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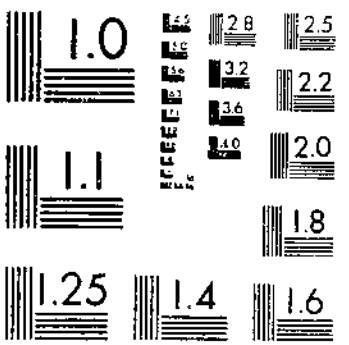
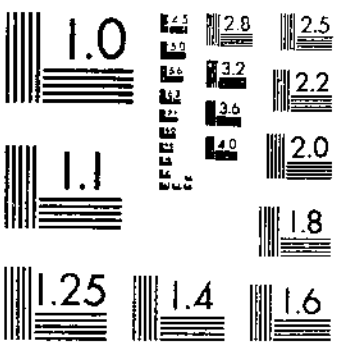


MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS-1963-A



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WASHINGTON, D. C.

CLUBROOT OF CRUCIFERS[1]

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INTRODUCTION

Clubroot of crucifers, caused by *Plasmodiophora brassicae* Wor., has probably given gardeners concern for well over 200 years. The first historic mention of the disease is that by Ellis (*16*),[3] who stated that he first noted it in 1736 on some of his travels in England. It was attacking turnips and was considered a very serious and contagious disease at that time. It was not until 1878, however, that Woronin (*55*) described and named the causal organism.

The disease, which attacks members of the Cruciferae only, is induced by a parasite which penetrates underground tissues of the host. These infections produce characteristic irregularly hypertrophied subterranean organs to which the descriptive names for the disease, such as anbury, finger and toe, maladie digitoire, Kohlhernie, Kapoustnaja kila, and clubroot, are applied. Very often the first symptom of the disease aboveground is not seen until after the host passes the seedling stage. Seedlings, which are one of the most potent means of distributing the trouble, may often be diseased but have apparently healthy foliage parts. Unless the roots are carefully examined after they have been pulled from an infested seed

[1] Investigations carried on cooperatively by the Office of Horticultural Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the department of plant pathology, University of Wisconsin.

[2] The writer is indebted to many persons at the college of agriculture, University of Wisconsin, for aid and advice in the execution of this work; but especially to J. C. Walker, under whose direct supervision the work was carried on and who has given invaluable help in the preparation of the manuscript, and to L. R. Jones for stimulating interest and for suggestions during the course of the experiments.

[3] Reference is made by italic numbers in parentheses to "Literature cited," p. 28.

bed, many slightly affected individuals will be passed over as being normal. Recently infected roots often appear to be healthy, and in many cases roots not actually infected carry infested soil on them. Later in the season, however, as the diseased plant develops, its roots will be found to be characteristically swollen and malformed. (Fig. 1, B.) Actually the first aboveground sign of clubroot infection usually occurs after the plant has attained considerable size. It consists of a flagging or wilting (fig. 1, A), which is decidedly marked on warm, bright days. Before the disease has become too far advanced a plant thus wilted will often recover fully during the cooler part of the day and will appear quite normal during cloudy and wet weather.

The writer's investigations of the disease were prompted by the increasing economic loss to cabbage growers in the Middle West. Additional information was needed concerning the life history of the

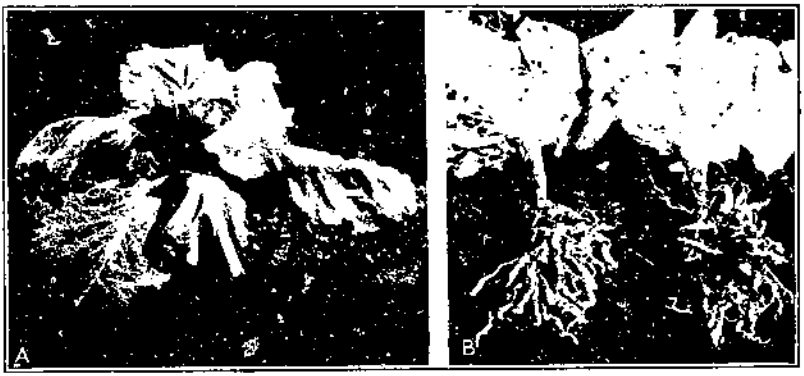

FIGURE 1.—Symptoms of clubroot on half-grown cabbage plants in the field: A, Wilt or flagging of the foliage, the first indication of the trouble in the field; B, malformed and swollen or clubbed roots found when a plant such as in A is pulled

organism, and control measures had not been studied adequately. Previous students had given attention to mycological and cytological details, but few had experimented with the physiological phases of the disease under controlled conditions. It is the purpose of this bulletin to review briefly the known salient features concerning the malady and the causal organism, apart from its cytology, and to present in detail the results of the present researches.

EARLY HISTORY, IMPORTANCE, AND GEOGRAPHICAL DISTRIBUTION OF CLUBROOT

Ellis (*16*) reported having seen in 1736 the "Turnep Disease, called in Norfolk and Suffolk, Anbury." He believed it to be contagious and probably due to an excess of barnyard manure, especially "the long undigested ranker sort." In Scotland from 1829 to 1831 Farquharson (*17*), Abbay (*1*), and Birnie (*6*) described the disease and attributed it to unsatisfactory soil conditions or unbalanced fertilizing practices. Abbay stated that he saw the disease first in 1801. In 1855 Anderson (*2*) asserted that the trouble first appeared about 1813. At about the time Anderson wrote, American and English

students, among them Curtis (*15*), and later Slingerland (*44*). studied the trouble and concluded that it might be due at least in part to insects. Henderson, in his gardening book (*28*) published in 1867, discussed observations made on clubroot in the northeastern part of the United States many years previously. He believed the disease was caused by the attack of the cabbage maggot. In 1874 Sorauer (*45*) attributed the disease in part to insects. In one of his papers Ravn (*40*) wrote of the history of the disease and included in his bibliography citations of 18 articles published before Woronin's final description of the nature of the causal organism. Woronin began his studies of the malady in 1873, and in 1875 (*54*) he published a preliminary report on the organism, but did not name it at that time. His paper in which the nature of the organism and its host relationships were carefully described and illustrated did not appear until 1878 (*55*). He published a total of eight papers on this disease, of which three were in Russian and five in German.

The fact that for nearly two centuries botanists have been studying the nature and control of clubroot, and that now, as in the past, interest is not wanting, is ample proof of the importance and difficulties of the problems involved. Economically, the effects of a plant disease are hard to gauge, and because of the nature of the trouble it is peculiarly so in this case. Woronin (*55*) estimated that in 1869, in the vicinity of St. Petersburg, Russian gardens lost approximately half their cabbage plants, and in 1918 it was reported[4] that in the United States New York sustained a loss of several thousand tons of cabbage. The important fact is that the disease spreads readily and that, once established in a field, it may completely destroy, for an indefinite number of years, the usefulness of the plot as ground on which to grow crucifers.

Geographically the disease is very widely distributed. In the Old World it occurs in nearly all regions where cruciferous crops are important. In the United States it has been reported as occurring in 36 States and as important in 21. It also occurs in Alaska. No trucking sections growing crucifers intensively appear to be incapable of becoming infested with the trouble.

CERTAIN PHASES OF THE LIFE HISTORY OF THE CAUSAL ORGANISM

Certain facts of the life history of the organism have been well established. The spore germinates in the soil as a uninucleate zoospore with a single anteriorly placed flagellum. Through movement in the soil water these bits of naked protoplasm come in contact with subterranean portions of the host. The organism penetrates, grows in the tissue, and forms a true multinucleate plasmodium, which may migrate as a whole or separated into smaller parts from cell to cell. Through toxic action of the parasite, hypertrophic and hyperplasic reactions of irritable host cells about the infecting plasmodium produce the typical swollen regions characterizing the disease. Derangement of the health of the host upon the development

[4] HASKELL, R. J., and MARTIN, G. H., JR. SUMMARY OF PLANT DISEASES IN THE UNITED STATES IN 1918—DISEASES OF FIELD AND VEGETABLE CROPS. (Continued.) U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Bul. Sup. 3, p. 84–118. 1919. [Mimeographed.]

of the swollen roots probably results largely through the disturbance of the systems absorbing the soil solution and conducting it away from the place of entry. In the enlarging regions of infection, plasmodia develop rapidly and live for a time in intimate union with host cytoplasm without killing the cells. The plasmodia mature within the lumina of the host cells, produce no capillitium or peridium characteristic of truly saprophytic myxomycetes, but simply break up into spores which mature and lie packed within the unbroken host cell walls. Disruption of these walls by the action of secondary decay organisms allows the spores to be deposited in the soil. Here they germinate upon the advent of proper moisture and temperature conditions. Kunkel (*34*) described the tissue invasion by *Plasmodiophora brassicae*.

