

EFFECT OF MINERAL NUTRITION
ON INVASIVENESS OF PLASMODIOPHORA BRASSICAE WOR.
AND DEVELOPMENT OF CLUBROOT

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INTRODUCTION

Clubroot of crucifers was one of the first plant diseases described and has been the subject of a great deal of experimentation. More than 1200 papers have been published on various aspects of the clubroot disease. Many of these reports have dealt with control of the disease by the addition of lime to infested soil. When sufficient lime has been added to raise the pH of the soil to 7.2, the incidence of disease usually has been markedly reduced. In field trials it has been observed that lime and boron may exert a definite influence on the extent and severity of this disease. Little is known, however, of the mechanisms involved in clubroot control. For example it is not known whether control by addition of lime to soil is through the reduction of infection or retardation of neoplastic development, nor is it known whether the principal effect of lime is through its changing the pH or through the action of calcium that is added.

To understand the influence of various elements on the clubroot disease, one must be able to distinguish between their effects on parasitic establishment and on neoplastic response of the host. This investigation was

conducted to determine the effect of several mineral elements on infection of crucifers by Plasmodiophora brassicae Wor. and on subsequent development of the club-root disease. Particular emphasis was placed on the effect of calcium, potassium, boron and hydrogen ion concentration on clubroot. Some of the problems encountered in developing a method to study the effect of mineral nutrition on initial infection have previously been published (34).

LITERATURE REVIEW

The first outstanding research on clubroot of crucifers was carried out by Woronin (51) who in 1877 identified the pathogen causing the disease as a member of the Myxomycetes and gave it the name of Plasmodiophora brassicae. He described the disease and the pathogen causing it and suggested several means of control, most of which had been used for several years by progressive farmers. For the most part Woronin's research and results have withstood the test of time and the report of his work has been translated and published as a Phytopathological Classic.

The Disease

The first visible above-ground symptom of the clubroot disease is a slight wilting during the heat of the day. By the time this symptom is evident, the roots of the plant will be swollen and distorted. If the disease is not checked by some means, the swellings will increase in size until eventually the root is greatly swollen, and the above-ground portions of the plant turn yellow. Eventually permanent wilting causes death of the plant. Many of the cells in the swollen roots are filled with the resting spores of the fungus which may overwinter in

the soil or even survive for several years. These spores are about 1.6 to 4.3 microns in diameter (23) and are resistant to freezing. In fact the spores have been found to germinate more readily after being subjected to frost than otherwise (2).

Each resting spore germinates to form a single zoospore (49) which is biflagellate (27). The zoospore swims in the soil solution and eventually comes to rest on a root hair or other epidermal cell of a cruciferous plant. The flagella disappear and the pathogen takes the form of uninucleate amoeboid body. The amoeboid body penetrates the cell wall and enters the cell cavity where it develops into a plasmodium (7). More than one amoeba can enter a cell but they never fuse. After the plasmodium is developed the organism may follow any one of several different methods of infecting additional cells of the host.

(a) In one case the young plasmodia can penetrate from cell to cell directly through the cell wall or the plasmodia can break up to form several smaller plasmodia which can spread from cell to cell in the same manner (7) (24). The host cell which is penetrated by the amoeboid body swells after being infected. The nucleus of the infected cell usually enlarges to more than twice its normal size. Progressively the cells surrounding the

infected cell begin to enlarge, and as the pathogen spreads in all directions from the initial infection point the roots begin to show a visible swelling. While the pathogen is confined to the cortical region of the root, the swelling is due mainly to cell enlargement, but when the pathogen reaches the cambium the enlargement of the root is due to cell division as well as to cell enlargement (24). The pathogen eventually penetrates into most of the soft tissue of the root, at least in the immediate vicinity of the initial infection. The fungus spreads up and down in the cambium and eventually causes a spindle-shaped swelling of the root. Often the swelling is more on the infected than on the uninfected side of the root (24).

(b) The other means of spread through the host plant is by division of the host cells. When the protoplasm of the host cell divides to form two daughter cells, the plasmodium is broken into two pieces and a portion goes to each daughter cell (33). There is still the question of how much spread is by direct penetration of the fungus through cell walls and how much is due to spread by division of the host cells.

(c) Cook and Schwartz (12) observed the plasmodia in the root hairs and other epidermal cells to behave in a manner different from what had been described by earlier

investigators (24) (7) (33). They reported that the plasmodium in an initially infected cell enlarges and undergoes division to form several zoosporangia, each of which has 4 to 8 nuclei. Later they observed from 4 to 8 uninucleate zoospores in each zoosporangium when temperature and moisture conditions were suitable. These zoospores were found to be smaller than those coming from the resting spores. Cook and Schwartz were unable to determine the significance of these zoospores in the life cycle of the fungus or in the disease cycle. The formation of zoosporangia and zoospores was confirmed later but their function was not determined (2). They were observed to have a long and a short flagellum. Cook and Schwartz suggested that the zoospores are actually gametes, which fuse to form a zygote, but they did not offer any experimental evidence that this occurred. The suggestion has also been made that the zoospores swim down to the tips of the root, penetrate into the meristematic cells and then are spread through the young tissue by means of division of these cells (12).

Several workers have studied the life cycle of Plasmodiophora brassicae and various schemes have been proposed. They will not be discussed here as Karling included a lengthy discussion of this subject in his book on the Plasmodiophorales (23).

P. brassicae is not restricted to invading the tips of the roots or the zone of root hair formation (7), although these have been considered as the usual places of entrance for the pathogen (2). This fungus has been observed to invade older parts of the root and stem (24), especially where wounds have been made either by use of an instrument or caused by the development of adventitious roots (25). Evidence has been obtained that the fungus is systemic under certain conditions (45). When stem inoculations were made, the pathogen would move down to the hypocotyl region where a typical club was produced. No clubs developed in the region above the hypocotyl.

Until 1949 P. brassicae was believed to be parasitic on cruciferous plants only. In that year Webb (48) reported that he had observed zoosporangia which closely resembled those of P. brassicae in the root hairs of a grass, Holcus lanatus and a strawberry. Since then several other plants have been reported to be susceptible to root hair infection (29). It has been suggested that these hosts play a part in the survival of the pathogen in soil for long periods of time in the absence of a cruciferous host.

Even though it has become fairly well established that P. brassicae can invade the root hairs and other

epidermal cells of non-cruciferous hosts, there has been no evidence which would indicate that this fungus causes clubbing of any of these hosts.

Effect of Various Environmental Factors on Disease Development

As one would expect with an organism that is dependent on a delicate zoospore for its infection, this pathogen is very sensitive to certain environmental factors such as moisture, hydrogen-ion concentration, and mineral composition of the soil. A complete review of the literature on all these factors will not be given here but the high-points will be reviewed. Of course, in studying the effect of various environmental factors on a disease such as clubroot, it is well to consider that these factors can affect any one of several parts of the disease cycle such as spore germination, initial infection, spread through the host tissue or neoplastic response of the host. In earlier studies all of these possible effects were grouped into one general effect, which was usually called the effect on disease incidence, disease severity or disease development. These possible effects should be considered individually since a better understanding of the disease and the effects of environmental factors on it will be attained when this is done.

Resting spores have been found to germinate at a wide range of temperatures. Wellman (49) reported 6° and 28° C. to be the minimal and maximal temperatures for spore germination and 18 to 25° C. optimum. Chupp (7) found 27 to 30° C. to be optimum with no germination occurring between 16 and 20° C. unless a host plant was present, indicating that the host exerts an influence which to a certain extent takes the place of that offered by a greater amount of heat.

The effects of temperature on infection and on disease development have never been separated. The minimum for both has been reported as 12° C., the optimum as 18 to 24° C., and the maximum as 27° C. (49). Ayers (2) reported a minimum of 54 to 57° F. and a maximum of 90 to 92° F. The temperature range for both spore germination and disease development are approximately the same. Monteith (31) reported that the temperature range for clubroot development follows very closely the range for development of the host with a minimum of 9° C. and a maximum of 30° C. He concluded from his results that temperature is probably not a direct limiting factor in clubroot development under field conditions.

Soil moisture has been found to be a limiting factor (31). Clubroot developed on plants grown in soil maintained at a moisture content of 60 to 100 percent of the

water-holding capacity of the soil but failed to develop in soil kept at 45 percent or less of the capacity. Plants did not develop as well at 45 percent capacity as they did at higher levels. Further study (49) has shown that a continuous high level of soil moisture was not required for clubroot development. Exposure of plants to soil moisture of 80 percent of the water-holding capacity for as short a time as 10 hours resulted in clubroot infection and development when the plants were transplanted to soil held at 40 to 47 percent of the water-holding capacity.

Effect of pH and Mineral Nutrition

Several workers have reported greater spore germination in acid media than in alkaline media although exceptions to this generalization have been reported. Bremer (4) reported that hydrogen ion concentration is not the only factor involved. He showed that strong alkalinity inhibits germination without killing the spores. He also showed that germination occurs over a pH range of 5.4 to 7.5 but not at pH 8.0.

Several who have studied the effect of hydrogen ion concentration on clubroot development have found a fairly close correlation between incidence of infection and hydrogen ion concentration of the soil. One of the first

significant contributions on the effect of hydrogen ion concentration was published in 1928 (6). In greenhouse tests, a trace of the disease was observed in soil ranging from pH 7.2 to 7.4. Only one diseased root was found in soil that tested higher. Between pH 7.2 and 6.0 there was a rapidly increasing amount of clubroot until below pH 6.0 often 100 percent of the plants were infected.

Many workers had found before 1928 that the addition of lime or some other alkaline substance to the soil was often effective in controlling clubroot (turf ashes by Farquaharson (14), lime by Hunter (22), Somerville (42), Voelker (43), Halsted (19) and Atkins (1).

Farmers were using calcareous preparations in treating soil to control clubroot long before an alkaline soil reaction was known to give control. Prior to 1744 farmers were using marl for dressing their diseased fields before planting turnips (13).

In general the investigators cited, and numerous ones not cited, noted that an increase in alkalinity resulted in a decline in infection. However, a great deal of the evidence reported would indicate that soil alkalinity is not always effective in preventing clubroot. Lindfors (28) observed that clubroot developed in soil up to pH 7.5 but not at pH 7.8 to 8.0. Naumov (32) reported an optimum for the disease at 6.0 to 6.5 but found the disease

occurring where the soil pH was from 5.2 to 8.4. In 1927 he reported an experiment where he added barium oxide to soil and noted an increase in disease incidence as the pH of the soil was raised. At pH 6.8 no infection occurred while at pH 7.4, 20.9 percent of the plants showed evidence of clubbing. Clubroot has been reported to occur in soils that ranged in pH from 4.1 to 8.8 (3). Wellman (49) made a survey of 116 different soils infested with clubroot and found clubroot occurring in soils which ranged in pH from 5.0 to 7.8. In a greenhouse experiment he artificially changed the pH of the soil toward the alkaline side with several substances. Potassium carbonate was added to the soil until the pH was 8.1 but clubroot severity was not reduced. Sufficient calcium carbonate was added to adjust the soil pH to 7.9 before a reduction in the disease was noted. However, the addition of calcium hydroxide to raise the pH to 7.2 resulted in prevention of clubroot. Wellman concluded that hydrogen ion concentration alone should not be considered an important factor in the occurrence of the disease.

Haenseler (18) added various alkaline substances to heavily infested soil and observed the percentage of cabbage plants infected with clubroot. He found a reduction of the disease in all soils when the pH was raised to 7.0 or above, regardless of whether potassium,

sodium, calcium or magnesium was used. Unlike the results of Wellman (49) carbonates gave better control than oxides or hydroxides. Sodium and potassium carbonates gave more effective control than calcium or magnesium carbonates.

In field trials the addition of calcium hydroxide and calcium or magnesium carbonate in amounts sufficient to raise the pH to 7.1 and higher did not generally inhibit development of clubroot in silty clay loam soils (26). In greenhouse tests, however, infection was reduced by the addition of the same materials in sufficient quantities to raise the pH to 7.0, and at pH 7.2 and above infection was completely prevented. Larsen and Walker (26) observed a relation between soil moisture and the effect of alkalinity on infection. As reported above there was good correlation between soil reaction and infection by the clubroot organism in greenhouse pot experiments. In field experiments evidence was found which indicated that when the soil moisture fluctuated at relatively low levels, abundant infection occurred at soil reaction values which gave no infection under more constant high moisture level conditions. Colhoun's (8) results did not agree with Larsen and Walker's (26). He found that infection occurred in soil with high constant or fluctuating soil moisture at pH 7.8 in pot experiments but did not occur at low constant or fluctuating moisture levels.

Whitehead (50) found the disease to be generally less prevalent in alkaline soils, but a high percentage of susceptible plants were infected in soil with a pH range of between 7.45 and 7.81. Infection of root hairs has been obtained in a soil and sand mixture at pH 7.7 at 25° C. but only with high spore concentration (40).

