest.

DISCUSSION

We have confirmed many reports that lime may control clubroot (2,11) and extended them by showing a high degree of control from a single application of lime and by correlating soil pH and extractable cations with control. For this discussion, cations will refer to calcium and magnesium. Measurement of extractable cations includes the exchangeable plus the water-soluble cations available to the plant and is preferred to measurement of total cations. We propose a revised hypothesis on the mode of action of lime. The related laboratory tests are reported in a companion paper (12). We propose that pH and cation concentration are the two important, interacting factors that are affected by liming and that affect disease control. Furthermore, we hypothesize that in the

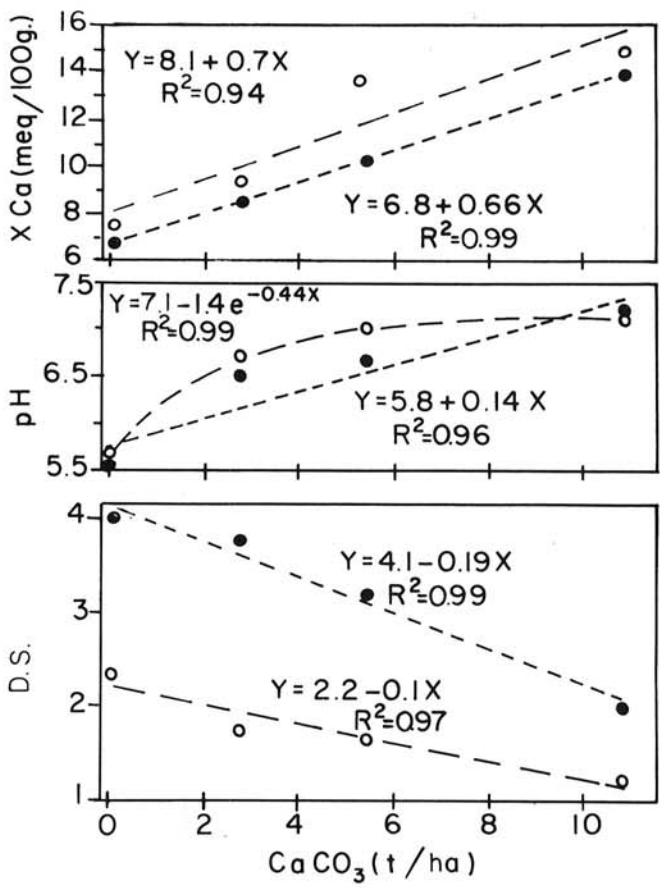

Fig. 3. Relationship of lime ($CaCO_3$) to severity of clubroot of broccoli, soil pH, and extractable calcium in Trial IV on Elder sandy loam soil: o——o, 1981 crop; ●——●, 1982 crop. Disease severity scale from 1 = no clubs to 4 = >50% of taproot clubbed is described in Table 1.

pH range from about 6.7 to about 7.2, the balance between extractable cations in the soil and pH affects the degree of control achieved by liming. Based on trials in Placentia sandy loam soil, we propose that the minimum extractable cation level needed for good control of clubroot is 14 meq/100 g (12 meq XCa + 2 meq XMg in our plot). This figure doubtless will need correction as more soils are tested. From pH 6.7 down to the lower limit for clubbing at about pH 4.0 (9), the pH is generally favorable for fungal development and the cation concentrations must be very high, such as from heavy applications of $CaSO_4$ (6), to reduce clubbing. A pH above 7.2 is unfavorable for abundant primary infection or the initiation of cortical infection and low cation concentrations are adequate to control clubbing (12).

This hypothesis is consistent with the views that there is no single pH value that can be used to predict clubroot control (2,6,11,19) and with the results of Fletcher et al (6) who showed a calcium effect from gypsum applications. This hypothesis does not negate the emphasis placed on pH in other studies or the importance of pH, but it permits an explanation of the variable results from lime applications at pH levels near pH 7.2. Although the cations play a smaller role than pH, their relative importance may differ in the complex environment of field soils. Inherent differences among soils in available cations and the application of lime at rates below the 5–10 t/ha rate, which gave good control in our small-plot trials, could account for many of the reported failures of liming. Other factors, such as high inoculum concentration and moisture levels (2), apparently were much less important as evidenced by the control we achieved in the severely infested fields that were irrigated weekly. Finally, the efficacy of lime for control of clubroot probably varies among soil types (13). We had excellent control in Trials I and II on Placentia sandy loam and much poorer control in Trial IV on Elder sandy loam.