There are portions of the life cycle of *Plasmodiophora brassicae* that have not been thoroughly studied. It came within the field of the present investigations to study some of them with especial emphasis on limiting factors, and these results are herewith presented. The method of germination was observed, some of the conditioning factors for germination were studied, and the minimum period required for infection of the host at a favorable soil moisture was determined.

SPORE GERMINATION

PREVIOUS STUDIES OF SPORE GERMINATION OF MYXOMYCETES

Spore germination of myxomycetes was first studied by DeBary, who in 1854 described the main facts of this process. In his monograph (*4*) published in 1864 he described further observations on the process and physiological factors involved in its consummation. His findings were partly included in a general work, the English translation of which was published in 1887 (*5*). Woronin's complete report of studies on the spore germination of the parasite, *Plasmodiophora brassicae*, was published in 1878 (*55*), and was soon confirmed by the work of numerous students following him. The works of Jahn, Lister, Pinoy, and others should be mentioned in connection with this general subject, but space does not permit citations or specific reviews. The somewhat monographic publication of Constantineanu (*13*), however, deserves especial note. In this were treated many factors governing development of myxomycetes and germination of their spores. He found, of course, much variation between the different genera and species studied. Usually he obtained good germination at room temperature in 30 minutes to 20 days. In general, a maximum temperature was established at 35° to 40° C., an optimum at about 30°, and a minimum at below 18°. In some species plasmodia developed below 5°, but in general growth was poor below 12°. An optimum for growth was obtained usually at about 25°. At 30°, or slightly above, the plasmodia encysted, and they were usually killed a few degrees above that point. This maximum temperature for inactivation of the plasmodia was in some cases lower than the maximum for spore germination.

Kunkel (*33*) described observations of others along with his own relative to spore germination of the myxomycetous parasite of the potato, *Spongospora subterranea* (Wallr.) Johnson. Chupp's (*10*)

researches on the clubroot organism supplement those of Woronin by the use of modern cytological killing and staining methods. Recently Gilbert reported a series of studies on spore germination and feeding habits of saprophytic myxomycetes. In his study of spore-germination processes (*21*) he concluded they could be divided into two general types. It appears, however, that spore germination of *Plasmodiophora brassicae* does not fall under either of Gilbert's two generalized schemes of myxomycete spore germination. In spite of all the work that has been done on the spore germination of myxomycetes, many interesting phases are still untouched. It is evident, therefore, that even aside from its economic aspect a fuller knowledge of the process and attending phenomena in *P. brassicae* is worthy of attention.

SPORE GERMINATION OF PLASMODIOPHORA BRASSICAE

Spores were teased out from previously frozen clubbed roots of cabbage into sterile distilled water. Excess débris was removed, and the suspension was centrifuged. The supernatant water was then decanted and a fresh supply poured on the pasty mass of spores. This was then stirred up from the bottom of the centrifuge tubes, and the process was repeated several times. Spore suspensions were made in distilled or tap water, incubated, and observed in hanging-drop cultures. Because of the minute size of the spores and zoospores the oil-immersion objective was used.

The ripe spores of *Plasmodiophora brassicae* are smaller than those of typical saprophytic myxomycetes and are spherical, with a smooth, colorless episporium which appears somewhat membranous under the oil-immersion objective. Freshly matured spores studied by the writer averaged about 1.7 μ in diameter, while older spores from the same source which showed the first stage of germination averaged slightly more than 2 μ in diameter. This increase in size, which was first described by Chupp (*10*), in 1917, would seem to be due to water absorption. The factors upon which this depends and its duration before actual germination occurs have not been ascertained.

In the large number of germination studies made by many students no actual observations of emergence of the zoospore have been reported. Woronin's (*55*) illustrations of the process, which were apparently partly based upon his knowledge of the saprophytic myxomycetes, have been copied by many. It has been assumed since that time that spore germination produces a single zoospore, but no absolute proof has ever been presented. Jones (*32*) in a recent paper stated that the spore germinates into one or more zoospores which act as gametes. His evidence obtained from a limited amount of material seems to the writer to be inconclusive. Of the multitude of actively germinating spores observed by the writer, from a large number of sources during three seasons, no evidence has been noted of the production of more than one zoospore from a resting spore.

Hundreds of germinating spores were studied, but in only nine cases has the writer seen actual emergence of the zoospore. In each such case it resulted in only one zoospore. After a spore swells it appears that an irregular break occurs in the spore wall, and the spore contents become vigorously appressed behind it. The germi-

nating spore becomes lopsided, and, accompanied by a slow peculiarly irregular series of motions, the zoospore emerges, probably by means of a combination of mechanical pressure upon the epispore and its dissolution. Microscopic observations indicate that 9 to over 24 hours elapse from the time the epispore appears to be cracked until germination is completed. At the moment of zoospore emergence consequent activity is often so heightened that under the oil-immersion objective of the microscope germinating individuals frequently are temporarily lost to view. In many such cases when the individuals were found again just after germination the empty episporium and a single zoospore could be seen lying more or less quietly side by side for a few moments before the zoospore swam away. Furthermore, a newly emerged zoospore is considerably smaller than the diameter of the swollen spore from which it germinates, but it is very nearly the same size as dormant spores before being placed under conditions favoring germination.

Germination itself does not suggest either of the two typical methods described by Gilbert (*21*) for saprophytic myxomycetes, but it resembles more closely that described by De Bary (*4*) for Stemonitis. Spores of the clubroot organism germinate differently from any myxomycete the writer has thus far observed or found reported, in that an actively lashing flagellum is typically produced very soon after the first portion of the zoospore body emerges from the epispore and long before the process of germination is complete.

Near the edge of a hanging drop the germinating spore often presents a peculiarly characteristic twirling or spinning motion[5] which appears to be counterclockwise under the microscope. De Bary (*4*) seems to have noted something similar to this twirling motion in his study of myxomycetes, for in a general discussion he stated that when the zoospore has difficulty in emerging from the spore wall it may whirl around in its efforts to complete emergence. Gilbert (*22*) in a study of feeding habits of zoospores found that they moved in two ways, "an active rotating movement and * * * a slower, more or less undulatory, creeping movement." The twirling activity of *Plasmodiophora brassicae* is not an invariable accompaniment of germination, nor does it always continue without interruption. In many cases slight "trembling" or "jigging" as described by Chupp (*10*) is all that is noticeable before emergence. As he and others found, activity usually increases greatly as the time approaches for the zoospore to leave the epispore. Often a few seconds before emergence the spore may be seen to spin so fast as to appear almost a blur; then it suddenly ceases whirling, and the single, naked zoospore struggles out of the spore wall and swims off. The excessive activity of these last few moments, unless the organism is surrounded by inactive spores or débris, is the greatest hindrance to observation of the actual emergence process.

DESCRIPTION OF THE ZOOSPORE

Woronin (*55*), with whose observations the writer is in accord, described the organism after germination as a round to spindle-

[5] A. H. R. Buller, in a personal interview during which he was shown spores acting in this way, stated that he had noted the same type of activity in the spermatozoa of sea urchins. A review of zoological literature relative to this question was made and this movement of spermatozoa was found not to be confined to sea urchins.

shaped myxamoeba having an elongate, anteriorly located beak with a single flagellum. The lashing of the flagellum, coupled at times with the doubling back and forth of the motile beak, pulls and jerks the zoospore body along. It is also able to move in an amoeboid fashion by the protrusion of posterior pseudopodia even while still retaining its flagellum. Chupp (*10*) found the zoospore to be spherical or pyriform with an anteriorly placed flagellum. This had been previously observed by Woronin, but Chupp did not see the narrow spindle-shaped bodies his predecessor saw, nor was he able to see suggestions of amoeboid movement.

In the writer's experiments numerous uniflagellate zoospores were observed. The zoospore gains in size rapidly after germination and becomes capable of changing its form readily. Speed of movement ranged from a barely perceptible rate to such rapidity that a single zoospore could not be followed under the microscope. In studying a single flagellate zoospore, from a culture several days old, it was seen to change readily from a rather globular or pyriform individual to a narrow spindle-shaped body. Its property of changing form readily was further exhibited by the pushing out of rounded pseudopodia almost immediately when it touched glass. Flagellate zoospores were found as soon as the spores in the cultures began germinating. True myxamoebae (swarm cells lacking flagella and moving only by pseudopodia) were only found when the cultures were several days old. No indications of the fusion of zoospores or myxamoebae were noted.

INFLUENCE OF TEMPERATURE ON GERMINATION

Chupp (*10*) failed to get spore germination at room temperature (16° to 21° C.), but stated the optimum to be between 27° and 30°. He obtained root infection at room temperature and concluded that "the presence of the host seems in some manner to exert an influence which to a certain extent takes the place of that offered by a greater amount of heat."