After reviewing the papers cited above and those not cited, one must conclude that the hydrogen-ion concentration of the soil is not the sole factor involved in the prevention of clubroot when lime or certain other alkaline substances are added to the soil. Haenseler (17) suggested that an uneven distribution of soil acidity may be adequate to account for the occurrence of field infection in soil giving with composite samples a pH of 7.5 to 7.8 but including small local areas of pH 5.73 to 8.45.

Temperature has been found to have a greater effect on infection in alkaline than in acid soils (11). Very severe clubroot development occurred in alkaline soils at a mean air temperature of 23° C. In one experiment where a constant temperature of 23° C. was maintained, 41 percent of the plants were infected at pH 7.8 while at a temperature of 14.7° C. only 3.3 percent of the plants were infected. Further experiments showed that when plants in heavily limed soil (pH 6.8 to 7.0) were maintained at greenhouse

temperatures of about 23° C. for periods up to 12 days and then at approximately 12.5° C. for 21 days only slight attacks of clubroot developed. Whereas when plants were exposed to 23° C. for at least 6 days and then placed at about 16° C. severe attacks developed. Very severe infection occurred at 23° C. while at the lower temperature the plants remained healthy.

Colhoun (9) found also that fungicides were generally less effective in controlling clubroot in limed soil than in unlimed soil.

After Wellman (49) published his paper the suggestion that mineral nutrition might be a factor in the development of clubroot was considered to be a distinct possibility. However, to study the effects of mineral nutrition on clubroot development, it was necessary to be able to produce the disease in a medium that could be purified, such as sand or nutrient solution. Not until 1940 was a satisfactory method developed to secure clubroot on plants grown in a sand culture (36). This technique made it possible to study the effects of various ions on clubroot development. Pryor (36) reported that the percentage of infected cabbage plants growing in a balanced solution was increased somewhat over that of a balanced solution with an increase or a decrease of the nitrogen concentration or with an increase of the potassium concentration.

The percentage of clubbed plants was markedly reduced with the omission of potassium. The absence of sulfur increased the percentage of clubbed plants. None of the elements tested by Pryor increased the susceptibility of a resistant variety of turnips, Purple Top Milan. There was no correlation between clubroot incidence and sulfur oil content of the plants.

Gries et al. (16) carried out a series of experiments in soil in which the calcium and potassium concentrations were varied while holding the pH constant at 3 different levels with calcium sulfate to maintain an acid pH, with calcium carbonate to maintain a medium pH and with calcium hydroxide to maintain an alkaline pH. They found less clubroot in alkaline soil than in acid soil. Clubroot was found to decrease as the calcium-potassium ratio was reduced by increasing the potassium concentration while holding the calcium concentration constant. Clubroot incidence increased when potassium concentration was held constant with decreasing concentration of calcium. An interesting sidelight was observed by them. They found clubroot of cabbage and scab of potatoes caused by Streptomyces scabies to react as mirror images of one another as far as their relation to pH and potassium-calcium ratios was concerned.

Walker and Hooker (46), in one of a series of studies on the effects of plant nutrition on disease development, investigated the effect of salt concentration in a balanced solution, the effect of potassium concentration and the effect of nitrogen concentration on clubroot development, using a disease index method of comparing plants. In general their results were similar to Pryor's in that a high potassium concentration increased the severity of clubroot, while a low concentration markedly decreased severity of the disease. Either an increase or decrease in nitrogen concentration increased disease severity. An increase in phosphorus had little effect while a decrease in phosphorus usually decreased the severity of clubroot. An increase in salt concentration in a balanced solution tended to increase the disease index, but this was much less pronounced when conditions were favorable for good growth of the host as in late spring, compared to poor conditions for plant growth in midwinter.

From a thorough review of the literature, it was obvious that much information was needed on the effect of mineral nutrition on the clubroot disease. Evidence that certain elements do affect the severity of the disease had been reported but no detailed studies had been made of the effect of mineral nutrition on initial infection and

subsequent invasion. In particular an investigation of the role of the element calcium should be conducted since calcium is applied in high concentrations every time lime is applied to the soil to control clubroot.

METHODS AND MATERIALS

Initial Infection

Shogoin turnip seedlings were used as test plants in all these experiments. This plant was selected because of its fast rate of growth as well as its extreme susceptibility to Plasmodiophora brassicae Wor. (36). Plants were grown in a medium-fine grade of washed white silica sand (flintshot grade of Ottawa silica sand) which was further purified by being soaked for 24 hours in 18 percent hydrochloric acid solution, rinsed in running tap water until all acid was removed and then rinsed in distilled water. After the sand was thoroughly washed, it was placed in an oven set at 80° C. until dry.

Nutrient Solution

For the experiments the sand was moistened with a nutrient solution containing analytical reagent grade potassium nitrate, calcium nitrate, monobasic potassium acid phosphate, dibasic sodium phosphate, magnesium sulfate and trace elements prepared according to Robbins (38). The pH of the nutrient solution was adjusted to pH 5.9 by means of the 2 salts, monobasic potassium acid phosphate and dibasic sodium phosphate. In order to maintain a pH of about 5.9 throughout an experiment,

0.00025 Molar ammonium sulfate was added in the nutrient solution.

Where the calcium content was to be varied, the sand was moistened with a basic nutrient solution which contained 0.002 M. sodium nitrate, 0.002 M. potassium sulfate, 0.002 M. magnesium nitrate, 0.00178 M. monobasic sodium phosphate, 0.00022 M. dibasic sodium phosphate, 0.00025 M. ammonium sulfate and trace elements as recommended by Robbins (38). Calcium was added by replacing the sodium nitrate with calcium nitrate up to 40 milligrams of calcium per liter and calcium sulfate thereafter. When the concentration of potassium was varied, the basic solution contained no potassium sulfate. Desired concentrations of potassium were obtained by adding the required amount of potassium sulfate to the basic nutrient solution. The concentration of boron was varied by adding different amounts of boric acid to the nutrient solution.

Inoculum

Inoculum was added to the nutrient solution at the rate of approximately five million resting spores per milliliter. Inoculum was obtained from clubbed roots freshly harvested or held in storage for less than six months at 0 to 5° C. The clubs were macerated in a Waring Blender for about three minutes, the suspension was

filtered through cheesecloth to remove host plant debris, and the spores were washed at least 3 times by centrifugation and resuspension in distilled water. The number of spores was estimated with a haemocytometer. Sufficient spores were then added to the nutrient solution to give the desired concentration.

Plant Culture

Plants were grown in 50 milliliter pyrex beakers filled with acid-washed sand with the exception of the experiments where the concentration of boron was varied. In those experiments soft glass beakers were used. In either case the methods were similar. The beakers were filled with acid-washed sand to one-quarter inch from the lip of the beaker, after which $19\frac{1}{2}$ milliliters of nutrient solution containing suspended P. brassicae spores were poured in the beaker to moisten and inoculate the sand. A quarter-inch piece of glass tubing which was two inches long was placed in each beaker, with sand around it to allow air to escape when nutrient solution was added to the beaker. If this were not done, an air lock would often develop and the sand would be unevenly moistened. In preliminary experiments, this procedure was found to give a better distribution of infection among the seedlings in each beaker.

Early experiments were carried out by maintaining the cultures in moist chambers on a laboratory bench. Illumination was supplied by means of fluorescent lights. At the same time attempts were made to maintain cultures in a greenhouse but without much success because of the variability of temperature inside the moist chamber over a 24-hour period. Finally a specially-designed moist chamber was constructed with a removable aluminum shade. During the day the aluminum shade was placed on the moist chamber to prevent most of the heat waves from striking the plants. By this means it was possible to maintain the turnip plants in an atmosphere which was approximately 10° C. cooler than the rest of the greenhouse. This method proved to be satisfactory and allowed the laboratory bench to be used more effectively.

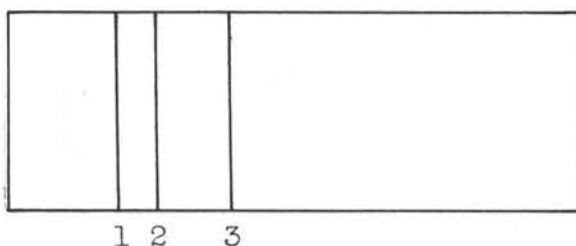
Twenty-four hours after the sand was moistened, 14 Shogoin turnip seeds were placed on the sand in each beaker. Earlier experiments showed that seed size had a decided effect on the amount of infection (34), therefore seeds measuring 1.5 to 2.0 millimeters in diameter were used. After the seeds were placed on the sand in the beakers the seeds were covered with a thin layer of sand. Although infection could be detected within 2 to 4 days following planting, the seedlings were allowed to grow for 6 days. It was usually necessary to add 2 to 3 milliliters

of distilled water at least twice during the growth period of the plants. The water was added through the glass tube in each beaker by means of a polyethylene wash bottle.

Data Assessment

Seedlings were removed from the sand cultures 6 days after exposure to the inoculum, washed in tap water to remove sand particles and placed in 1 percent aqueous solution of acetocarmine to fix the roots and stain the pathogen (40). The seedlings were left in the stain from 1 to 30 days or until they could be examined under the 16 mm. objective of a microscope for plasmodia inside the root hairs and other epidermal cells.

The roots were prepared for counting by washing them in tap water to remove the surface stain and were then placed on a slide designed especially for this type of counting. Lines were etched on the slide $\frac{1}{2}$ and 1 centimeter apart, as diagrammed:



The location on the stem where the seed was attached was placed on the line to the left. One-half centimeter

of root was left uncounted and data were taken on the length of root between lines 2 and 3. Therefore data were taken on the number of infections in the root hairs and other epidermal cells in the zone 0.5 to 1.5 centimeter below where the seed had been attached.

The lengths of the stem and the primary root were measured with a metric ruler.

Data were taken on the five largest plants in each culture. Five different cultures were grown for each treatment. Thus a total of 25 plants were counted for each treatment.

Field Experiment

A preliminary experiment was carried out to determine the effect of lime in combination with various concentrations of boron on clubroot development under field conditions. The trial was conducted in a field where clubroot had been established for several years. Hydrated lime was added to the soil at rates of 0, 250, 1000, and 4000 pounds per acre and with each rate of lime, sodium borate was added at rates of 0, $12\frac{1}{2}$ and 50 pounds per acre. The hydrated lime and sodium borate were applied in strips $1\frac{1}{2}$ by 15 feet with 9 inches left between treatments. Boron was added to the soil and mixed in with a hoe before the lime was applied. Each treatment was replicated

6 times. Following the soil treatment, seeds of Shogoin turnip were sowed using a Planet Junior Planter. The experimental area was watered frequently and was weeded by hand to prevent spreading soil from treatment to treatment.

About 90 plants were harvested from each plot 6 weeks after sowing the seed. Plants were washed free of soil and data were taken on the wet weights of the roots, tops and clubs.

Analysis of Plants for Potassium

Shogoin turnip plants were grown as described in previous section in 50 milliliter beakers for 6 days at which time the roots were harvested, dried, weighed and analyzed for potassium.

Plants that were to be harvested at the end of 6 weeks were grown in sand contained in $\frac{1}{2}$ -gallon glazed pots in a greenhouse. During the growth period, the plants were watered 3 times weekly with 200 milliliters of a complete nutrient solution. Diseased plants were obtained from cultures that had been inoculated while healthy plants were obtained from uninoculated cultures. When the plants were harvested, the clubs were approximately 0.75 inches in diameter. After the plant materials were harvested they were dried in an oven at 80° C. They were then ground in a Wiley Mill.

For the analyses approximately 100 milligrams of oven-dry powdered plant material were placed in Microkjeldahl digestion tubes, digested with 4 milliliters of concentrated sulfuric acid and oxidized by a dropwise addition of 30 percent hydrogen peroxide until clear. The contents of the tubes were diluted to 100 milliliters with glass-distilled water and this dilution was analyzed for potassium with a Beckman DU Flame Photometer at 768 millimicron wavelength. Readings were made from a standard curve constructed with potassium sulfate.

Assays were made on diseased and healthy 6-day old plants and 6-weeks old plants. Only the roots of 6-day old plants were analyzed while the tops, roots and clubs of the 6-weeks old plants were analyzed.

Greenhouse Experiments

Cultures of Shogoin turnip plants were maintained in sand cultures in a greenhouse. The plants were grown in a medium-fine grade of silica sand (flintshot grade of Ottawa silica sand) which was washed in tap water and rinsed in distilled water prior to being used for plant culture.

Turnip seeds were planted in the washed sand, which was moistened at daily intervals with a complete nutrient solution. About 3 days after planting, the seedlings

were inoculated with P. brassicae spores. A suspension of spores was poured around the seedlings. When the seedlings were 7 to 10 days old, they were removed from the sand and placed in a suspension of spores for 2 to 3 minutes. The seedlings were then transplanted to washed sand in $\frac{1}{2}$ -gallon glazed flower pots. After being transplanted the seedlings were watered 3 times weekly with 200 milliliters of nutrient solution. Excess nutrient solution was collected in a saucer in which each pot was placed in order to prevent the sand from drying between waterings. To prevent an accumulation of salts in the cultures, the sand was washed weekly with tap water followed by a rinse with distilled water. The seedlings were reinoculated 7 to 10 days after transplanting to be sure all of the plants were inoculated. Uninoculated control plants were included in the experiments.

