Effect of Calcium & Boron Nutrition of Flax on Fusarium Wilt

ABSTRACT

M.Sc. Eunice Keane Plant Pathology

Flax plants inoculated with Fusarium oxysporum f. lini and grown in 0 boron solutions developed very severe wilt irrespective of the calcium levels supplied to the plants. Plants given 40 ppm calcium developed more severe wilt at 0 and 2.5 ppm than at 0.5 ppm boron. Wilt severity in plants at 0.5 or 2.5 ppm boron decreased with increasing calcium from 40 to 480 ppm.

Plants grown in boron deficient solutions before inoculation developed more severe wilt than plants transferred from normal to deficient solutions after inoculation. Plants deficient in calcium after inoculation developed more severe wilt than those deficient before inoculation.

F. oxysporum f. lini grew rapidly in xylem of plants inoculated when 21 days old regardless of the nutrient level. When inoculations were made at 28 days its growth was restricted in plants given high (480 ppm) calcium and normal (0.5) or high (2.5 ppm) boron.

THE EFFECT OF CALCIUM AND BORON NUTRITION OF FLAX ON FUSARIUM WILT

by

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A

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I. INTRODUCTION

Sackston and Laganière (1964) reported on the occurrence of an apical wilt of flax plants in a field near Magog, Quebec, in 1962. The symptoms of this disease closely resembled those of calcium and/or boron deficiency. The fact that a deficiency of these elements in Quebec soils had been reported, led them to investigate in greenhouse experiments, the effects of different levels of these elements on the growth of flax plants. Calcium deficiency produced a collapse and necrosis of the shoot tips, the typical withertop disease symptoms observed by Millikan (1944) and boron deficiency, a stunting and necrosis of root and shoot apices (Laganière and Sackston, 1967).

In 1964 outbreaks of Fusarium wilt on Marine flax were reported in Quebec, near Montreal (Sackston and Laganière, 1964). This variety of flax was known to be resistant to wilt in other flax growing regions of Canada, and it was assumed that the hot dry summer had influenced the susceptibility of the plants to infection. It is also possible that there may have been some correlation between the decreased resistance to wilt, and the calcium and boron deficiency in Quebec soils.

This thesis involves an investigation of the effect of calcium and boron nutrition of flax on the development of Fusarium wilt. Plants were grown in nutrient solutions of varying calcium and boron composition, and the effect of these elements on the growth, and resistance and susceptibility of the host, the aggressivity and invasiveness of the pathogen, and pathogenesis, were studied. The influence of the nutritional status of the host before and after infection, on disease development, was also investigated.

II. LITERATURE REVIEW

A. History and Economic Importance of Fusarium Wilt of Flax

At the end of the nineteenth century, there was a recession in the development of the flax fibre industry in Northern Europe and North America, as a result of the devastation of flax crops. The causal agent was then unknown, but the destruction had been attributed to "flax sickness" of the soil. Lugger (1890), a Belgian agriculturalist, in his appraisal of the situation said, "It is well known in Europe that Flax is unkind to its own offspring". In Belfast, Ireland, J.B. Taney (1895) reported that "Ireland had been flaxed out", while in North America, the consensus of opinion was that "flax makes heavy growth on virgin soil, but the crop fails gradually after persistent culture".

Some additional explanations for this "flax sickness" were: a depletion of soil nutrients; that flax is unkind to flax, leaving some substance in the soil which is detrimental to the health of subsequent crops; and that it was caused by an infectious disease.

Lugger (1890) working at the Minnesota Experimental Station attempted to control the disease and to identify the causal agent. He applied various fertilizers

to the soil, but obtained no effective control of the disease, nor did he identify the cause. His experiments proved, however, that, "Exhaustion of the soil by previous flax crops could not be the cause of the disease, as all of the nutrients formerly removed by the plants had been replaced in a soluble state". In subsequent experiments, he used fungicides which had been effective in controlling other crop diseases. These included sodium hypophosphate, lime, sodium sulphide and copper sulphate. Again, failing to obtain control, he concluded that, "It is not a specific vegetable disease which affects the plants, because one or other of the fungicides would have shown the effects of its application. A cure for the disease does not seem possible". When he observed that the disease was most severe in areas where flax straw had accumulated, he proposed that, "We have not to deal with a disease, but the straw of flax itself is the cause of the trouble."