Suspensions of spores from frozen clubbed roots of cabbage were placed in constant-temperature chambers arranged at approximately 3-degree intervals from 3° to 35° C. The suspensions were in open culture cells in moist chambers, and the relative germination was determined by microscopic examination at 24-hour intervals. Hanging drops were removed from the culture cells with a flamed platinum loop both before and after the suspensions in the culture cells were stirred. Hanging drops of spore suspensions were also prepared and carried through at different temperatures, but they did not give such satisfactory results as the culture cells. All of the six series that were run gave practically the same results, and as a consequence a single one of these was chosen as a typical example. Spore germination (Table 1) is furthest advanced after a 4-day incubation period and begins to drop off after five days. The maximum temperature for germination appears to be just below 28°, and the minimum occurs at about 6°. The optimum range extends from 18° to a little over 25°, with the peak probably at 25°. In some cases germination occurred at 27°, but never at 28° or above.

TABLE 1.—*Results of a typical experiment showing relation of temperature to germination of spores of Plasmodiophora brassicae*

Temperature (° C.)	Germination at end of indicated period				Temperature (° C.)	Germination at end of indicated period			
	2 days	3 days	4 days	5 days		2 days	3 days	4 days	5 days
4	0	0	0	0	18	0	Good.	Good.	0
5	0	0	0	0	22	0	Fair.	Good.	Trace.
9	0	Trace.	Slight.	Slight.	25	Good.	Very good.	Very good.	Good.
11	0	Slight.	Fair.	Fair.	28	0	0	0	0
14	0	Fair.	Fair.	0	30	0	0	0	0

COMPARISON OF TEMPERATURE RANGES OF SPORE GERMINATION AND DISEASE DEVELOPMENT

Monteith (*36*) in his soil-temperature studies demonstrated that the disease developed at 9° to 30° C., though in one case he found slight clubbing at 35°. However, he stated that this clubbing produced at 35° was on the main stem at the surface of the soil where its contact with the air may have resulted in a somewhat lower temperature. He found that the disease was most severe at about 25°, which condition he considered due in great measure to host reaction. Up to the time of Monteith's work it was commonly believed that outbreaks of the disease were most severe in cool countries and during the cool seasons in warm regions. He concluded that the temperature range over which the disease occurred would be practically parallel with that required by the host and that temperature itself could not be considered a limiting factor in disease production.

Monteith's soil temperature range as it affected disease production was not quite in agreement with the writer's spore-germination findings and was consequently reinvestigated. In the present study a 2-inch layer of insulating material was placed on top of soils held at constant temperatures, and the water when added to the pots was at the exact soil temperatures being studied. The writer found (Table 2) that no clubbing resulted below 12° and above 27° C. The optimum temperature for percentage of disease production was at a range extending from 18° to 24°, with the peak for severity of clubbing occurring slightly above the latter figure. In the 12°, 15°, and 27° soil temperatures a fair percentage of plants became infected, but comparatively slight development of swollen roots resulted.

TABLE 2.—*Results of two representative experiments, showing the relation of soil temperature to the production of clubroot of cabbage*

Temperature (° C.)	Experiment No. 1		Experiment No. 2		Temperature (° C.)	Experiment No. 1		Experiment No. 2	
	Plants observed	Per cent diseased	Plants observed	Per cent diseased		Plants observed	Per cent diseased	Plants observed	Per cent diseased
9	35	0			21	35	100	14	100
12	35	74	14	29	24	35	100	14	93
15	35	75	14	36	27	35	20	14	29
18	35	100	14	100	30	35	0	14	0

In the light of these new facts it is of interest to consider the question as to whether the temperature influence upon disease development is one of direct effect upon the host or the parasite or both. Tisdale (*46*) studied healthy cabbage-root development over a growing period of seven and a half weeks. He found that excellent conditions for growth occurred through a range of 14° to 20° C., the optimum point being about the latter temperature. A rapid drop in growth occurred as the temperature rose, and at 23° the roots produced about 50 per cent less dry weight than at 20°. The roots grew poorly at a higher temperature than 23° and only very slightly at 35°. Comparison of Tisdale's studies of the host temperature relationships with the writer's investigations of the spore-germination and disease-production temperature ranges show significant differences. At 14° vigorous root development was accompanied by fair spore germination and slight clubbing. At 18° about the peak of root development took place along with fairly good spore germination and only fairly serious clubbing. At 23° the rate of root development took a decided drop, while very good spore germination resulted, together with an approach to almost optimum disease production. At 25°, where root growth was about the same as at 23°, there occurs optimum spore germination and the most malignant disease development. At 27°, where the rate of root growth was again about the same as at 23°, slight clubbing occurred, together with poor spore germination. At 29°, where roots grew as well or slightly more successfully than at 23°, no spore germination nor clubroot could be found.

It is difficult to state a critical conclusion as to what part temperature plays in disease development in clubroot of cabbage. *Plasmodiophora brassicae* has never been studied in pure culture, and the fragmentary data at hand concerning temperature as it affects the organism only cover spore germination. It is hardly possible to determine directly whether the effect is the result of host reaction or a stimulation produced by the temperature as it affects the growth, motility, or production of irritating substances by the parasite within the host tissues. It is evident, however, that the optimum temperature for host-root development, 20° C., is distinctly lower than the optimum temperature for spore germination and disease development, 25°. It would seem that these facts indicate at least that the effect of temperature upon disease production is in a great measure due to its influence upon the causal organism.

SOIL MOISTURE AND THE INFECTION PERIOD

Monteith (*36*) demonstrated that the production of clubroot requires high soil moisture. He was able to grow plants free from clubroot in thoroughly infested soil by keeping the soil moisture content down to 45 per cent of the water-holding capacity. At 60 per cent of the water-holding capacity the disease would again be uniformly present. He concluded that failure of clubroot to develop on plants growing in infested soils with low moisture content was probably due to insufficient moisture for spore germination. Observations show that often the plants appearing to be most seriously

diseased in infested fields occur in low spots and in what appeared to be the most poorly drained portions of infested areas. In neighboring fields, however, malignant disease development has been found on the higher, well-drained soils and in fields carefully underdrained with tile. Monteith (*36*) discussed observations of investigators in this and other countries who found that the employment of good soil-drainage measures was an actual curative agent, though he believed that it could not be relied upon in itself as an inhibitor. It seemed evident that complicating soil factors such as humus content and relative acidity entered into the question of the efficacy of drainage as a curative measure. It occurred to the writer that the question of the part drainage played as a preventive measure would depend largely upon the length of time required for the existence of high soil-moisture conditions about the host roots before infection takes place. Studies were carried out to determine this infection period, with clubroot-infested soils from Wisconsin fields.

Plants from disease-free soil were transplanted into infested soil which was kept at 40 to 47 per cent of the water-holding capacity, determined according to Monteith's methods (*36*). This had been found to be below the minimum soil moisture for infection. The plants were watered twice a day until they became adjusted to growth after the transplanting process and showed signs of new top and root growth. This required seven to nine days. The soil moisture was then increased to 80 per cent of the water-holding capacity, and at stated time intervals series of 5 to 10 plants were removed, the roots washed, and replanted in relatively dry infested field soil. This was held at 40 to 47 per cent soil moisture in a greenhouse in which the air temperature was held at 15° to 22° C. After one month the plants were removed and the roots examined for evidence of clubroot.

These experiments were performed repeatedly and show (Table 3) that, in the soils described, clubbing results quite generally in roots which have been exposed for 18 hours to soil having relatively excessive soil moisture. This period was reduced in some cases to 10 hours. These data indicate that even in an otherwise dry season a single heavy rain, or a few moderate rains at short intervals, might raise the soil moisture sufficiently and for a long enough time to insure clubroot infection. Therefore it seems reasonable to conclude that while an adequate system of drainage might in some cases reduce the severity of the disease in lightly infested sandy soils, it should be expected neither to inhibit absolutely infection by *Plasmodiophora brassicae* nor to offer, of itself alone, a practical remedial measure.