The effect of the different elements on clubroot development was investigated by carrying out at least 3 experiments in which each element was varied. Each treatment was replicated 4 to 5 times with 3 plants being used in each replication.

In early experiments the severity of clubroot was assessed by classifying the diseased and healthy plants into various groups according to the severity of the symptoms--healthy, mild disease development, moderate

disease development and severe disease development. This method did not prove satisfactory however, because an experiment carried out at one time could not be compared with an experiment carried out at another time.

Another method that was used to estimate the severity of clubroot development was to count the number of spores in the diseased plants. The diseased plants were macerated with a Waring Blender. The host plant debris was removed by filtering through cheesecloth. The spore concentration in a suitable dilution of this spore suspension could be estimated using a haemocytometer. The spores of P. brassicae are very small and difficult to identify except when using very high magnification. This method of assessing disease severity was soon abandoned when carrying out large scale experiments because of the time involved in counting the spores and the difficulty involved in identifying the spores.

Instead of classifying the diseased plants into various classes or counting the spores in each plant, the plants were cut into 3 parts: foliage, roots and clubs. Wet weights and in some experiments dry weights of these plant parts were taken. Using these weights it was possible to calculate the percentage of a plant that was

diseased. This was a simple, yet quantitative way of measuring the degree of clubroot development. Except for the early experiments, this method was used exclusively.

EFFECT OF MINERAL NUTRITION ON INVASIVENESS
OF PLASMODIOPHORA BRASSICAE

Effect of Calcium on Initial Infection

The effect of calcium on initial infection was studied using 7 levels of calcium i.e., 0, 20, 30, 40, 60, 80 and 160 milligrams per liter of nutrient solution. The greatest amount of infection occurred when 40 milligrams of calcium was present per liter, while infection was reduced more than 50 percent when 80 milligrams of calcium were present (Figure 1).

Plant growth was not reduced at the highest concentrations of calcium used. There was a proportional increase in the growth of roots up to about 60 milligrams of calcium per liter, and of tops up to about 20 milligrams per liter. Beyond these concentrations of calcium, the growth curves of the roots and of the tops were nearly level. These data indicate that calcium selectively inhibits clubroot infection without inhibiting growth of the host plant at the same concentrations. The usual level of calcium that is used in nutrient solutions ranges from 120 to 200 milligrams per liter (21). The level of calcium that was inhibitory to the maximum infection attained in these experiments was considerably below these concentrations.

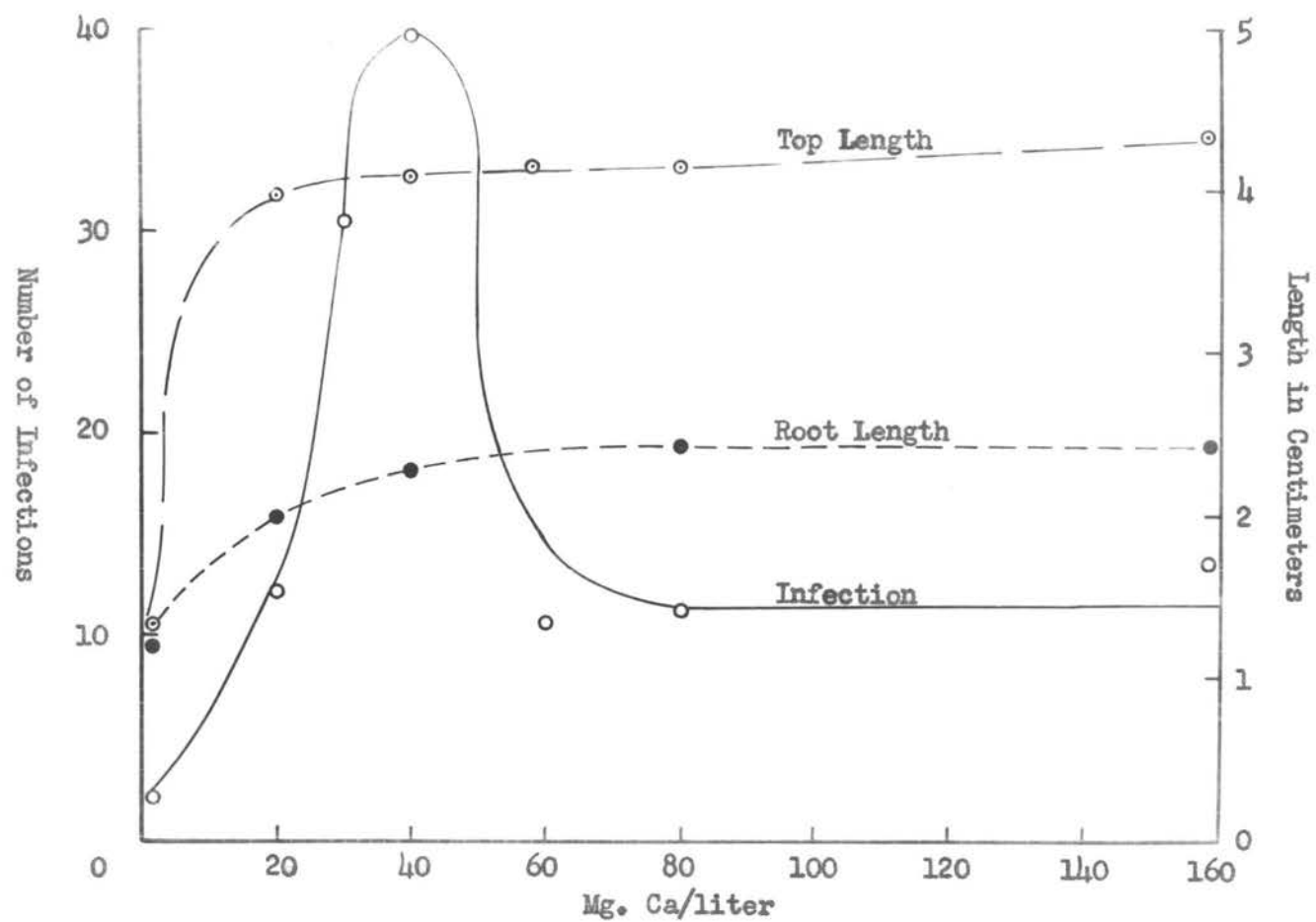


Figure 1. Effect of Calcium Concentration on Initial Infection and Growth of Host.

It is pertinent to note here that these results agree with the field observations of Gries et al. (16) who studied the effect of different ratios of calcium to potassium on potato scab and clubroot. Their results showed that calcium reduced clubroot development under field conditions.

Effect of Potassium on Initial Infection

Experiments were carried out on the effect of potassium concentration on the incidence of infection (Figure 2). The concentration of potassium was adjusted to 12 levels over the range 0 to 640 milligrams per liter. Infection was consistently greatest at 160 to 280 milligrams of potassium per liter but declined at concentrations of 320 milligrams per liter or more. The usual range of concentrations of potassium used in nutrient cultures is from 80 to 320 milligrams per liter (21). Therefore potassium does not appear to inhibit initial infection of turnip plants at concentrations usually used in nutrient culture work.

Seedling growth, as measured by the length of tops and roots, was excellent over a range of 160 to 360 milligrams of potassium per liter. Both infection and growth declined slightly beyond these concentration limits. However, infection of plants receiving 320 to 400

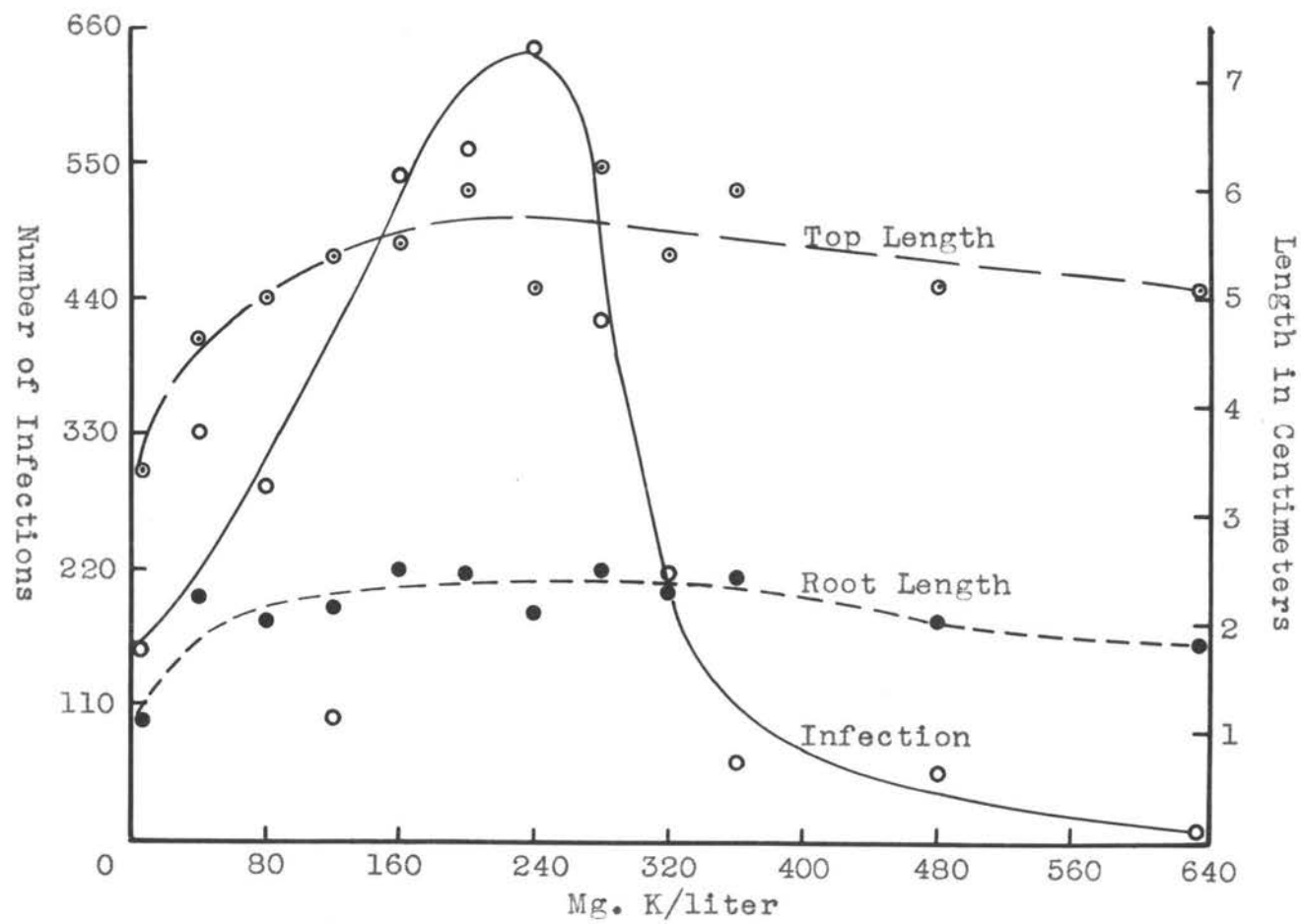


Figure 2. Effect of Potassium Concentration on Initial Infection and Growth of Host.

milligrams of potassium per liter proportionately declined much more than growth. These data indicate that the growth response of the host alone will not explain the severity of infection.