A basic premise of our hypothesis is that the interaction of cations and pH may be at least as important for cation uptake (12) and effect within the plant as for their effect on the fungus in the soil. Thus, our use of finely divided lime applied during the summer or fall to dry, friable, easily workable soil was beneficial because the calcium was uniformly incorporated into the soil and readily available to the plants, even when sowing occurred a day after application. In this respect, we agree with the interpretation of Dobson et al (5) that the uniformity of incorporation of lime is important and that a large variation in pH of microsamples may reflect nonuniform incorporation. On the other hand, we do not agree with their premise that the effect of lime is on resting spores in the soil. This premise would be tenable only if it could be shown that there was a corresponding localized effect on uptake and distribution of cations within the root.

There are other recommendations that would be greatly diminished in importance by our hypothesis. For example, lime in the carbonate form was effective in our trials, but some authors consider it to be inferior to hydrated lime, and it has not been used as extensively (2,11). The forms of lime have not been compared in our field trials because spent lime is readily available and low in cost. Although lime application is recommended at least 6 wk before planting in New York (16), this may not be necessary because control was obtained with lime applied 1 day before planting in one trial.

The efficacy of lime is supported by our observations on the progress of the clubroot infestation in the Salinas Valley. The known infested area, in which the disease had become severe enough to cause visible, reportable plant damage, increased from about 1 ha in 1978, to 24 ha in 1979, to 44 ha in 1980, and to 80 ha in 1981. By 1981, the results of the present trials stimulated extensive, precautionary application of lime to commercial fields regardless of whether they were known to be infested. Only one more infested field was reported in 1982 and 1983. We attribute the slowing of the infestation to the widespread use of lime. Furthermore, growers have achieved good to excellent control by liming their infested fields. No nutrient deficiencies induced by heavy lime applications were observed in any of the trials.

The cost effectiveness and residual effect of lime make it unlikely that PCNB or Perlka will be competitive. However, Perlka did not control weeds which indicates the trial may have been faulty. Even so, the reduction in the severity of clubroot by Perlka in our trials was approximately of the same magnitude as that reported by Dixon (4) in trials conducted for 3 yr.

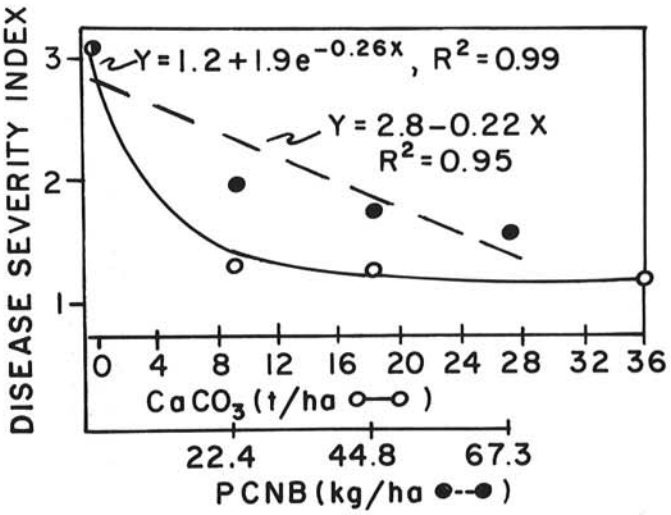

Fig. 4. Control of clubroot by pentachloronitrobenzene (PCNB) or lime ($CaCO_3$) incorporated into beds one day before planting broccoli, Placentia sandy loam soil, 1980. Disease severity scale from 1 = no clubs to 4 = >50% of taproot clubbed is described in Table 1.

Clubroot in irrigated crops of direct-seeded broccoli, and to some extent cauliflower, differs from the disease in transplanted crops or root crops. This is reflected by the skewed distribution of plants into disease severity classes based on those used by others (1,6,9). If the inoculum concentration was high and there was no control of clubroot, most plants were in disease severity class 4. Usually in this class, the entire primary root was clubbed, apparently from early infection as the seed germinated, and the plant was severely stunted or dead by harvest. If the lime treatments were effective, most plants were free of clubs (disease severity class 1). Very few plants fell into the intermediate severity class 3. For practical purposes this class could have been omitted from our field trials. Plants with few clubs (disease severity class 2) were more frequent than those in disease severity class 3. These plants were epidemiologically significant because they would maintain or increase inoculum without obvious growth or yield depression.

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