TABLE 3.—*Results of exposure of cabbage for various periods to moist soil thoroughly infested with clubroot*

Hours of exposure	Occurrence of disease in the indicated experiment			
	No. 1	No. 2	No. 3	No. 4
0	Healthy	Healthy	Healthy	Healthy.
1	do			
2	do			
3	do	Healthy		
4	do			
5	do			
6		Healthy	Healthy	Do.
7	Healthy			
9		Healthy		
10	Healthy		Diseased	Do.
12		Healthy	do	Do.
15		Diseased		Do.
18	Diseased	Healthy	Diseased	Diseased.
21		Diseased		
24	Diseased	do		
27			Diseased	Do.
36	Diseased.			
48	do			
72	do			
96	do			

SOIL REACTION IN RELATION TO CLUBROOT

REVIEW OF LITERATURE

The physiological ecology of soil-inhabiting organisms as affected by the H-ion concentration relationships is still incompletely understood. Historical or theoretical treatment of this complex question is beyond the scope of this work, but it is discussed in such papers as those by Fisher (*18*, *19*), Truog (*47*), and Pierre (*39*). Wherry (*52*), after a long series of studies, found that certain chlorophyllous plants thrive in soils of a relatively narrow pH range. Investigations, one of the purposes of which was to determine whether changes brought about in soil reaction might be useful in preventing or reducing the various diseases caused by the organisms studied, are reported by Peltier (*38*), Sherwood (*43*), Hopkins (*29*), Hawkins and Harvey (*27*), Gillespie (*23*), and Waksman (*48*).

Webb (*49*) in 1921 and Wolpert (*53*) in 1924 reviewed literature on the relation of the H-ion concentration of media to the action of fungi. They found in their own studies that in general OH ions were more toxic than H ions. Webb noted that the pH range for a specific organism was not the same under all conditions, though the reason for this was not always explainable. Wolpert concluded that it was not possible to name a marked optimum pH value for an organism or even a narrow range in which the optimum would invariably fall, and that the pH range was dependent on various environmental factors.

It has long been asserted that *Plasmodiophora brassicae* is most destructive in acid soils, and liming has been used with varying success in combating clubroot. In a number of papers from Denmark, Ravn and his associates presented and discussed evidence which they believed demonstrated that the action of lime as an inhibitor of clubroot was due to the reaction of the organism to the basic condition induced in the soil, rather than to the toxic action of the lime itself.

Ravn (*42*) in 1911 reported results from liming experiments on infested soil over a period of nine years. He used in this series of trials a finely divided "vaporized" lime. This consisted of a powder which was seven-eighths lime, all in the form of $CaCO_3$. At the beginning of the experiment the infested soil showed a "normal" content of calcium, was practically neutral to litmus solution, effervesced slightly upon treatment with dilute HCl, and sustained slight growth of Azotobacter in some cases, though in others the bacteria failed to grow. For these reasons Ravn considered the soil practically neutral. In the light of recent knowledge it is known that the change of color in litmus occurs over such a wide range of pH values that it has been discarded as an indicator for denoting exact neutrality. Gainey (*20*) in 1922 showed that *Azotobacter* spp. grow well at pH 6.0 and also in media strongly alkaline.

Ravn found that it required the application of vaporized lime ($CaCO_3$) at the rate of at least 1.23 tons per acre each year for four years, a total of 4.92 tons per acre, before any appreciable effect could be noted in litmus reaction or in consistency in occurrence of azotobacterial growth on cultures inoculated with soil. With this treatment, however, he obtained no control of the disease. After he had applied 1.64 tons of vaporized lime every year for four years, a total of 6.56 tons to each acre of land, he obtained fairly strong alkaline litmus reaction, a good growth of Azotobacter on cultures inoculated with soil, and a notable reduction in the amount of clubroot. His heaviest application of vaporized lime, 2.47 tons per acre each year for four years, a total of 9.88 tons per acre, induced vigorous alkaline litmus reaction, a consistently abundant growth of Azotobacter on samples of soil, and in practically every plot an almost normal crop of healthy roots, though in some cases, for reasons he could not explain, serious infection still occurred. To a certain extent he carried on parallel tests in which he used air-slaked instead of vaporized lime. The largest quantity of the slaked material that he applied was 1.23 tons per acre for four years, which made a total of 4.92 tons to the acre. Though he did not apply a greater quantity than this, his data show that ton for ton the air-slaked lime, composed of a mixture of one-half CaO and one-fourth $CaCO_3$, was much more efficient as a disease inhibitor than the vaporized lime, containing six-sevenths $CaCO_3$. Both limes seemed to change the soil reaction with equal effectiveness.

Bramer (*7*, *8*) found that strong alkalinity inhibited the germination of *Plasmodiophora brassicae* spores without killing them. He believed that the hydrogen-ion concentration was in itself a limiting factor for the organism, though unexplained exceptions were observed. Germination resulted over a pH range of 5.4 to 7.5, but not at pH 8.0. Lindfors (*35*) noted in pot tests that with increase in alkalinity of the soil there was a decline in infection until at pH 7.8 all plants were healthy. Naumov (*37*) studied the effect of various metallic salts on the control of clubroot. He concluded that inhibition depended not on the character of the metal ion so much as upon the presence of free hydroxyl ions in the substratum.

Chupp (*11*) in 1928, working with a naturally acid soil, applied calcium hydrate and sulphur and studied the effect of H-ion concentration on clubroot incidence. He found that applications which

served to raise the pH value to slightly more than 7.0 inhibited the disease. At a pH of 7.2 to 7.4 only a trace of clubroot was evident. The amount of trouble increased rapidly between pH 7.0 and 6.0. At pH 6.6 he found 80 per cent of the host plants diseased; at below pH 6.0 it was possible to get almost 100 per cent diseased.

METHODS USED IN DETERMINING SOIL REACTION

The double-wedge comparator described by Barnett and Barnett (*3*) and later adapted to soil-acidity determinations by Wherry (*51*) was used for H-ion concentration measurements after being found to check within pH 0.1 with a standard Clark & Lubs colorimeter set. The apparatus was found to be simple to manipulate and sufficiently accurate for the work herein discussed. Soil samples were obtained occasionally by shaking soil from the roots of plants but usually by the use of a 6-inch soil auger. The samples while still moist were crumbled with the fingers and thoroughly mixed and sifted. In no case was a sample crushed or ground in a mortar or forced through a sieve. Wide-mouth bottles of a little over 30 c. c. capacity with screw caps were graduated at 12.5, 15, 27.5, and 30 c. c. The new bottles were first cleaned with soap powder and weathered for a number of days, first in concentrated H_2SO_4 and then in 20 per cent NaOH. Before each test the bottles were washed by first being scrubbed with soap powder and then soaked in 20 per cent HCl for about one hour. They were then rinsed under the tap and placed in a bath of water made strongly alkaline with NH_4OH. The alkaline bath was rinsed off, first under the tap and then repeatedly with distilled water, and the bottles were allowed to drain. Just before the bottles were used they were rinsed carefully with conductivity water. The aluminum screw caps were always carefully washed in soap, rinsed, and dried. None of the solutions to be tested stood in contact with the metal cap, its main use being to exclude air and serve as a cover when the soil and water were shaken in the bottles. Samples of distilled and conductivity water shaken in containers treated in this way and kept covered at room temperature for 12 hours did not change in pH values.

In testing soils a bottle was filled with conductivity water up to the 12.5 c. c. mark. Soil from the sifted and mixed sample was put into the bottle until the water level reached the 15 c. c. mark, when it was capped, shaken 50 times, and set aside to settle. The particles of some soils showed no signs of settling out of suspension after a few minutes. Conductivity water was added to the bottle of such a suspension, bringing the level up to the 27.5 c. c. mark. Soil from the same sample was added until the 30 c. c. mark was reached; the bottle was shaken again 50 times and set aside to settle. In this quantity of solution the soil particles had a longer column in which to settle. In many cases this helped in obtaining a sufficient quantity of clear solution for pH determination.

In a few cases the pH value was determined as soon as enough clear liquid was obtainable to fill the test cells of the comparator. In most cases, however, the soil solutions remained overnight in the capped bottles in an evenly heated room at approximately 22° C. and were tested for pH value within 12 to 15 hours. No radical

differences were noted in identical solutions 3 to 15 hours after settling, and the greater ease of color determinations in the clearer liquids with indicator dyes in them made using the longer period of settling the more satisfactory method. The conductivity water used ranged in pH from 6.7 to 7.2, depending upon storage conditions. To offset any such possible variations in results, the same stock of water was used in testing a given series of soil samples.

RESULTS OF SURVEY OF INFESTED SOILS

The writer visited more than 200 fields of cruciferous crops affected with clubroot in Wisconsin, Illinois, and Indiana. The types of soils were noted, and 116 samples were obtained for acidity determinations. It was found that clubroot apparently occurred with equal severity in any type of soil capable of producing a cruciferous crop in the regions studied. These soils were largely loams and sands, though silts and clays were also commonly encountered. Fields close to limestone outcrops and those on the bottom of prehistoric Lake Chicago along the west shore of Lake Michigan, which contained large numbers of gastropod shells, were seriously diseased. Subsoils were also found of gumbo, limestone, and marl. Disintegrating peat and other newly reclaimed marsh lands in which no particles of lime were noticeable were also seriously diseased. Subsoils in some infested fields were of noncalcareous nature and consisted of pure sand, clay, and glacial deposits of sand, clay, and pebbles.