Effect of Elements Related to Calcium and Potassium on Infection

Since calcium and potassium exercised such a strong influence on the invasion of turnip roots by P. brassicae, a series of tests were made with the sulfate salts of cesium (Figure 3), rubidium (Figure 4), barium (Figure 5), beryllium (Figure 6), strontium (Figure 7), and lithium (Figure 8). A complete nutrient solution was used in this series of tests. Calcium and potassium were used at rates of 40 milligrams and 160 milligrams per liter, respectively. Low concentrations of all of the elements except strontium inhibited infection. The number of infected root hairs and other epidermal cells declined directly in proportion to the concentration of the elements used. Of the elements tested in this series, beryllium was most inhibitory to infection. At a concentration of 2 milligrams of beryllium per liter the number of infections was reduced from about 24 to 4 or 5. All of the elements inhibited infection at a much lower concentration than was inhibitory to root or top growth. This is further evidence that infection by P. brassicae is greatly influenced by the

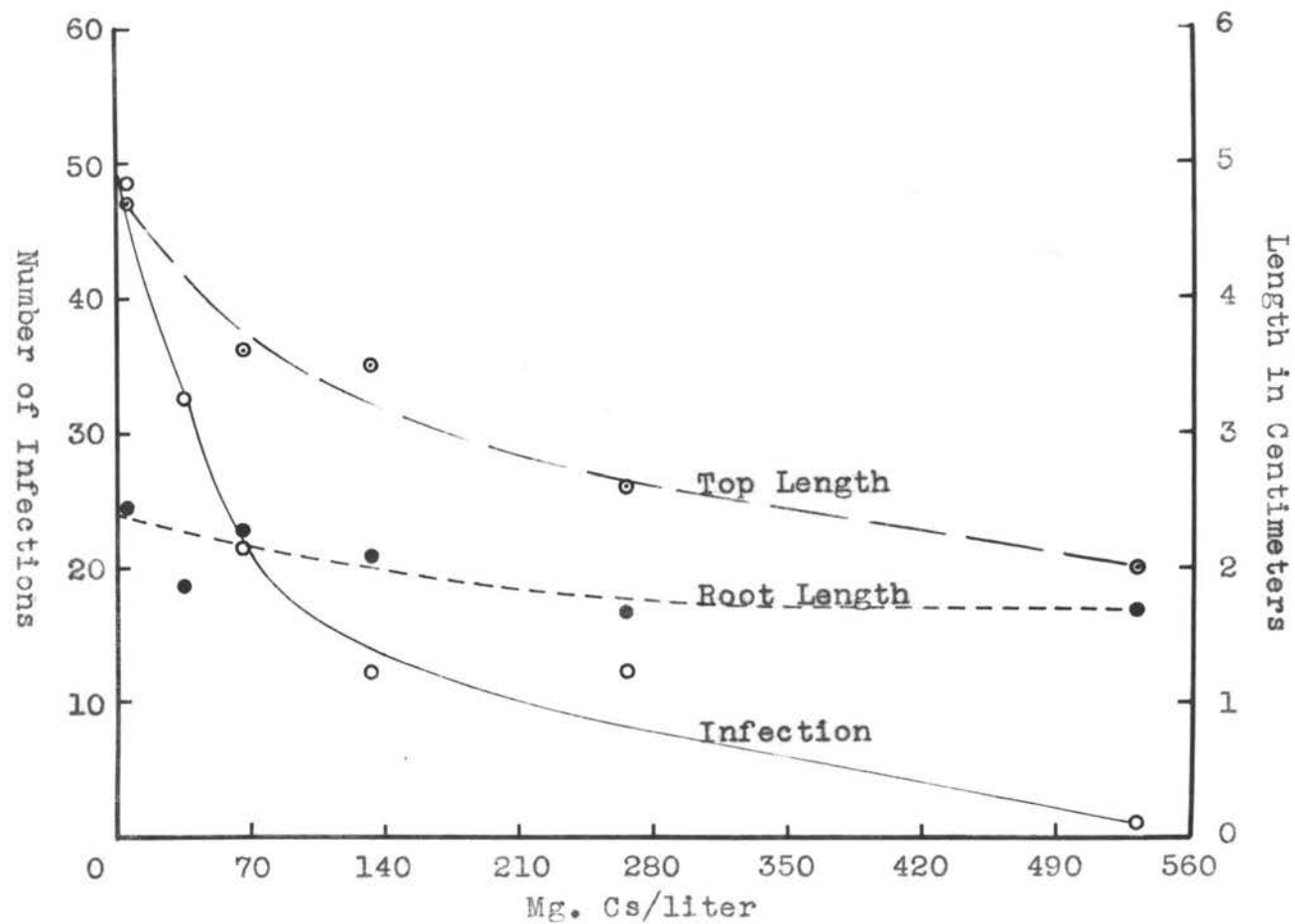


Figure 3. Effect of Cesium Concentration on Initial Infection and Growth of Host.

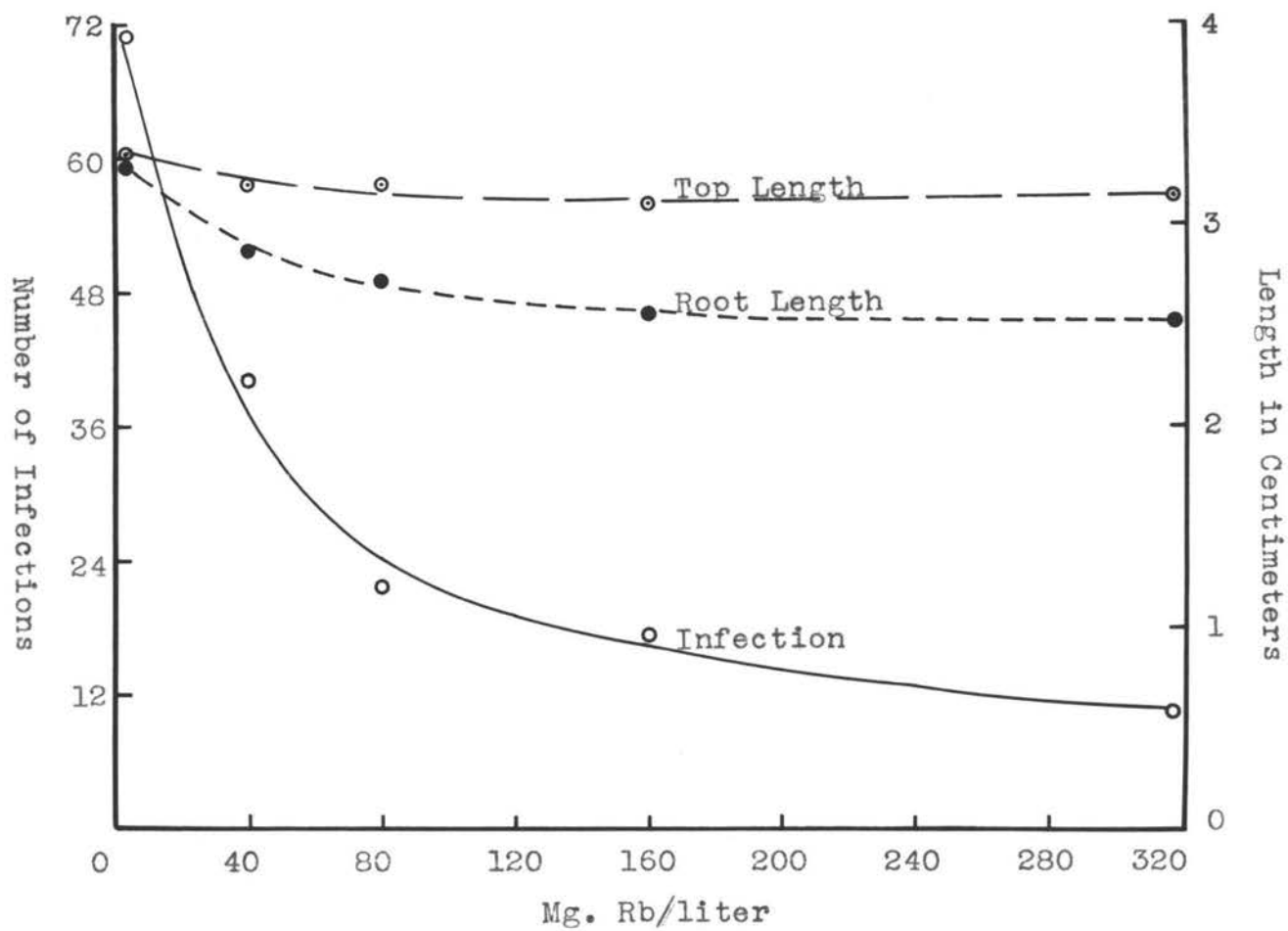


Figure 4. Effect of Rubidium Concentration on Initial Infection and Growth of Host.

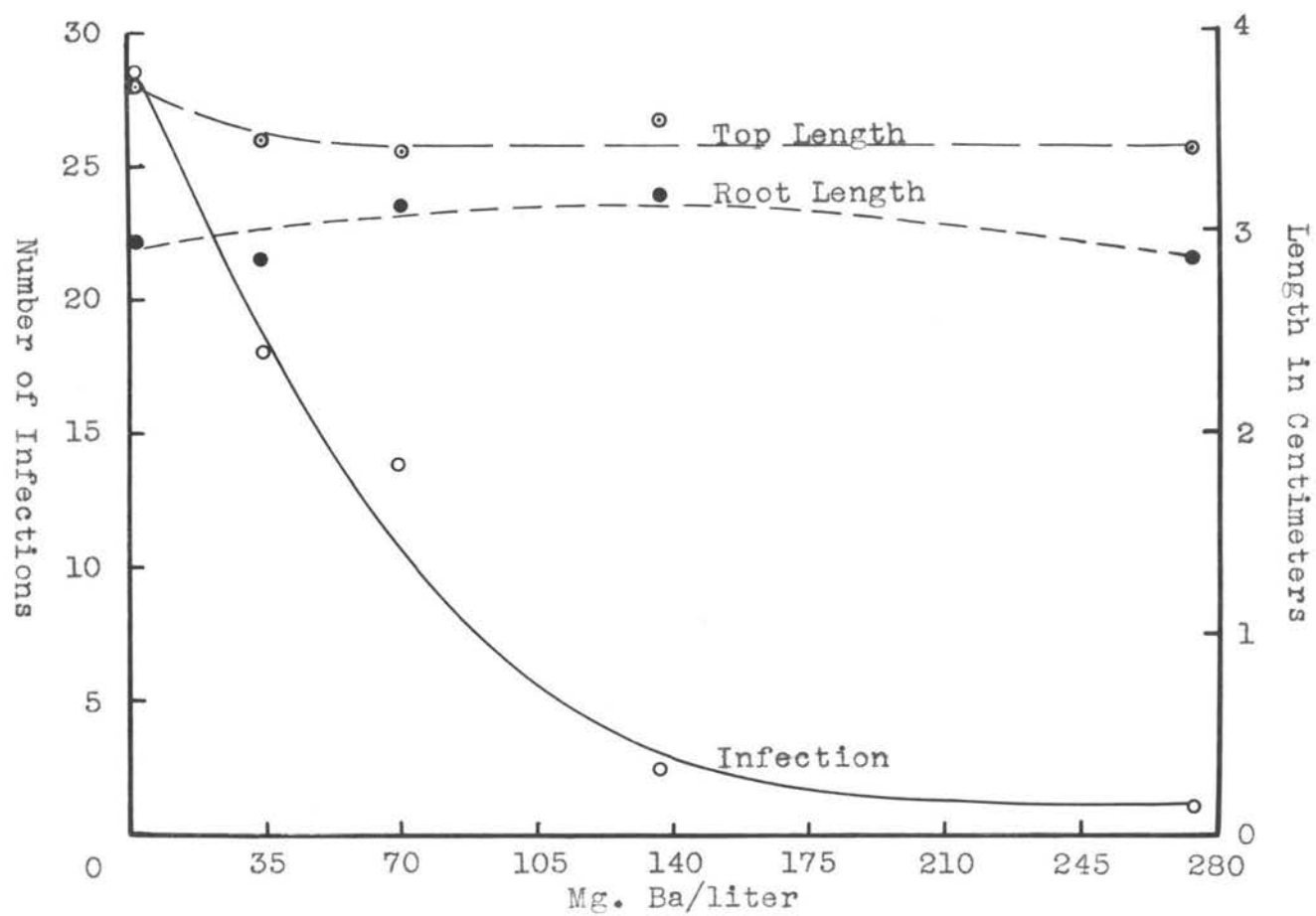


Figure 5. Effect of Barium Concentration on Initial Infection and Growth of Host.

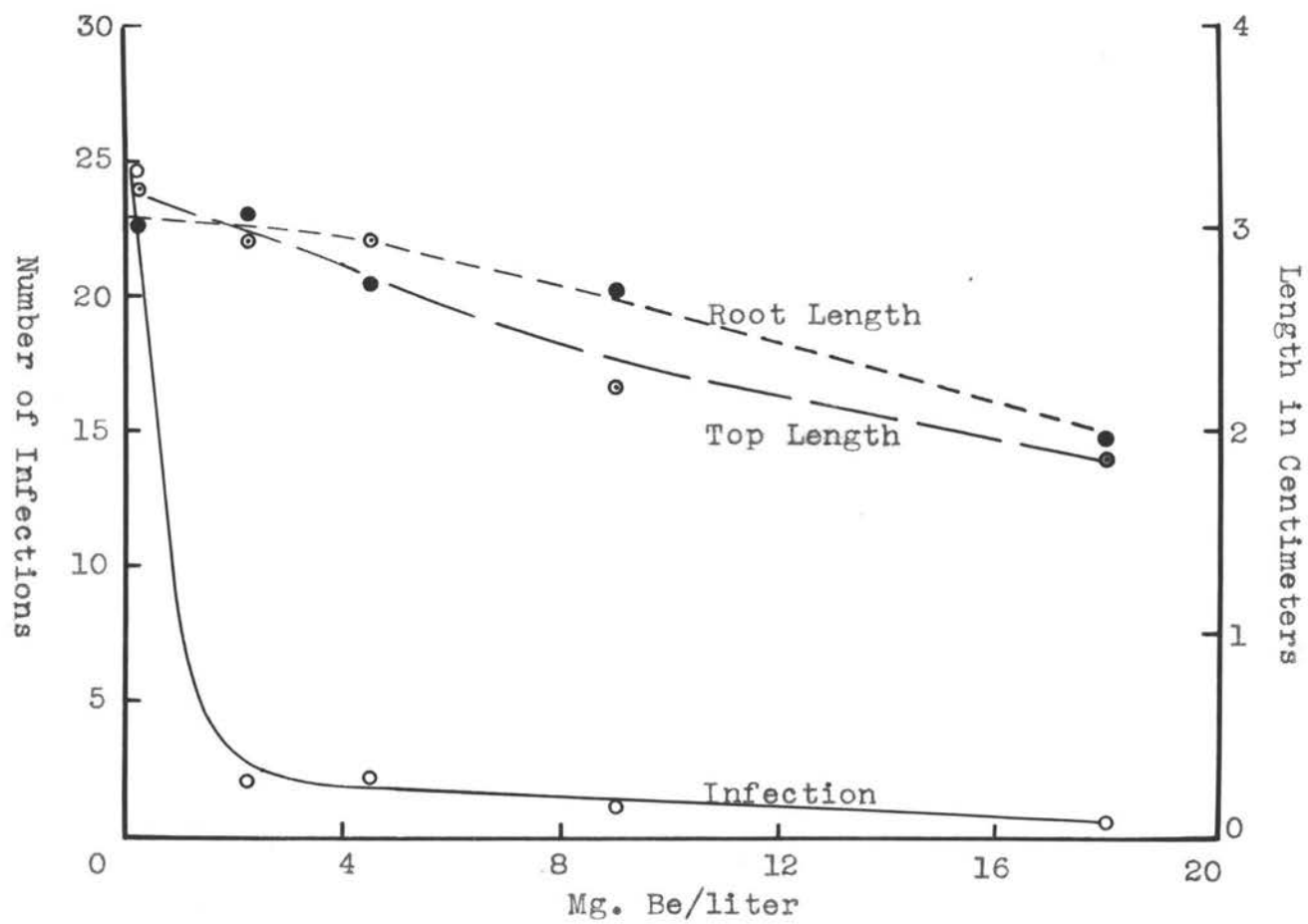


Figure 6. Effect of Beryllium Concentration on Initial Infection and Growth of Host.