In 1900-1901, H.L. Bolley recorded some symptoms of the disease on flax plants in fields at the North Dakota experimental station (Bolley 1901, 1902). These included a pre-emergence killing of seedlings, seedling blight, chlorosis and necrosis of mature plants and root rot. He observed that the disease affected large numbers of plants over extensive areas, and that it spread rapidly and

uniformly through infected fields. The plants sometimes showed a unilateral blighting, and the areas of most severe disease were located near plant debris, and in fertile and highly alkaline soils. These observations in collaboration with intensive experimentation led him to isolate a fungus from infected flax plants and from the soil. He described the fungus as Fusarium lini. He established that the pathogen was a soil inhabiting facultative parasite, which infected the host through its roots, and that it was also disseminated on infected seed. For the control of the disease he recommended various soil and seed treatments, crop rotation, and proper cultural practices, all of which effected a marked decrease in the severity of the disease, but did not eliminate the problem (1906).

Bolley noticed that some flax plants grown on "flax sick soil" showed no symptoms of flax wilt. He selected these resistant plants, and in cooperation with other North American workers bred the wilt resistant varieties Bison and Buda, which were subsequently shown to be resistant to other diseases affecting flax, and to retain their resistance under different environmental conditions (Bolley, 1907).

In 1941, Snyder and Hanson grouped all Fusaria causing vascular wilts of plants under Fusarium oxysporum,

and each pathogen specific to a given host was designated by a trinomial. The pathogen causing wilt of flax is now called Fusarium oxysporum f. sp. lini (Bolley) Snyder & Hanson (1941).

The disease has virtually disappeared from the important flax growing areas of the world, and plants showing symptoms of wilt are observed only in years when a hot dry growing season favours the growth of the pathogen, and predisposes the host to infection (Flor, 1964). Reduction in the yield of wilt resistant oilseed flax, due to fusarial infections is difficult to assess because these infections produce no visible disease symptoms, though Fusarium lini is often isolated from apparently healthy flax stems. Of the diseases affecting flax in North America, wilt causes the least significant damage (Flor, 1964).

B. The Relationship between the Nutritional Status of the Host Plant and its Resistance and Susceptibility

The control of disease by the use of resistant varieties is not always accomplished if there is a deficiency, excess or imbalance of any of the essential nutrients in the growth medium or in the plant tissues. According to Stakman and Harrar (1957), the effects of

different nutrient elements on pathogenesis are most pronounced in plants that are neither highly resistant nor highly susceptible. Mineral deficiencies of any kind may reduce vigour and make plants more susceptible to some diseases, and less congenial to others. The fate of the invading parasite is determined by the nutritional and inhibitory environment within the host tissue.

Garber's nutrition-inhibition hypothesis postulates that in the absence of an effective inhibitory environment, i.e., in susceptible hosts, a parasite in an adequate nutritional environment will in general be virulent. In the presence of an effective inhibitory environment, i.e., in a genetically resistant host, the parasite will be avirulent, though a nutritional imbalance which either favours the invasiveness of a pathogen or reduces the vigour of the host will predispose the host to disease. Conversely, a nutritional environment which increases host plant vigour but is inimical to the pathogen, will in general increase the resistance of the host to disease (Garber, 1956).

Yarwood (1959), in his studies of factors which predispose plants to disease, defined predisposition as the tendency of non-genetic conditions, acting before infection, to affect the susceptibility of plants to disease. If plants grown in different nutrient regimes before infection

and then transferred to similar regimes after infection show any differences in disease development, predisposition may be inferred.

C. Role of Calcium and Boron in Disease

The literature contains no references to the mineral element nutrition of flax and its possible relationship to Fusarium wilt. For this reason, references to similar diseases in other host plants will be cited.

1. Effects on the pathogen

The effects of different nutrient elements on pathogenesis are largely a function of their effects on the host rather than on the pathogen (Stakman and Harrar, 1957). This may be due partly to the fact that microorganisms, fungi in particular, have mineral requirements different from those of higher plants. Calcium and boron, for example, are of major importance in higher plant nutrition, but are apparently non-essential for the growth of fungi.