In testing for the pH values of the 116 soil samples studied it was found that clubroot occurs in soils with a pH of 5 to 7.8. Fifty-seven per cent of the soils tested between 6.5 and 7.4, which is near the neutral point for soils;[6] 35 per cent tested between pH 5.5 and 6.4; 4 per cent were distinctly acid, testing between pH 5 and 5.4; and 4 per cent were quite alkaline, testing between pH 7.5 and 7.8.

At first glance these results seemed significant in giving the preponderance of soils a pH of below 7. It is also to be noted, however, that healthy crucifers grew in fields over the same range of H-ion concentrations. In a number of partially infested fields studied (Table 4) the pH values usually differed slightly in diseased and healthy areas, though the number of shifts toward the acid and toward the alkaline side was practically equal. When the distribution of acidity was studied in certain infested fields (Table 5) the pH values were found to vary over a wide range, and diseased areas were not necessarily confined to the more acid regions. From these observations it seems logical to conclude that the percentage distribution of diseased soils with regard to H-ion concentration is just what might be expected in samples taken from fields in the trucking areas studied without respect to occurrence of clubroot. Probably soils slightly more acid than pH 5 and more alkaline than pH 7.8 might be found growing cruciferous crops, and it is to be expected that the range of clubroot occurrence might also be extended. In the soils investigated the H-ion concentration does not appear to be a limiting factor in the occurrence of clubroot of crucifers.

[6] Wherry (*58*) used the term "circumneutral," which includes slightly acid, absolutely neutral, and slightly alkaline reactions.

TABLE 4.—*Comparison of pH values of soils free from and infested with Plasmodiophora brassicae in well-defined regions within the same field*

Field No.	pH values of soil in the indicated region		Difference in pH value	Field No.	pH values of soil in the indicated region		Difference in pH value
	Infested	Disease free			Infested	Disease free	
1	5.9	6.8	+0.9	9	7.3	7.4	+0.1
2	7.1	6.8	−.3	10	5.6	7.0	+1.4
3	6.8	6.8	0	11	6.1	5.9	−.2
4	7.1	6.9	−.2	12	6.3	6.5	+.2
5	6.5	7.6	+.5	13	5.7	6.4	+.7
6	7.4	7.3	−.1	14	5.2	5.5	+.3
7	6.7	6.9	+.2	15	7.1	7.1	0
8	6.7	6.5	−.2				

TABLE 5.—*Irregularities in pH values shown by soils of three clubroot-infested fields*

Location of sample	Soil type	Drainage condition	Infection (per cent)	pH value
Field A:				
High land	Black loam	Excellent	95	7.2
Shoulder	Dark sand	do	100	6.9
Bottom land	Black silt	Fair	100	6.2
High land	Light sand	Excellent	95	6.6
Field B:				
High knoll	Light loam	do	100	6.4
Shoulder	Dark loam	Good	100	6.5
Edge of bottom	Black loam	Fair	100	7.7
Shoulder	Dark loam	Excellent	100	6.6
Bottom	Black loam	Fair	100	7.6
High land	Dark loam	Excellent	100	6.7
Field C:				
High knoll	Light clay	do	100	5.9
High land	Sandy clay	do	100	6.8
Bottom	Black clay	Poor	100	7.1

INFLUENCE OF ADDITION OF VARIOUS CHEMICALS TO THE SOIL

After the wide range of pH values at which clubroot occurred in the field had been determined, the H-ion concentration was varied experimentally in clubroot soils by adding certain chemicals. Those used were of laboratory grade, "C. P." quality, and in a well-ground state. Quantities used varied increasingly from a little more than 1 to 11 gm. per kilogram (oven-dry weight) of infested soil. The soils used were from portions of fields which were known to be thoroughly infested with clubroot. These experiments were all performed in the greenhouse.

The soils were sifted and thoroughly mixed. Requisite quantities of alkali were added to weighed amounts of soil in a pile and mixed by being turned five times with a trowel and rubbed between the hands. Vigorous cabbage seedlings were grown in the treated soils at a high soil moisture content (70 to 85 per cent water-holding capacity). After a month the plants were removed and examined for clubbed roots, and the pH of the soil was then determined. Various alkalis were tested for their toxicity to cabbage and their effect on the clubroot disease. The H-ion concentration was shifted

with varying degrees of success, depending upon the soils employed. Four soils used in the experiments tested, respectively, pH 6.6, 7.2, 7.1, and 6.7, all close to neutrality. As all four gave essentially the same results, the data from only one are presented. (Table 6.) In the case of this soil, which tested originally pH 7.2, the H-ion concentration was raised by the use of K_2CO_3 to pH 8.1 without inhibiting the occurrence of the disease. One treatment with $Ca(OH)_2$ inhibited disease production but only increased the pH 0.1. A large excess of chemically precipitated $CaCO_3$ inhibited the disease, but it affected the H-ion concentration only slightly in comparison with $Ca(OH)_2$. At a higher H-ion concentration than was produced by the $Ca(OH)_2$ treatment, and when three times as much reagent was employed, the carbonate raised the pH 0.1 above the point at which inhibition occurred when the hydroxide was used, but did not reduce disease production. It was not until the pH was raised to 7.9 that $CaCO_3$ inhibited clubroot.

TABLE 6.—*A representative experiment, showing the comparative effect of the addition of certain chemicals to the soil upon the pH value and upon the occurrence of clubroot in cabbage*

pH value	$Ca(OH)_2$	$CaCO_3$	K_2CO_3	pH value	$Ca(OH)_2$	$CaCO_3$	K_2CO_3
7.1	Diseased	Diseased	Diseased.	7.7	Healthy		
7.2	do		Do.	7.8		Diseased	
7.3	Healthy		Do.	7.9		Healthy	
7.4		Diseased		8.0	Healthy		
7.5	Healthy	do		8.1	do		Diseased.
7.6	do	do	Diseased.				

The pH of naturally infested field soil was found to be as high as 7.8, and it is to be noted that the disease was inhibited at much below this (Table 6) by the use of $Ca(OH)_2$, while K_2CO_3 increased the pH to well above 7.8 without inhibiting disease development. On the other hand, several trials showed that $Ca(OH)_2$ inhibited the disease without raising the pH more than 0.2 or 0.3 above the approximate neutrality shown by untreated soil.

The pH determinations in an experiment on one seriously infested field (Table 7), to which different quantities of several types of commercial liming materials were applied, ranged from 6.4 to 8.1. The larger percentages of plants that died of clubroot occurred in soils having a pH of 6.4, 6.5, 6.7, 7.1, or 7.5. The highest percentage of normally developing plants occurred in soils having a pH of 6.7, 7.1, 7.5, 7.8, 7.9, 8, or 8.1. On the other hand, plants were so seriously diseased that they were not able to head in plots showing a pH of 6.5, 6.7, 6.8, and 7.6. In plots showing a pH of 6.8 and 8 no plants were killed by *Plasmodiophora brassicae*. From these data no correlation appears between severity of disease and increase in active alkalinity of the soil.

TABLE 7.—*Relation of pH values to clubroot of cabbage in plots of thoroughly infested field soil treated with lime*

pH value	Plants headed (per cent)	Plants dead from clubroot (per cent)	pH value	Plants headed (per cent)	Plants dead from clubroot (per cent)
6.4	1	47	7.5	1	23
6.5	0	51	7.5	21	49
6.5	7	52	7.6	0	15
6.7	23	2	7.7	7	16
6.7	0	40	7.8	62	
6.7	4	54	7.9	20	7
6.8	0	0	7.9	3	7
7.1	49	5	8.0	78	0
7.1	27	22	8.1	36	3

From the foregoing data it appears that, in the soils studied, *Plasmodiophora brassicae* is a disease-producing agent over such a wide range of naturally occurring and artificially induced H-ion and OH-ion concentration in the soil that to consider OH-ion concentration alone as a limiting factor is questionable. A limiting influence is exerted, however, which may be interpreted as actual toxicity of the chemical molecules themselves. This question needs further investigation. The pH relationship may be indicative of a condition of chemical dissociation in the soil solution, but it appears that this should not be considered the only limiting toxic element. It seems that to control the disease effectively materials must be applied which will alter the soil solution in such a way as to inhibit the action of the parasite while altering of the pH value in itself is of secondary importance.