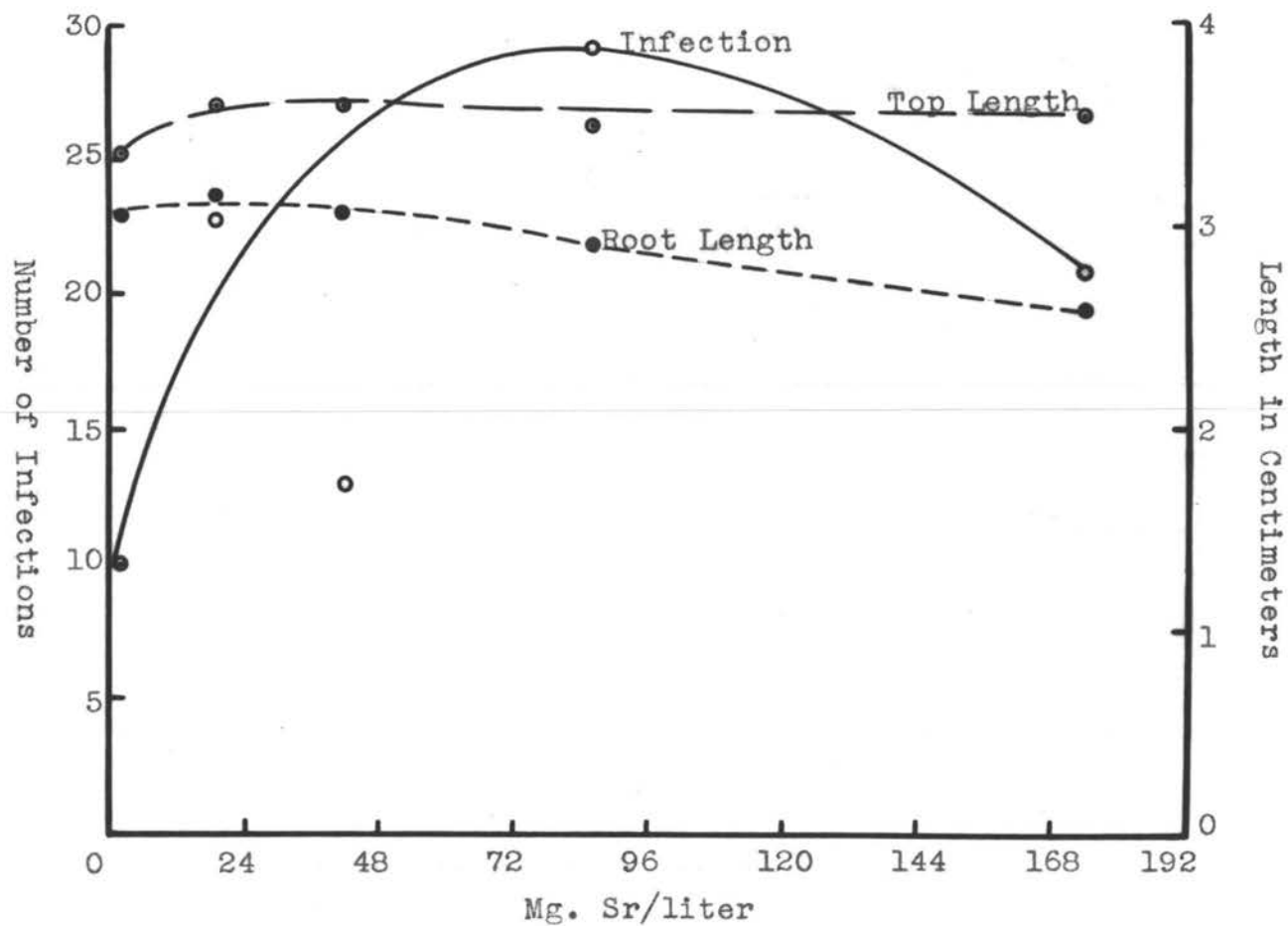


Figure 7. Effect of Strontium Concentration on Initial Infection and Growth of Host.

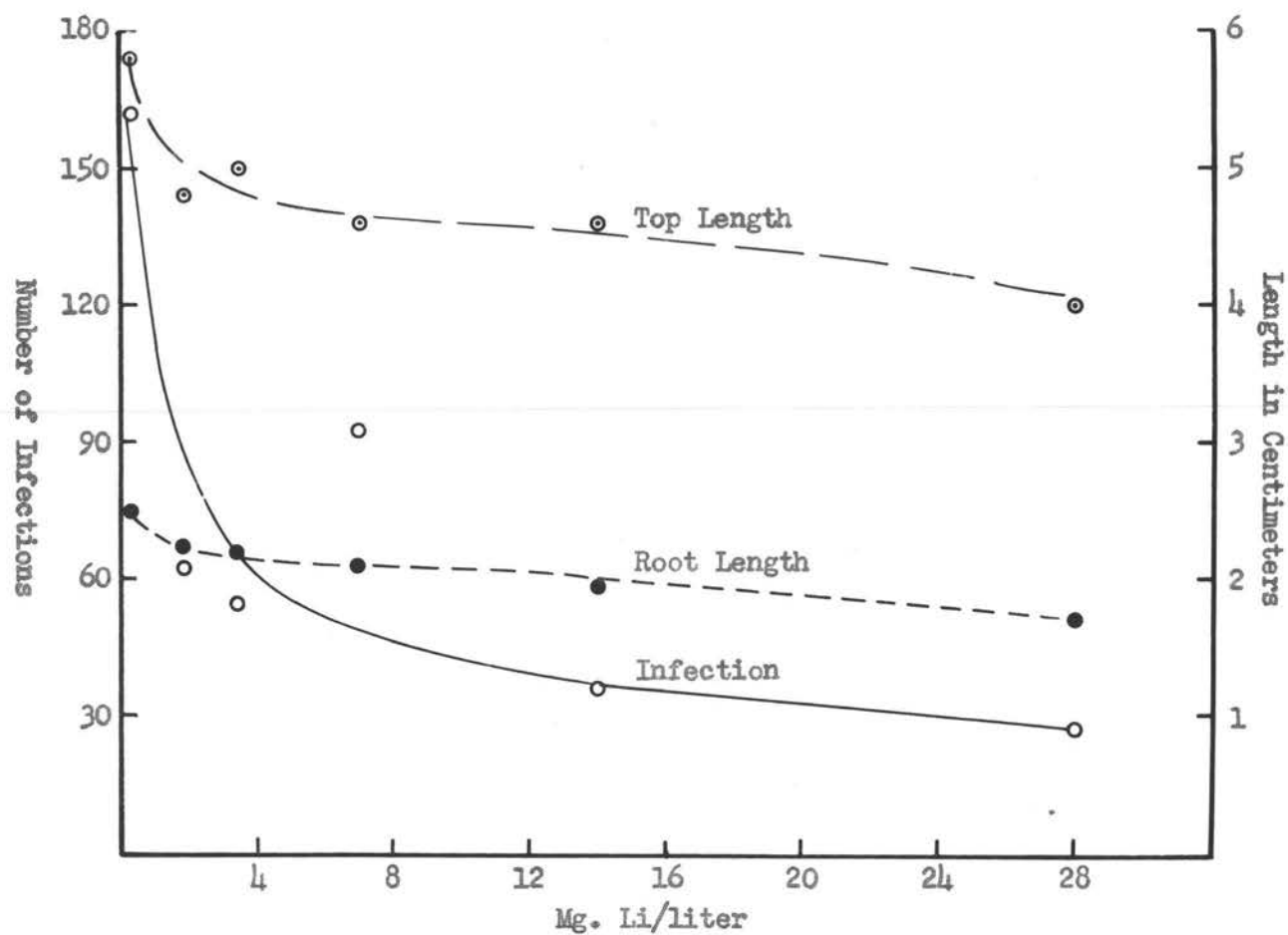


Figure 8. Effect of Lithium Concentration on Initial Infection and Growth of Host.

mineral content of a nutrient solution. Strontium appeared to stimulate infection at concentrations up to 100 milligrams of strontium per liter. At the highest concentration tested strontium appeared to inhibit infection.

An additional test was made with lithium to determine if there was any relation between the effect of potassium on infection and that of lithium. Lithium was present at rates of 0, 14 and 28 milligrams per liter in combination with potassium which was present at rates of 0, 80 and 160 milligrams per liter. Lithium reduced the incidence of infection but did not entirely offset the increase in prevalence of the pathogen in plants receiving 80 to 160 milligrams of potassium per liter (Table 1). It is interesting to note that when no potassium was present, 14 milligrams of lithium per liter reduced infection 52.8 percent, when 80 milligrams of potassium per liter was present 39.8 percent and when 160 milligrams of potassium was present per liter 51.4 percent.

Effect of Boron on Initial Infection

The effect of boron on initial infection was studied by using 8 levels of boron over the range of 0 to 64 milligrams of boron per liter (Figure 9). Boron was added to the nutrient solution as boric acid. Infection was consistently greatest when 1 milligram of boron per liter

Table 1

The Effect of the Ratio of Lithium to Potassium
on the Incidence of Infection
by Plasmodiophora brassicae

Concentration of elements mg/l		Number of infections per centimeter of root
<u>Potassium</u>	<u>Lithium</u>	
0	0	17.6
0	14	8.3
0	28	2.0
80	0	433.4
80	14	260.3
80	28	53.4
160	0	943.0
160	14	457.8