In 1928, Davis, Marloth and Bishop, using calcium and boron salts which had been recrystallized and purified, showed that when Aspergillus niger and Penicillium italicum

were grown in media deficient in calcium, there was a significant decrease in yield as compared with cultures grown on non-deficient media. Spore formation was shown to be dependent on the presence of the calcium ion in the medium. In the absence of boron, Dothiorella sp. showed a 50% decrease in yield as compared with the yields obtained from cultures grown on medium prepared from unpurified salts. When the organism was grown on medium containing 1.5 ppm boron, the yield increased to 90%. They concluded that calcium and boron did not merely stimulate growth and sporulation of the fungi, but were essential for their proper growth and development. Their findings were not supported by subsequent investigations.

Steinberg (1946) showed that growth and reproduction of A. niger were not retarded when the culture medium was deficient in calcium and boron. Foster (1949) also found that these elements were not essential for fungi, and observed a wide variation among species and strains with respect to their tolerance to boron. Aspergillus niger seemed to be more sensitive to boron during spore germination and the early stages of growth than in the later stages of development.

Bowen and Gauch (1967) studied the boron requirements of a number of fungi including Saccharomyces

cerevisiae, Aspergillus niger, Neurospora crassa and Penicillium chrysogenum. They concluded that boron was non-essential because they obtained equal growth rates for cultures grown in boron deficient media and those grown in media with as much as 5 mg/l of boron.

The yields of cultures of N. crassa containing up to 100 mg/l boron, did not differ significantly from those of the zero-boron controls. Boron at 250 mg/l significantly reduced the yields as compared with the controls. Similarly boron was non-essential for A. niger, but at 100 mg/l the formation of pigmented conidia was inhibited, and at 1,300 mg/l, the yield was reduced significantly.

The toxicity of high boron concentrations to Saccharomyces cerevisiae was due specifically to the inhibition of aldolase. Thus the cells were unable to utilize carbohydrates at a rate sufficient to maintain the metabolic processes involved in growth and reproduction.

According to Woltz and Jones (1968), boron deficiency in the culture medium increased the growth, sporulation and virulence of Fusarium oxysporum f. lycopersici, as compared with control cultures receiving 0.1 ppm boron.

Corden (1965) observed in in vivo studies that a calcium concentration of 380 µg/ml in the xylem elements of tomato stems was toxic to F. oxysporum f. lycopersici.

The toxicity was attributed to inhibition of the activity of fungal polygalacturonase. These results were substantiated by in vitro studies, in which similar concentrations of calcium had similar inhibitory effects. He proposed that the inhibition of fungal polygalacturonase, which may normally function to provide a carbon source (host galacturonic acid) for the pathogen, could account for the observed retardation of growth of the pathogen in its host.

2. Effects on the host

Boron is known to function primarily in the differentiation and maturation of the plant cell. It is a non-labile element, and although it is required for the formation of plant tissues, it is apparently not required for the maintenance of tissues already formed. Gauch and Dugger (1952) have proposed that the major function of boron is in the translocation of sugars. One role involves the reaction of boron with sucrose to form a sugar-borate complex which moves through the cellular membranes more readily than non-borated, non-ionized sugar molecules. Sucrose in combination with 5 ppm boron was shown to increase the respiratory rate of pea root tips by 35% as compared with root tips treated with sucrose alone. They postulated that the increased respiration rate may have

been the result of an increase in the movement of sucrose to the respiring cells.

A boron deficiency in a plant, therefore, may prevent the translocation of sugars to the growth sites. Typical symptoms of boron deficiency include an initial chlorosis of the root and shoot apices, stunting of the primary shoots, purpling of the veins in some leaflets, production of numerous secondary shoots, and eventually necrosis of the shoot apices (Laganière and Sackston, 1967, and Edginton and Walker, 1958). Boron toxicity produces a marginal and tip necrosis first of the lowest leaves, but progressively spreading to the upper leaves (Millikan, 1948, and Laganière and Sackston, 1967).