LIMING FOR CONTROL OF CLUBROOT

PREVIOUS INVESTIGATIONS

Ellis (*16*) reported that before 1742 farmers were using clay or marl for dressing their diseased fields before planting turnips. About 75 years later the Highland Agricultural Society of Scotland offered prizes for essays concerning the nature and control of the turnip disease known as "finger and toe." Farquharson (*17*) believed the disease to be due to abnormal growth of host roots induced by the use of inadequately decayed manure and suggested that the mixing of quantities of powdered lime shells in manure heaps would hasten fermentation and produce well-rotted manure, which would obviate future excess stimulation. At the same time Abbay (*1*) recommended, after careful trials on diseased land, the application of a particular type of lime known as "Knottingley" at the rate of 256 bushels per statute acre. His general conclusion was that "bone manure affords no relief from the disease; and different kinds of lime have been tried without success."

In 1855 Anderson (*2*), chemist to the Highland Agricultural Society, reported detailed analyses of soils from diseased and healthy fields. He concluded that the chemical nature of the ground could not be correlated with occurrence of the disease, though it occurred most severely on light "deafish" soils which would not

respond readily to manuring practices. He recommended, after field experimentation, the use of lime at the rate of 60 bolls per Scotch acre two or three years before the crop was to be grown, but found that for some unexplainable reason it did not always prove effective. At about the same time Hunter (*30*) found that on his farm "lime applied to the young plants [turnips] was quite ineffective; phosphates in the drills equally so; lime worked in whilst preparing the land very slightly beneficial." He recommended the use of 14 to 16 tons of "hot" (probably burnt) lime per Scotch acre applied on the "lea" and plowed under. Henderson (*28*), a gardener near New York City, reported in 1867 observations that crucifers grown in soils containing excessive numbers of disintegrating oyster shells were not subject to attack by the insect causing clubroot. He found that on lime-free seriously infested land heavy dressings of lime were both expensive and only temporarily effective. He procured successful control, however, by the use of 2,000 pounds of "flour of bone" per acre. Halsted (*25*) noted that gardeners of the eastern United States were using lime as a preventive of clubroot. He concluded (*26*), after seven years of field experimentation, that air-slaked lime at the rate of 75 bushels per acre was a commercially satisfactory remedy for the disease.

Christensen, Harder, and Ravn (*9*) through a series of laboratory and field experiments came to the conclusion that the more a soil needed a base the greater the possibility of malignant infection of the crop. They believed that the quantity of lime required to inhibit the organism depended materially upon the nature of the soil. Ravn (*42*) reported the results from liming experiments over the period 1902 to 1911. He used calcium carbonate and a mixture of calcium carbonate and calcium oxide for the liming materials, in quantities varying from about two-fifths of a ton to nearly 10 tons per acre. His conclusions were that the largest treatment was the most successful as a disease inhibitor. Infection still occurred in spite of this quantity of lime, but crop returns were usually commercially satisfactory.

Though Ravn did not believe in the intrinsic toxicity of lime itself, it is noticeable that the results from his experiments show that he obtained more effective clubroot inhibition with some types of limes than with others. In 1910 (*41*) he reported the successful inhibition of clubroot by the use of one application of air-slaked lime at the rate of 2 tons to the acre. A year later (*42*) he found it required four yearly applications of vaporized lime totaling 9.88 tons per acre to procure successful inhibition of the disease. The air-slaked lime tested about 51 per cent CaO and 25 per cent $CaCO_3$, and the vaporized lime tested a total of about 89 per cent $CaCO_3$, which indicated that CaO was a much more efficient disease inhibitor than $CaCO_3$. The differences which appeared in the effectiveness of air-slaked limes were probably due to the differences in the contents of oxides or hydroxides and carbonates. Halsted (*26*) found 75 bushels of air-slaked lime per acre a successful clubroot inhibitor, but Cunningham (*14*) reported that it required from 75 to 150 bushels for effective inhibition of the disease.

Calcium may be more readily obtained and applied to the soil in the carbonate form than in any other. Its traditional use as a remedial measure for soil troubles is well known. Carbonated forms of lime have, therefore, become very popular as material for clubroot treatment. Other forms of lime have been used, some cases of which have already been mentioned. Chloride of lime was reported as an unsuccessful remedy by Cunningham (*14*). Jones (*31*) applied stone lime (CaO) at the rate of 80 bushels per acre to the surface of the soil, where it was allowed to slake and was worked in with a rake. The field was planted, and the limed areas showed much less disease than the untreated. Hall (*24*), writing in 1904 in a general text on soils developed out of English experience, suggested 3 or 4 tons of quicklime (CaO) to the acre as a curative measure for clubroot.

As has been pointed out, liming has proved effective as a treatment against clubroot in many instances, although exceptions have occurred. The purpose of the present investigations was to gain some knowledge of the part lime played in the clubroot treatment, what forms were efficient disease inhibitors, and why liming operations have not always been effective.

GREENHOUSE POT TESTS

Soils for greenhouse pot experiments were obtained from thoroughly infested fields. Weights were calculated on an oven-dry basis, 3,750,000 pounds per 9-inch acre being arbitrarily used as the average weight of cabbage-growing soils. These experiments were all carried on under greenhouse conditions. Calcium compounds of "C. P." grade and commercial types of limes were applied at rates of 1, 1½, 2, and 6 tons per acre. The materials were carefully mixed with moist soil and allowed to stand in pots for 24 hours before cabbage seedlings were planted in them. After planting, the soil was held at 80 per cent water-holding capacity for two weeks, after which it was allowed to dry out slightly but held at approximately 60 to 70 per cent water-holding capacity to keep the plants growing thriftily. After six weeks the plants were examined for clubroot.

From these experiments (Table 8) it may be seen that chemically pure $CaCO_3$, raw ground limestones of either high calcium or dolomitic types, and gypsums are not effective clubroot inhibitors. Commercial air-slaked lime and a compound in which air-slaked and ground limestone were used together, if applied in large quantities, 6-ton rate at least, in some cases showed a tendency toward checking the disease. Chemically pure CaO, quicklime, or ground burnt or stone lime are effective clubroot inhibitors. Chemically pure $Ca(OH)_2$ and commercial hydrated limes are also potent preventive agents.

TABLE 8.—*Relative value of liming materials as preventives of clubroot of cabbage as determined by pot tests*

Predominant chemical compound	Material applied	Rate used (tons per acre)	Result as to disease development
CaO	CaO, C. P.	2	Healthy.
	Quicklime (lot A)	2	Do.
	Quicklime (lot B)	2	Do.
	Milk of lime	2	Do.
$Ca(OH)_2$	$Ca(OH)_2$, C. P.	2	Do.
	Hydrated lime (lot A)	1½	Do.
	Hydrated lime (lot B)	1½	Do.
	Hydrated lime (lot C)	2	Do.
$CaCO_3$	$CaCO_3$, C. P.	2	Diseased.
	High calcium limestone	2	Do.
	Dolomitic limestone (lot A)	2	Do.
	Dolomitic limestone (lot B)	6	Do.
	Dolomitic limestone (lot C)	2	Do.
	Dolomitic limestone (screenings)	6	Do.
	Marl (high quality, ground)	6	Do.
	Marl (natural, unground)	6	Do.
$CaCO_3$, CaO, and $Ca(OH)_2$	Air-slaked lime (lot A)	2	Do.
	Air-slaked lime (lot B), (fresh)	6	Some healthy.
	Air-slaked lime (lot C)	6	Diseased.
	Mixed air-slaked lime (lot B) and limestone (lot A)	2	Do.
	do	6	Some healthy.
$CaSO_4.2H_2O$	$CaSO_4.2H_2O$, C. P.	2	Diseased.
	Gypsum (lot A)	6	Do.
	do	2	Do.
	Gypsum (lot B)	6	Do.

FIELD EXPERIMENTS

SEED-BED TREATMENTS

Clubroot-free seedlings are of utmost importance to growers of cruciferous crops that are transplanted. The roots of seedlings having incipient infections or infested soil clinging to them distribute the disease and insure crop failure the first year. In regions where the Brassicas are grown intensively in the United States it is usually easily possible to obtain clubroot-free plants for transplanting; yet these conditions may not always exist. For that reason, and because of its general interest, seed-bed treatments were carried on. Various laboratory and proprietary compounds of copper, mercury, and calcium were used in powder and in variously concentrated water solutions. Copper carbonate and sulphate did not appear to inhibit clubroot even in concentrations sufficiently strong to be decidedly toxic to the plant. Experimentation with mercury compounds following Clayton's (*12*) method, which was reported as successful in New York, did not inhibit disease production under Wisconsin conditions. Further trials with mercury compounds in Wisconsin showed them to be capable of inhibiting the disease, but only when they were applied in sufficient quantities to be poisonous to the host; but such quantities are too expensive to be practicable. Carbonates and sulphates of calcium were not toxic to the clubroot organism. Calcium hydrate, however, gave promise of being useful.

Seed-bed treatments with lime were carried on for three years in the greenhouse and in thoroughly infested fields. In the field the material was applied to freshly plowed ground and thoroughly worked into the soil with hoe and rake. Cabbage seed was sown

immediately, and after six weeks the seedlings were removed from the soil with a digging fork and examined for clubbed roots.