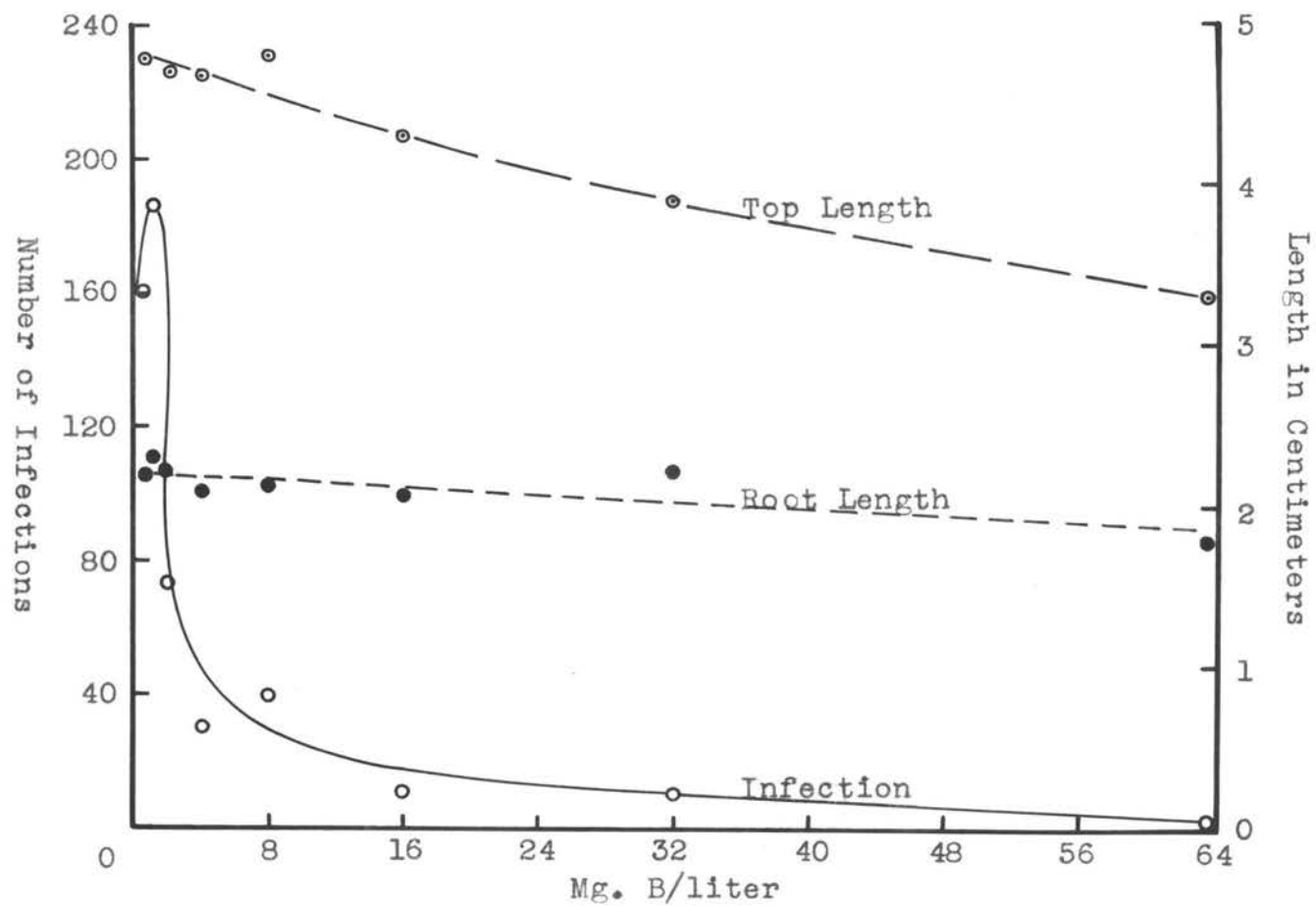


Figure 9. Effect of Boron Concentration on Initial Infection and Growth of Host.

or less was present in the nutrient solution. Two milligrams of boron per liter resulted in a marked inhibition of infection, however infection was not inhibited completely even when 32 milligrams of boron per liter were present.

Under the conditions of these experiments boron did not appear to be very toxic to root growth at the concentrations used as measured by the length of the roots. There was a progressive reduction of top growth with increasing concentrations of boron. These tests indicate that boron is a very important factor in initial infection and warrants further study. Boron and calcium have been known for a long time to interact in mineral nutrition of plants. For example, boron has been reported to stimulate the uptake of calcium by plants (20). It seemed logical at this point in these investigations to study the relation of boron and calcium to initial infection.

Effect of Boron and Calcium on Initial Infection

The relation between the effects of boron and calcium on initial infection were investigated. Five concentrations of calcium (0, 20, 40, 80 and 160 milligrams per liter) and three concentrations of boron (0, 0.25 and 8 milligrams per liter) were used in combination. When young seedlings were supplied with no boron an

increase in the amount of calcium did not reduce the number of infections (Figure 10). When 0.25 milligrams of boron per liter were added to the nutrient solution, a maximum number of infections was obtained with 40 milligrams of calcium per liter. The number of infections was reduced approximately 50 percent with 160 milligrams of calcium per liter. When 8 milligrams of boron per liter were added, a peak infection was obtained with 20 milligrams of calcium per liter with a reduction occurring at either higher or lower concentrations of calcium.

Effect of Calcium and Boron on Clubroot Development in a Field Experiment

An experiment was carried out to determine the effect of lime in combination with various concentrations of boron on clubroot development under field conditions. Hydrated lime was applied at rates of 0, 250, 1000 and 4000 pounds per acre and sodium borate at rates of 0, $12\frac{1}{2}$ and 50 pounds per acre in a field where P. brassicae had been established for several years. The addition of large quantities of lime resulted in good control of clubroot (Table 2). The most effective control was obtained with 4000 pounds of lime per acre. When lime was applied to the soil at the rate of 250 pounds per acre, clubroot severity was greater than when no lime was added or when greater quantities were added.

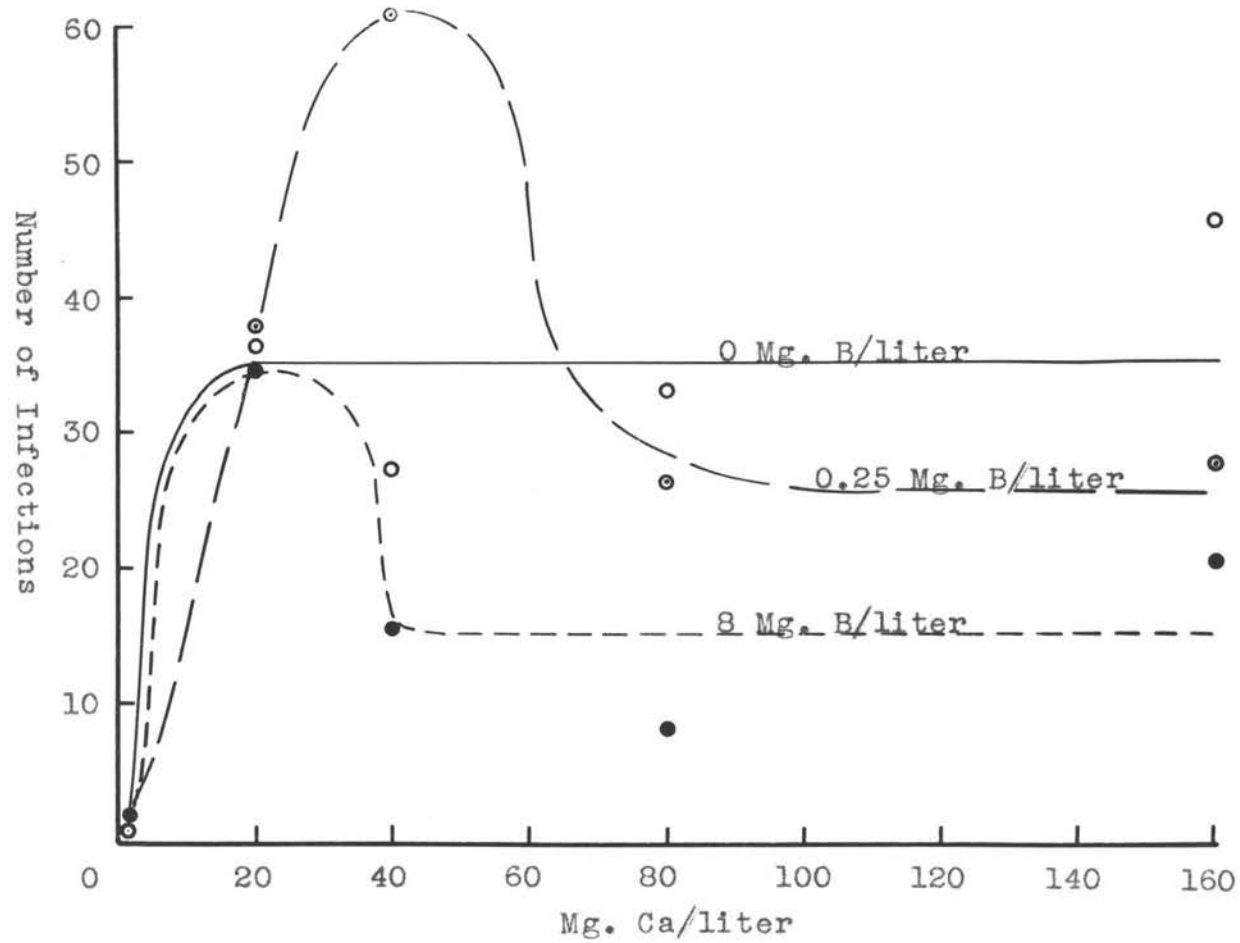


Figure 10. Effect of Calcium/Boron Ratio on Initial Infection.

Table 2

Effect of Various Combinations of Hydrated Lime
and Sodium Borate
on Clubroot in a Field Experiment

<u>Hydrated lime pounds/acre</u>	<u>Sodium borate pounds/acre</u>	<u>Percentage of plants healthy</u>	<u>Percentage of root weight healthy</u>
4000	0	66.1	73.8
4000	$12\frac{1}{2}$	68.1	76.4
4000	50	70.4	78.4
1000	0	39.4	51.4
1000	$12\frac{1}{2}$	46.4	55.8
1000	50	41.0	60.5
250	0	4.5	21.1
250	$12\frac{1}{2}$	21.1	37.6
250	50	27.0	49.7
0	0	36.5	47.2
0	$12\frac{1}{2}$	30.3	44.2
0	50	21.9	42.2

Sodium borate did not influence clubroot development appreciably but substantial control was secured with sodium borate at the rate of 50 pounds per acre compared with no sodium borate when lime was applied at the rate of 250 pounds per acre. Even when sodium borate was applied at the rate of 50 pounds per acre in this particular treatment series, the percentage of healthy plants was less than when no boron and no lime was applied.

Effect of pH of Nutrient Solution
on Initial Infection

To determine the effect of pH of the nutrient solution on infection it was necessary to use a different method for culturing the turnip plants. Cultures were maintained in washed silica sand in 2½ inch clay pots. Nutrients were supplied by a constant drip through a series of tubes coming from a reservoir. One liter per day of nutrient solution was allowed to drip on the sand in each pot. This was a large quantity of nutrient solution for such small pots but in order to maintain a nearly constant pH with sand cultures it was necessary to replace the nutrient solution in the sand constantly. The pH would change to a greater extent when an intermittent method of moistening sand was used. Even this constant drip method did not hold the pH constant as can be seen by examining Table 3.

Table 3

Comparison of pH of Original Nutrient Solution
and pH of Solution
After Percolating Through Sand Culture

<u>Original pH of nutrient solution</u>	<u>pH of nutrient solution after percolating through sand culture</u>
4.14	5.64
4.88	5.78
5.23	5.88
5.82	6.02
6.06	6.13
6.14	6.30
6.52	6.54
7.14	6.95
7.79	7.39

The pH of the nutrient solution was adjusted to 9 levels by varying the ratio of sodium dibasic phosphate to sodium monobasic phosphate. By this means the pH of the nutrient solution could be adjusted from about pH 4.8 to about 7.5. Beyond these limits, sodium hydroxide was added for a more alkaline pH while sulfuric acid was added for a more acid pH. The optimum pH for initial infection was about pH 5 with a rather sharp decline at higher and lower pH values (Figure 11). Only a very small number of infections occurred above pH 7. This substantiated data others have obtained from field and greenhouse experiments on the effect of pH on clubroot development.

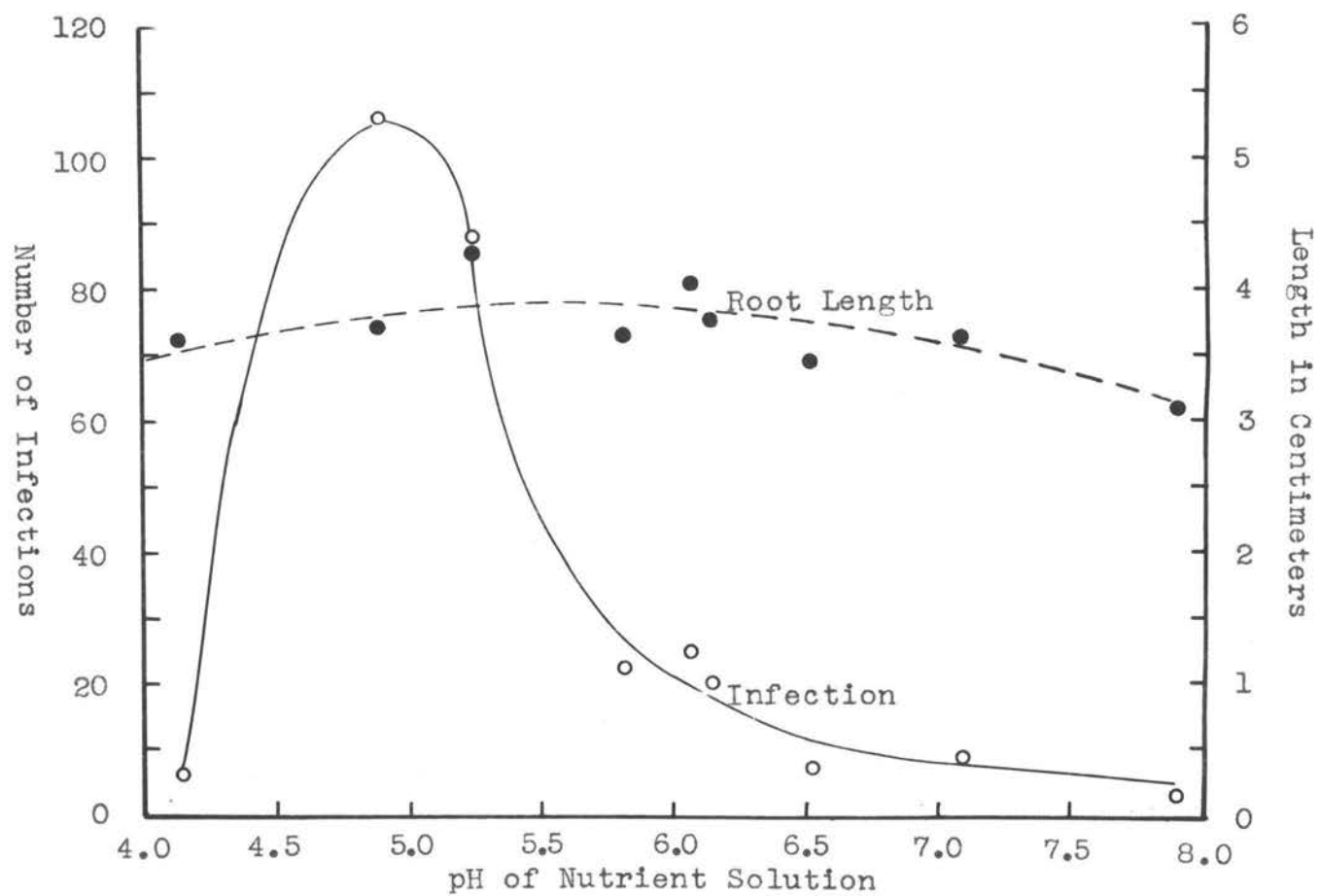


Figure 11. Effect of pH on Initial Infection and Growth of the Host.