An examination of data from a typical lime-treated seed bed (Table 9) shows that limestone at the rate of 6,000 pounds to the acre does not appear to reduce infection even slightly enough to be considered of any importance. A good grade of hydrated lime at the rate of 1,000 pounds per acre reduced infection to almost nothing. Five hundred pounds of hydrated lime per acre did not inhibit the disease, a treatment which admitted fairly large percentages of diseased plants in two trials in previous years. It was not until 1,500 pounds were applied that control was so perfect that there would be no danger of transplanting infected seedlings from the seed bed to the field. It appears from the data cited that hydrated lime well worked into the soil at the rate of 1,500 pounds [7] or more per acre is a practicable treatment for the control of clubroot in the seed bed.

TABLE 9.—*Effect of application of hydrated lime and ground raw limestone to seed beds on the control of clubroot of cabbage*

Treatment and pounds per acre	Plants diseased (per cent)			Treatment and pounds per acre	Plants diseased (per cent)		
	Bed No. 1	Bed No. 2	Average		Bed No. 1	Bed No. 2	Average
None	48	58	53	Hydrated lime:			
Limestone:				3,000	0	0	0
6,000	21	25	23	2,000	0	0	0
3,000	22	23	23	1,500	0	0	0
2,000	27	24	26	1,000	0	1	1
1,500	10	26	18	500	0	3	2
1,000	14	33	24	None	19	54	37
None	21	27	24				

In contrasting the above-described seed-bed findings with field data it is well to note several differences. From field observations it appears that in the same soil fewer individuals will be found with clubbed roots at the end of the seedling stage than will be found months later in matured plants at the close of the growing season. In seed-bed treatments the materials were applied by hand on a relatively small area. With the machine methods of more extensive field operations a deeper layer of soil is stirred, which results in the lime's being mixed into a larger quantity of soil. It should be noted, therefore, that in handling extensive seed beds by machinery disease control would probably require more than 1,500 pounds of hydrated lime per acre.

FIELD TREATMENTS

The principal field tests were conducted in Wisconsin upon badly infested soil in Kenosha County. This field had been abandoned by the owner for growing cabbage because of the severity of clubroot. The last year cabbage was grown on the field by the owner the crop was abandoned without having a head cut from it. This field was examined by the writer that fall. Except for a few individuals in one corner of the field, none of the plants pulled were free from the disease. The area was found to be as uniformly infested as

[7] At least 5 tons of hydrated lime to the acre are required in the Wisconsin soils studied to produce a sufficiently toxic effect on cabbage seedlings to be noticeable.

it could well be under natural field conditions. For the more critical field studies (Table 10) it was measured off into part-acre plots, and the limes were applied at ton-per-acre rates. These plots were studied for three years. Of the large number of data obtained only a few representative and significant cases are cited and tabulated.

Commercial grades of lime were applied to plowed ground the first two years by hand and the third by a fertilizer drill. The materials were disked and harrowed into the soil as soon after application as possible. Cabbage seedlings used for the field tests were all obtained from untreated seed beds known to be free from clubroot and were transplanted into the experimental field at the usual rate with a cabbage planter. Since the soil in the Kenosha County experimental field was known to be thoroughly infested with *Fusarium conglutinans* Wr., all data presented are based on the use of a commercial strain of cabbage resistant to the Fusarium disease. This strain was also grown on a near-by field whose soil was free from clubroot but known to be thoroughly infested with the yellow organism. The harvest count from the clubroot-free field was considered 100 per cent. In this way ordinary losses due to death at transplanting, yellows infection, and improper heading were not included under clubroot effects.

TABLE 10.—*Data from representative plots in a field experiment, showing the effect of lime applications on the control of clubroot of cabbage in Wisconsin*

[This field was known to be thoroughly infested with *Fusarium conglutinans* as well as *Plasmodiophora brassicae*. The method of determining clubroot effects is described in the text]

Plot No.	Liming treatment	Lime applied, per acre	Salable heads at harvest	Plot No.	Liming treatment	Lime applied, per acre	Salable heads at harvest
		Tons	*Per cent*			*Tons*	*Per cent*
6	No treatment		0	19	Hydrated lime	2	98
7	Air-slaked lime[1]	3	83	21	do	1½	78
9	do[1]	1½	0	22	No treatment		7
10	do[1]	2¼	7	24	Hydrated lime	1	75
13	Raw ground agricultural limestone[1]	9½	3	28	do	½	69

[1] This treatment was applied to the plot the year previous and a crop was grown on it and replanted.

LIMESTONE

The most popular liming recommendation of professional agricultural advisors has long been the use of raw ground limestone. This material is cheap, is considered a soil sweetener, and is widely used to prepare some soils for successful legume culture. It has been believed for many years that clubroot is found only in acid soils, which applications of ground limestone should effectively change. However, preliminary laboratory studies did not establish the usefulness of limestone as a clubroot inhibitor in Wisconsin soils.

Finely ground, raw, dolomitic limestone rock, spread under the writer's direction at the rate of 2 and 4 tons to the acre on commercial fields, did not inhibit the trouble. Heavier limestone applications were, therefore, studied in the experimental field. Fine screenings from a local dolomite limestone quarry were applied to one plot at the rate of nearly 4½ tons to the acre. These were ap-

plied late in the fall and lay in the soil about nine months before transplanting time. Cabbage grown on this plot produced 5 per cent salable heads, practically the same quantity as that produced on the control plot. Some investigators have thought that the longer limestone was allowed to remain in the soil the more effective it might become against clubroot. The limestone plot just described was, therefore, replanted to cabbage another year, 21 months after the ground limestone had been applied. No heads were produced from this planting.

In a plot adjacent to the winter-limed plot was an area treated with what seemed to the writer to be almost an excess of limestone. The stone was prepared for agricultural use, being ground to pass through a sieve with 10 meshes to the inch. It was presumably in a more readily available form. It was applied in the spring at the rate of over 9½ tons to the acre. That year not a single head was grown on that plot, and but 3 per cent were produced the next season. (Fig. 2, B.)

AIR-SLAKED LIME

The action of air-slaked lime on clubroot was also tried. In pot tests it had been previously found to differ in its effectiveness with regard to disease control. It is worthy of note that this form of lime varies as to relative amounts of hydrate and carbonate in its composition, depending upon the conditions under which the oxide is slaked. To this fact may be due its inconstancy as a disease inhibitor. In this series lime was obtained from two sources. In one case it had been freshly made; in the other it had been made some months previously. This lime was applied to the field at a number of different rates. The results from three plots, however, were illustrative of the rest and of especial interest. One application of nearly 2 tons of lime per acre and another of nearly 2½ tons per acre from the same source did not increase crop production significantly above the control areas. In another case fresh air-slaked lime from another source was applied at the rate of 3 tons per acre, and a commercially practicable crop resulted.

HYDRATED LIME

Hydrated lime was proved by laboratory and pot tests to be capable of completely inhibiting clubroot. Experimentation with this form of lime in the field was therefore believed to be of great importance. Hydrated lime is readily obtained. It is manufactured for the building trade and comes sealed in heavy paper bags to obviate carbonation. Although a large number of plots were treated with this, only a few will be discussed here. Different methods of application were tried, the question of residual effect was considered, and the most effective quantities to be used from the standpoint of disease control and economy were studied.

The lime was applied in different ways. Large quantities were thrown in around the roots of seedlings at transplanting time. This served to keep the taproot free from disease, but as soon as secondary roots pushed laterally into the lime-free soil they became seriously infected, and unproductive plants resulted. Heavy suspensions of

FIGURE 2.—Results of using lime in a field thoroughly diseased with clubroot: A, Portion of field treated with 2 tons of hydrated lime per acre; full crop was produced; B, portion of same field treated with over 9½ tons of finely ground agricultural limestone per acre; stand and appearance of foliage better than in untreated area, but practically no plants matured; C, untreated portion of same field; large percentage of plants died, and those living were badly stunted and had no chance of maturing

hydrated lime in water were also used to water seedlings at transplanting, with the same result. Lime applied on top of the soil after transplanting served to keep the roots free from clubroot at the surface, but this effect did not appear to extend down into the soil below. The only satisfactory way of applying lime was found to be working it into as much as possible of the soil through which the roots ramify. This was done both by machinery and by hand, and the more thorough the mixture the better the results obtained.

The residual effect of hydrated lime was tried by several plot experiments. Results varied slightly, but the conclusion was reached that in well-limed soil clubroot inhibition was distinctly noticeable even three years after the application. The third-season crop was not necessarily so perfect as the crop of the first season of the experiment, but it was good.