ANALYSIS OF HEALTHY AND DISEASED PLANTS FOR POTASSIUM

Healthy and diseased Foliage turnip plants were analyzed for potassium. Six-week old plants with clubs approximately 0.75 inches in diameter and 6-day old plants with no swellings were analyzed.

Stem and leaf tissues of healthy 6-week old turnip plants contained more potassium than similar tissues from diseased plants (Table 4). Analyses of roots and clubs revealed that the roots of healthy plants contained more potassium than the normal roots of diseased plants. On the other hand clubs contained more potassium than either the normal roots of a diseased plant or the roots of a healthy plant. These data suggest there may be a movement of potassium into the club from the rest of the plant or a lack of movement out of the clubbed tissue.

It was important to distinguish whether potassium was present because of the growth of the host or because of the presence of the pathogen. To distinguish between these two possibilities, 6-day old seedlings that were recently infected were analyzed for potassium (Table 5). These analyses revealed that recently infected seedlings in which clubs had not yet developed contained less potassium than healthy seedlings. These data indicate that the

higher concentration of potassium in the club than in the rest of the plant is not due merely to the presence of the pathogen.

Table 4

Milligrams of Potassium in 100 Milligrams
of Oven-Dried Plant Tissue
from 6-Week Old Shogoin Turnip Plants

Normal root		Club	Top	
<u>Inoculated plants</u>	<u>Uninoculated plants</u>		<u>Inoculated plants</u>	<u>Uninoculated plants</u>
2.40	3.02	4.46	4.66	5.01
2.21	2.67	4.19	3.44	4.66
2.12	2.20	4.54	3.82	4.28
2.10	2.22	4.21	4.42	4.53

Table 5

Milligrams of Potassium in 100 Milligrams
of Oven-Dried Root Tissue
from 6-Day Old Shogoin Turnip Plants

<u>Inoculated</u>	<u>Uninoculated</u>
0.619	1.168
0.655	0.723
0.663	0.993
0.618	0.845

EFFECT OF MINERAL NUTRITION
ON DEVELOPMENT OF CLUBROOT IN THE GREENHOUSE

Sand cultures of Shogoin turnip were maintained in a greenhouse to study the effect of calcium, potassium and hydrogen ion concentration on clubroot development. The chemical constitution of the complete nutrient solution and the method of varying its elemental composition were described in the methods section.

The seedlings were inoculated while still being watered with the complete nutrient solution in order to eliminate the effects of mineral nutrition on the initial establishment of the pathogen in the host. Seedlings were reinoculated when they were transplanted to the sand in the glazed pots, where the elemental composition of the nutrient solution was varied. The plants were reinoculated once after having been transplanted.

When harvested 4 to 8 weeks after being transplanted, the plants were washed free of sand, surface moisture removed, cut into foliage, roots and clubs. These plant parts were weighed and from these data it was possible to calculate the percentage of a plant that was diseased. The data reported in this thesis is from a typical experiment in all cases.

Effect of Calcium on Clubroot Development

Experiments were carried out to determine the effect of calcium on clubroot development in nutrient sand cultures in a greenhouse. Six levels of calcium were used i.e., 0, 25, 50, 75, 100 and 150 milligrams per liter of nutrient solution. Calcium appeared to stimulate clubroot development at increasing concentrations up to 100 milligrams of calcium per liter. At 150 milligrams per liter calcium appeared to inhibit clubroot development.

When the data showing the effect of calcium on initial infection (Figure 1) are compared with the data showing the effect of calcium on clubroot development (Figure 12), the inference can be drawn that the low concentrations of calcium that were inhibitory to initial infection were not inhibitory to clubroot development.

Effect of Potassium on Clubroot Development

The effect of potassium on clubroot development was studied using 0, 100, 200 and 300 milligrams of potassium per liter. With an increase in the concentration of potassium up to 200 milligrams per liter there was a corresponding increase in severity of clubroot (Figure 13). With 300 milligrams of potassium per liter there was no further increase in clubroot development based on the

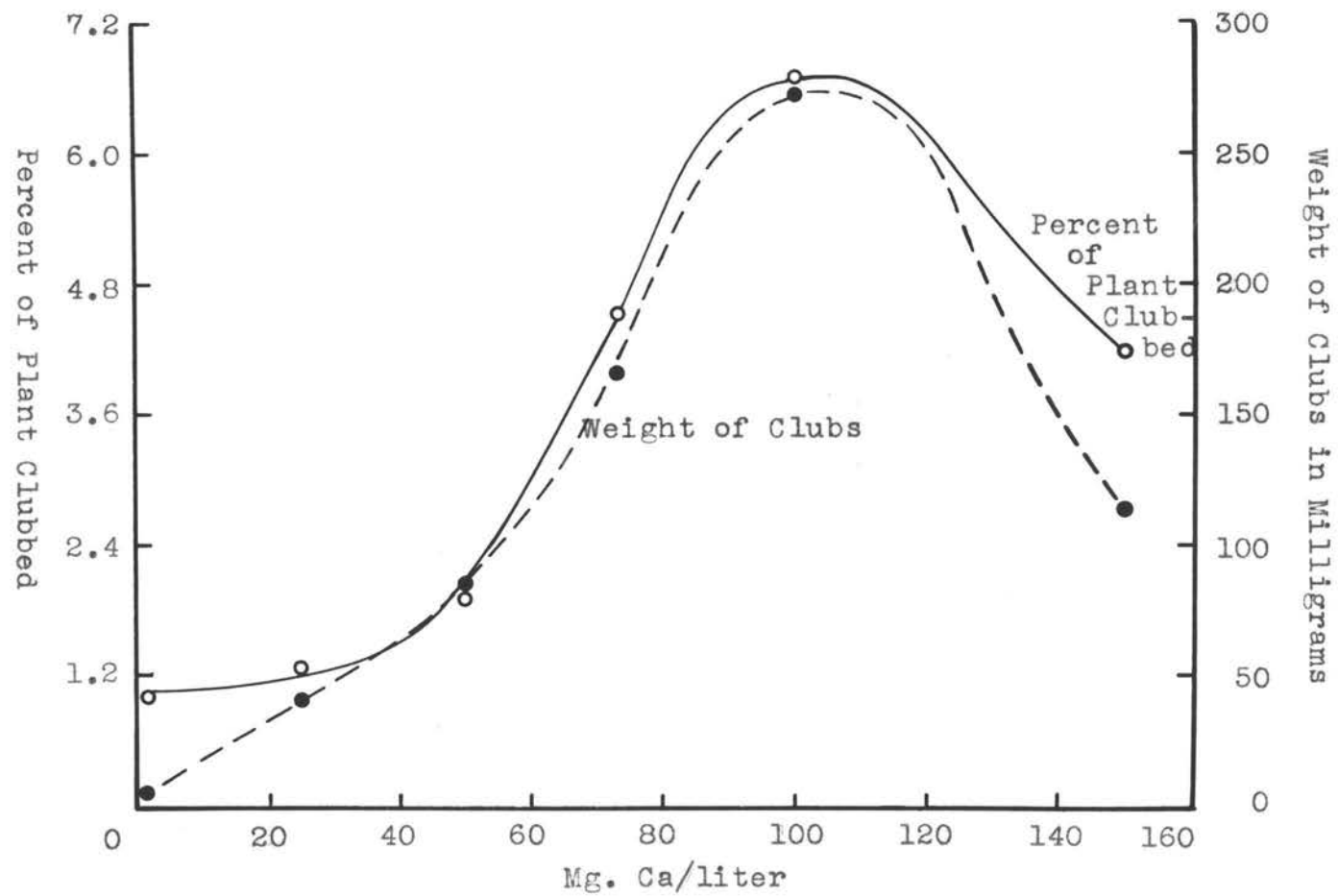


Figure 12. Effect of Calcium Concentration on Clubroot Development in Greenhouse.

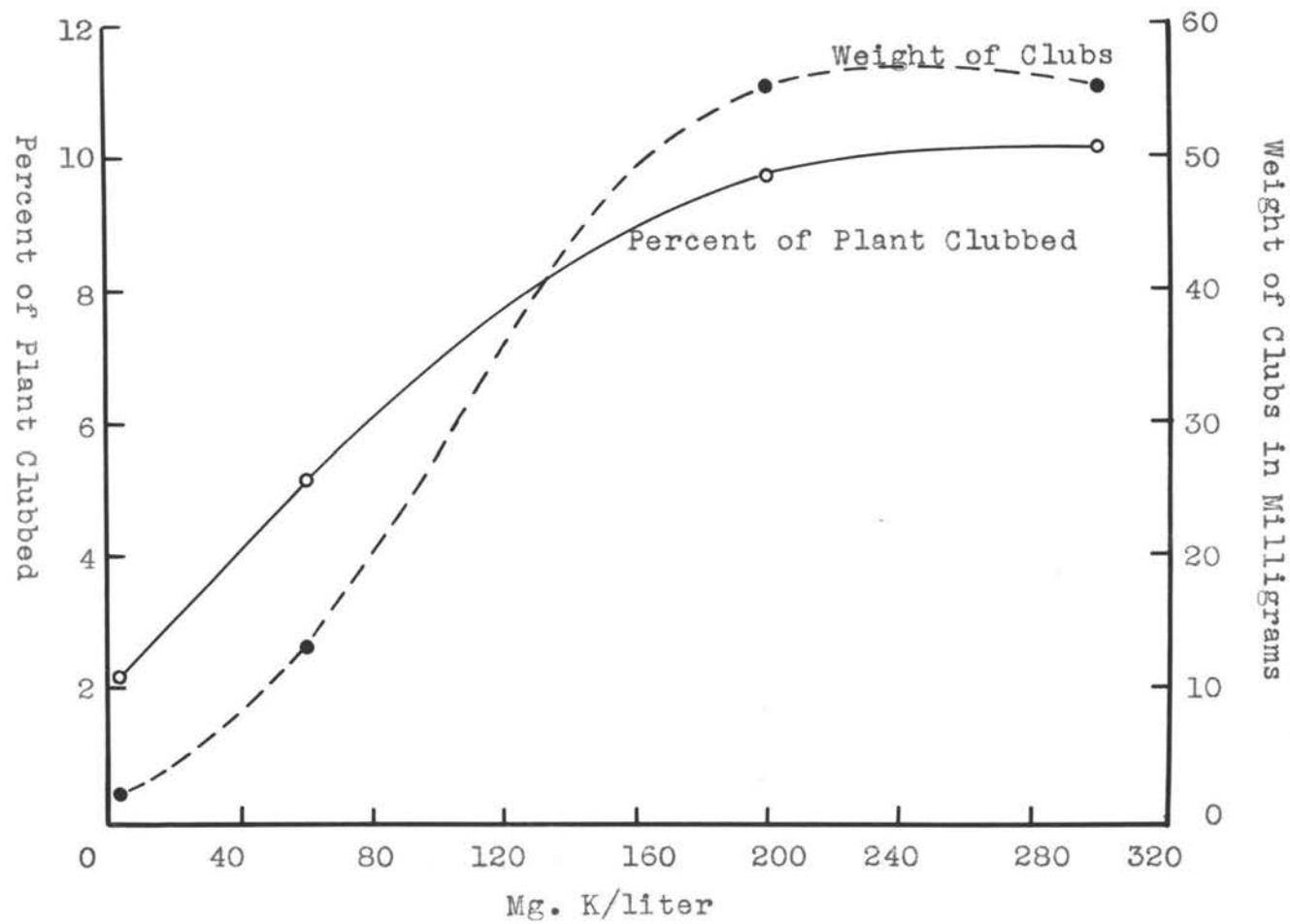


Figure 13. Effect of Potassium Concentration on Clubroot Development in Greenhouse.

weight of the clubs in each treatment and on the percentage of plant that was clubbed.

It is pertinent to note here that as the concentration of potassium was increased, both the incidence of infection and subsequent clubroot development increased.

Effect of Hydrogen Ion Concentration on Clubroot Development

Experiments were carried out to determine the effect of pH on clubroot development. Four levels of pH were used i.e., 4.8, 5.9, 6.8 and 7.5. The data show that maximum clubroot development occurred in the acid pH range (Figure 14). A pH level around neutrality or above markedly reduced clubroot development. The pH range for maximum clubroot development was in the same range as that for maximum infection (Figure 11). A pH of around 7.0 or above reduced both infection and clubroot development of plants growing in sand cultures in all of the experiments carried out.

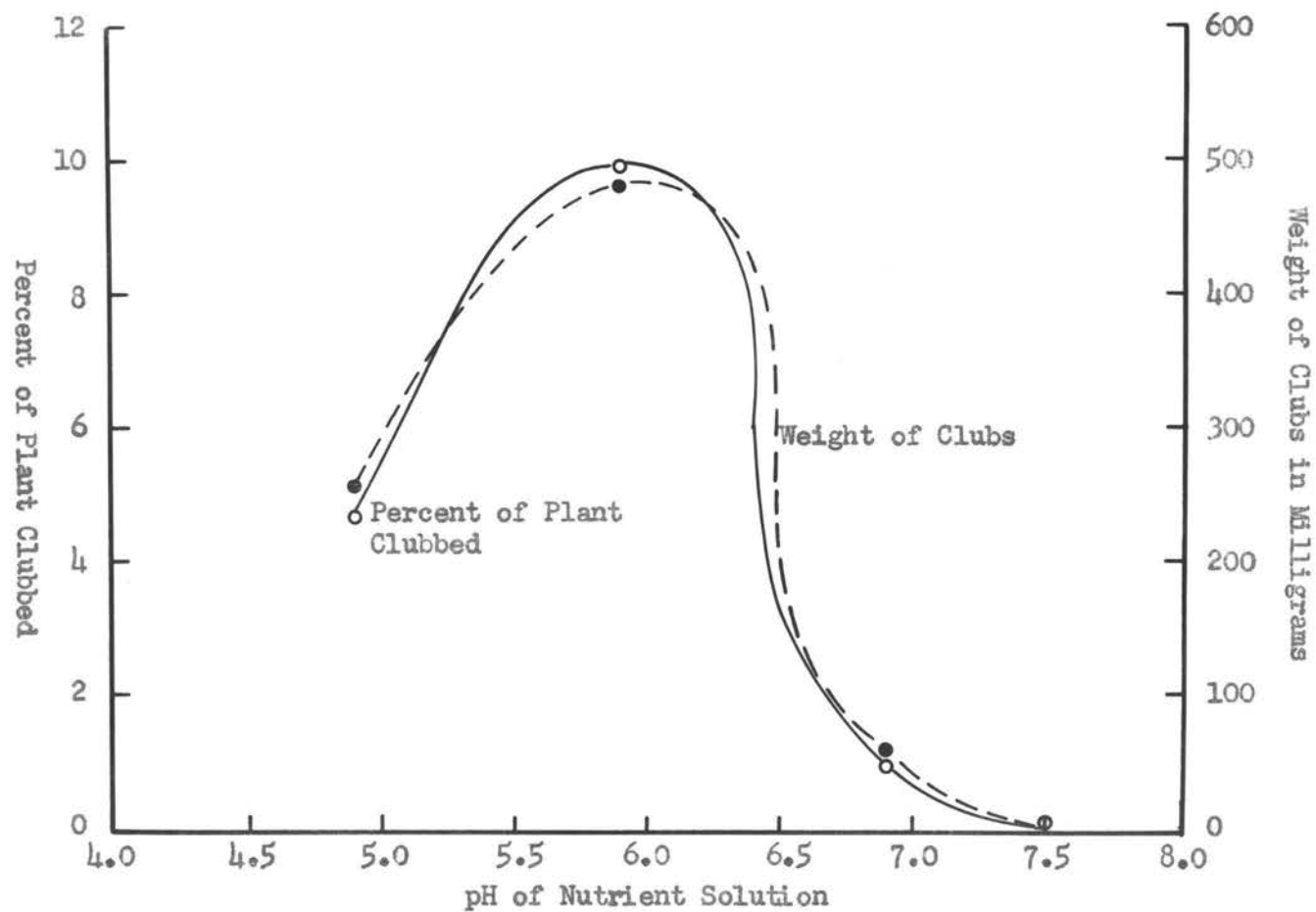


Figure 14. Effect of pH on Clubroot Development in Greenhouse.

DISCUSSION

The economic importance of clubroot has motivated considerable experimentation on various aspects of the clubroot disease. A great deal of the work has been concerned with control of the disease with lime. One of the most common control measures employed is to add sufficient lime to soil to raise the pH to 7.2. This is not always an effective means of control as Wellman (49) demonstrated experimentally, and as many farmers and home gardeners have probably known for centuries. When lime is effective in controlling clubroot, it usually proves to be economical and often improves the texture of the soil. An understanding of the mechanisms involved in clubroot control and of the effects of various elements alone and in combination on clubroot development are basic to the development of a consistently effective control program.

Calcium

At least two possible effects of lime on clubroot are obvious from this investigation. First as has been observed many times in field and greenhouse experiments lime influences the clubroot disease by affecting the soil pH. Secondly that calcium may be involved in decreasing the severity of clubroot when lime is added to the soil is suggested by the inhibitory effect of calcium

on initial infection. While concentrations of 30 to 40 milligrams of calcium per liter stimulated the initial establishment of the pathogen in the host plant, only slightly greater concentrations were inhibitory. The difficulty of properly interpreting effects of various concentrations of nutrient elements is well illustrated by the fact that quantities of calcium that inhibited infection were actually stimulatory to club development when infection had already occurred. Clubroot development progressively increased with an increase in the calcium concentration up to 75 to 100 milligrams per liter with a reduction in development at concentrations above that range. Calcium influenced both initial infection and clubroot development in the only successful field experiment. The addition of hydrated lime made the pH of the soil more alkaline and the effect of pH as a factor in the field experiment should not be discounted as undoubtedly it influenced clubroot control. Hydrated lime, applied at the rate of 250 pounds per acre increased clubroot incidence. In nutrient sand cultures, initial infection was more sensitive to the level of calcium than was clubroot development.

It was beyond the scope of this investigation to determine the mechanism by which calcium exerts its effect

on initial infection and to a lesser extent on clubroot development. Calcium is involved, however, in many chemical and physical phenomena that occur in plants (5) (35). As calcium pectate, it is a major constituent of the primary cell wall. Calcium is known to influence both the chemical and physical properties of the protoplasm. For example, a high concentration of calcium alters the permeability of the protoplasm. Depending on the concentration present, calcium accelerates or depresses ion accumulation. Calcium is involved in many other essential processes that occur in plants e.g., mitochondrial formation, cell division, water uptake, cell wall extensibility, detoxication of excess hydrogen ions and certain enzymatic activities.

P. brassicae is an obligate parasite, living in a very close relationship with the protoplasm of the host. It would seem likely, therefore, that calcium influences the clubroot disease by upsetting the establishment of this close host-parasite relationship. Growth of the host was not inhibited by the low concentrations of calcium which inhibited initial infection. Therefore, the mechanism involved in inhibiting initial infection appears to be different from that involved in the inhibition of growth in length of roots and stems.

Potassium

According to McNew (30) potassium fertilizers have alleviated damage from more diseases than any other nutritional treatment. As shown by these investigations, however, and to a certain extent by others (36) (46), clubroot is an exception in that potassium stimulates this disease. When the concentration of potassium in the nutrient solution was increased there was a proportionate increase in both initial infection and clubroot development. This increase was evident up to approximately 240 milligrams of potassium per liter for both initial infection and clubroot development. At concentrations of potassium above 240 milligrams per liter infection diminished as the concentration of potassium increased. Clubroot development was not inhibited when 300 milligrams per liter were used. The usual level of potassium in a nutrient solution ranges from about 80 to 320 milligrams per liter, therefore potassium did not inhibit either initial infection or clubroot development at the levels usually used in nutrient cultures.

The results from analyzing diseased and healthy plants for potassium suggest a role potassium may play in this disease. The clubs of 6-week old plants contained more potassium than the normal roots of diseased plants and also more than the roots of healthy plants. Likewise

there was less potassium in the normal roots and tops of diseased plants than in the roots and tops of healthy plants. These data indicate that potassium has accumulated in the club at a greater concentration than in the rest of the plant. Either the potassium has moved to the clubs from the rest of the plant or else the potassium has moved into the club from the soil or from normal root tissue distal to the club and failed to move up the plant. Either of these possibilities would explain the results obtained.

Potassium is usually present at a greater concentration in a region of the plant where growth is occurring most rapidly. For example, the growing apex of a stem usually contains more potassium than the portion of the stem lower down which has stopped growing or is growing at a much slower rate. When the plants were 6-weeks old, the clubs were actively growing and potassium was being used in the processes that bring about growth. Six-day old plants that were recently infected and exhibited no swelling contained less potassium than healthy plants, which indicates that the higher concentration of potassium in the club is not due to the presence of the pathogen. The inference can be drawn from the results that the higher concentration of potassium in the club than in the rest of

the plant is probably due to the actively growing region present in the club and not due to the presence of the pathogen.

Boron

The absence of boron is known to cause many physiological diseases of plants and has been shown to be related to the incidence of several pathological diseases of plants (15). Rohde (39) apparently was the first worker to recognize the influence of boron on the clubroot disease. He applied borax to the soil and noted a reduction in the incidence of clubroot.

In the present investigation boron was shown to bring about a marked reduction in the initial infection of turnip roots by P. brassicae. Very low concentrations of boron, 1 milligram or less per liter, appeared to stimulate infection whereas 2 or more milligrams per liter resulted in a marked reduction in the number of infections. On the other hand growth of the roots as measured by their length was affected very little by quantities of boron up to 64 milligrams per liter while the growth in length of the tops was progressively reduced with an increase in boron concentration above 8 milligrams per milliliter. In a field trial boron was found to have no influence on the

disease except when small quantities of hydrated lime were added to the soil.

The possibility that boron may be toxic to the spores of the pathogen should not be overlooked as a possible explanation for reduction in infection when the concentration of boron is increased. The mode of action of boron was not investigated in this particular study. Boron is known to influence many processes that occur in plants (15). For example, cell division, translocation of carbohydrates, water uptake, ion antagonism, metabolism of pectic substances and many other processes are affected.

Other Elements

The effects of rubidium, cesium, strontium, barium, beryllium, and lithium on initial infection and plant growth were studied. Some of these elements were very inhibitory to infection. For example, less than 2 milligrams of beryllium per liter of nutrient solution resulted in a 50 percent inhibition of infection. The elements can be arranged in the following sequence according to their relative inhibitory effect on initial infection: beryllium > lithium > cesium > barium > rubidium > strontium. These elements are not essential minerals for plant growth, but are present in many agricultural soils and some of them are toxic to many plants and microorganisms.

If an element such as beryllium were present in the soil in an available state at a concentration that would inhibit initial infection but have no toxic effect on the host it might prove an important factor in clubroot control. The effect of these elements may be directly on the pathogen rather than on the host parasite relationship. For example, they may inhibit spore germination.

SUMMARY

1. Some aspects of the effects of mineral nutrition on initial infection of Shogoin turnip plants by Plasmodiophora brassicae Wor. and subsequent clubroot development were investigated.

2. Initial infection was stimulated with increasing concentrations of calcium up to 40 milligrams per liter and clubroot development was stimulated at concentrations of calcium up to 100 milligrams per liter. At slightly higher concentrations than these both infection and clubroot development were reduced. Growth of the host was not inhibited at the same concentrations.

3. When the level of boron in the nutrient solution was high, calcium was more inhibitory than when the boron level was low or when no boron was added.

4. Two milligrams of boron per liter of nutrient solution markedly reduced initial infection while at least 8 milligrams of boron were required to reduce plant growth noticeably.

5. In a field trial hydrated lime, applied to the soil at the rate of 4000 pounds per acre gave good control of clubroot while hydrated lime at the rate of 250 pounds per acre stimulated the disease. Boron influenced disease control only when small quantities of hydrated lime had been added to the soil.

6. Both infection and clubroot development were stimulated with increasing concentrations of potassium up to 280 and 300 milligrams per liter respectively.

7. A greater quantity of potassium was found in clubs than in the rest of the plant or in a healthy plant, whereas in a recently infected plant in which no swelling had occurred, less potassium was found in infected roots than in healthy roots.

8. An acid pH range was optimum for both initial infection and subsequent clubroot development.

9. Several elements, that are not considered to be nutrients for plant growth, were tested against initial infection. All of them except strontium showed inhibitory action at low concentrations. The decreasing order of inhibitory activity was as follows: beryllium > lithium > cesium > barium > rubidium > strontium.

10. Possible mechanisms involved in the effect of various elements on the clubroot disease are discussed.

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