A separate series of applications of hydrated lime was made the third year, which is worthy of note. It had as its object the finding of the least effective and economical quantities of hydrated lime to use in field control. This lime of commercial quality was applied by machine at from one-half to 2 tons per acre. Immediately after application the lime was worked into the soil with a disk.

Comparative data were obtained at harvest time. Control plots produced practically nothing, every year, in all cases. The treatment of one-half ton of hydrated lime per acre did not inhibit clubroot sufficiently to justify recommending it as a control measure. Sixty-nine per cent of the plants produced salable heads, though they were neither solid nor of good quality. The stand was fair, but all roots were more or less seriously clubbed. In the plot treated at the rate of 1 ton per acre a suggestion of successful control was noted. By this treatment not quite 100 per cent stand of plants resulted, and up to the time of heading it seemed that a very good crop would be produced. Only 75 per cent of the plants finally headed well, however, and though the heads appeared to be of good quality, they were not heavy. All plants when pulled showed considerable clubbing of the roots. When 1½ tons of hydrated lime were applied per acre 78 per cent of salable heads were cut at harvest. This is only a slightly greater percentage than was produced in the 1-ton plot. Individual heads from this plot appeared to be about as heavy as those from the previous one, but the general appearance of the foliage was better. The stand of plants was perfect, but the roots seemed about as badly clubbed in this plot as in the one receiving the 1-ton application. In the last plot, on which 2 tons of hydrated lime were applied per acre, what appeared as full crop production resulted. (Fig. 2, A.) The stand was perfect, a normal percentage of plants produced salable heads, and the heads cut were heavy, solid, and of good quality. Nearly all plants in this plot showed slight swellings on the roots, but occasional individuals had root systems that were free from clubs.

DISCUSSION OF CONTROL STUDIES

In the case of turnips and other cruciferous crops that are grown for their roots alone, the only perfectly successful clubroot remedy is one that absolutely inhibits the disease. If an edible root is not only malformed but opened to secondary decay organisms, its value

is immediately greatly diminished. However, it is not necessary to inhibit the disease completely when growing members of the kohl group of Brassica which are useful for their edible aerial portions, as in the case of cabbage. This type of crucifer can mature with a diseased root system only relatively free from clubroot, provided a sufficient supply of readily available plant food is present in the soil for the remaining healthy roots to absorb for the use of the plant. Even with a reduced root system (fig. 3) the maturity of the aerial portions of the plant may be thus assured.

Hydrated lime appears to be a practicable material to use for application to clubroot-infested soil. It is much more toxic to the clubroot organism than any of the sulphate or carbonate forms of lime.

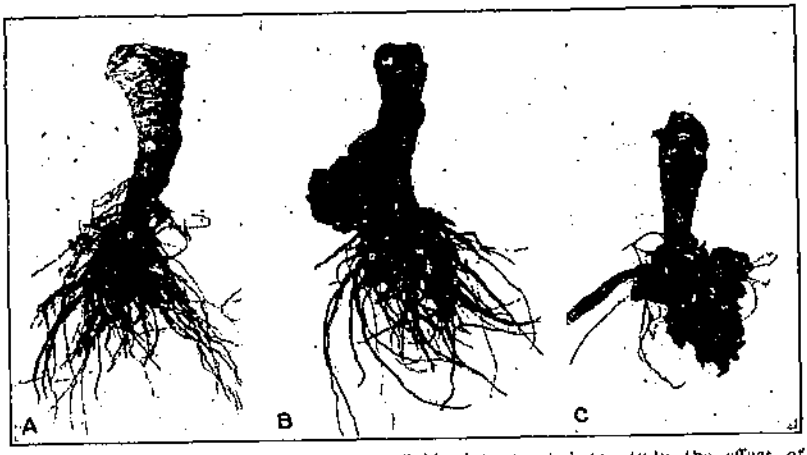

FIGURE 3.—Mature cabbage roots from field plots treated to study the effect of hydrated lime on control of clubroot. A, From soil into which lime had been well mixed. Lateral roots branched freely and had numerous fibrous rootlets with a few small clubs. Normal crop was produced. B, From soil treated with lime scattered on the surface just previous to plowing the land. Taproot and stem infections were common, and cordlike lower lateral roots had a few fibrous branches on them. Fair crop was produced. C, From untreated plot. The few living plants were severely stunted and had clubbed root systems almost wholly decayed

The toxicity to the parasite of calcium hydroxide seems to be due to a definite poisoning action on the organism by this chemical compound. The inhibiting effect produced on the disease by limes is apparently neither correlated with the amount of Ca ions applied or the number of active OH ions observed through pH-determination studies of the soil. Ground quicklime (calcium oxide) is unstable, but if it is incorporated into moist soil and left to slake it is apparently as successful an inhibitor of clubroot as is hydrated lime. In this case the moisture in the soil probably combines with the oxide, producing calcium hydroxide. The efficiency of burnt lime apparently depends upon whether it changes to carbonate or to hydrate. Hydrated lime is easily applied and does not cause the discomfort to the operator that air-slaked lime or ground quicklime does. A good grade of hydrated lime appears to have very definite inhibitive effects on the disease, whereas the efficacy of air-slaked lime can not be predicted.

Efficient control of clubroot of cabbage was obtained in the case of the present field experiments by the use of 2 tons of hydrated lime per acre. (Fig. 2, A.) The lime was spread by machine and immediately worked into the soil by disking and harrowing. In some soils a larger application of commercial hydrated lime may be desirable. It is impossible to make any general recommendation for all localities, all susceptible crops, and all soils, without much more extensive field study. The cost of hydrated lime in some cases may be prohibitive after the disease has become very severe. This is especially true on lower-priced land where cabbage is grown as a cash crop in a program of general farming. It is possible that in such cases long rotation with smaller applications of hydrated lime, between other crops several years before crucifers are planted, may be found to be practicable. In regions of higher-priced land where intensive cropping is practiced the cost of hydrated lime will not be so serious, and still less so where specialized crops such as cauliflower, kohlrabi, or Brussels sprouts are being grown. Considering the results from greenhouse and field studies, it appears that a long interval between the time of applying the lime and planting the crop is not necessary. If the proper quantity of hydrated lime is thoroughly mixed into the soil before the lime has had a chance to become carbonated by a few hours' exposure to the open air, the seed or seedlings may be planted as soon as convenient for the grower, with assurance that the treatment will be effective.

SUMMARY

The purpose of this bulletin is to report the results of several years' studies on the life history and control of *Plasmodiophora brassicae*, the cause of the destructive disease of crucifers known as clubroot.

The details of the process of spore germination are described.

The temperature range for spore germination occurs at a minimum of about 6° C. and a maximum of about 27°. Spores germinate well at temperatures ranging from 18° to a little above 25°, with the peak at 25°. Disease development occurs over a range of 12° to 27°, with the optimum from 18° to 25° and with most malignant disease development at about 25°. The temperature range for disease development and spore germination are practically identical, whereas, as shown by Tisdale, the temperature effect upon the growth of the host is different. This indicates that probably the temperature range of the disease development is the direct result of the action of temperature on the parasite.

It is shown that infection of the host occurs quite readily after 18 hours of exposure to infested soil held at a sufficient moisture content. This indicates that upon the occurrence of a heavy rain even the most adequate system for drainage could not necessarily be expected to inhibit infection by *Plasmodiophora brassicae*.

A survey of clubroot-infested fields in three States showed in some cases seriously infested soils which were naturally high in lime. A H-ion concentration survey of 116 disease-infested soils showed that they occurred at a range of pH 5 to 7.8, which was also found to be the range covered by samples of cabbage-growing soils selected

without respect to disease condition. The action of certain alkaline chemicals was studied by adding them at different rates to infective soils. In a soil with a pH of 7.2 the addition of K_2CO_3 produced a pH of 8.1 without inhibiting the disease, while by the application of $Ca(OH)_2$ the disease was completely prevented at a pH of 7.3. Data from field experiments have also shown that the H-ion concentration could not be considered a limiting factor in disease control.

Experiments carried on with different limes in pots and in the field confirm the above results. Limes consisting of $CaCO_3$ and $CaSO_4.2H_2O$ are not good clubroot inhibitors. The limes which are of CaO or $Ca(OH)_2$ composition controlled the disease well in plants grown in the clubroot-infested soils used.

In thoroughly infested seed beds it was found necessary to apply at least 1,500 pounds of hydrated lime per acre for satisfactory clubroot control. Unusually large quantities of raw ground limestone applied in the field did not inhibit the disease. Air-slaked limes were found to be of questionable value in their inhibitory effects. This form was therefore considered an unsatisfactory control material. Hydrated lime applied at the rate of one-half ton per acre was found to check the disease noticeably, but it was not until 2 tons per acre were used that a commercially satisfactory control was obtained on the soil in question.

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