Lee and Aranoff (1960) reported changes in the subcellular structure of sunflower seedlings approximately three days after their exposure to boron deficient solutions, and before any macroscopic deficiency symptoms had appeared.

The first changes were observed in the chloroplasts which appeared to contain larger volumes of starch than non-deficient cells. They suggested that this starch accumulation may have been due to a decrease in the enzymatic conversion of glucose-1-phosphate to starch. There was an increase in the number of mitochondria, and after six to seven days the nucleus was seen to contain rhombohedral osmophillic structures. The cell walls thickened

and became serrated. Finally, no subcellular structures were recognizable, and the cells were completely filled with unidentifiable structures.

Marinos (1962) observed similar microscopic changes in calcium deficient cells of tomato plants. At first, there was a vesiculation of the nuclear envelope, followed by swelling of the plasma membranes, the disorganization of other cell structures (mitochondria and golgi apparatus) and the appearance of dense structureless inclusions.

Calcium, like boron, is essential for normal cell function, and its various effects may be dependent on some fundamental structural role. It is somehow involved in the structure of and/or the maintenance of the plasma and vacuolar membranes. These membranes govern the permeability behaviour of the cell. A calcium deficiency in flax plants results in the necrosis and collapse of the shoot tips, a symptom described as "withertop disease" (Millikan, 1944).

A direct and intimate relationship between the absorption, accumulation and utilization of calcium and boron by plants has been reported. Brennan and Shive (1948) observed that an increase in the calcium level in nutrient solutions in which tomato plants were grown, tended to accentuate the symptoms of boron deficiency. At high boron

and calcium levels, there was a marked decrease in both the total and soluble boron in the tissues, and an increase in the severity of boron toxicity symptoms. These effects have been demonstrated in flax by Millikan (1948) and Laganière and Sackston (1967).

There is well documented evidence which suggests that an increase in disease resistance in some plants may be associated with an increase in the calcium and boron content of their tissues, and conversely, that a deficiency of these elements results in increased susceptibility to disease.

Corden (1965) showed that Fusarium wilt of tomato, caused by F. oxysporum f. lycopersici was increased by deficient calcium and decreased by excess. Deficient calcium after infection, regardless of the pre-inoculation calcium nutrition, significantly increased the severity of wilt disease and the growth of Fusarium in the host stems. Deficient calcium before inoculation, followed by normal calcium nutrition, significantly reduced disease. He suggested that increased wilt induced by calcium deficiency was not the result of a predisposition of the tomato plant to disease, but rather a stimulation of pathogenesis. Adequate calcium after infection interfered with pathogenesis by limiting the growth of the pathogen in the host

through the inhibition of fungal polygalacturonase. A calcium concentration of 380 µg/ml inhibited the enzyme activity about 93%, as compared with 58% for 73 µg/ml (level of deficiency in the host plant). Calcium, therefore, will slow down polygalacturonase activity, but does not block it completely, and susceptible plants will eventually succumb to disease.

Corden and Edgington (1960) reported a correlation between the presence of growth regulators and high calcium content in tomato plants, and their resistance to Fusarium wilt. Resistance to wilt was induced in plants treated with naphthalene acetic acid and 1000 ppm calcium. They postulated that increased resistance was associated with the ability of plant growth regulators to inhibit root elongation, and that these growth responses are physiologically related through increased calcium bonding of the pectic substances of the plant cell walls. Calcium pectates decrease cell wall plasticity (i.e., inhibit root elongation) and render cell wall materials relatively resistant to hydrolysis by fungal pectic enzymes. In the absence of sufficient calcium, rigid cross linkages fail to form and the pectic materials remain susceptible to fungal enzymes. If excess calcium is present, calcium bonding is enhanced if the number of bonding sites is increased by growth regulator treatments.

This evidence was supported by the observations of Bateman and Maxwell (1965), who found that the reaction of bean hypocotyls of different ages to infection by Rhizoctonia solani was dependent on the formation of calcium pectates in advance of the pathogen. Young susceptible bean hypocotyls had a low calcium content and highly methylated pectic compounds. Older, more resistant hypocotyls had a higher calcium content and their pectic compounds had a lower methoxyl content. Bateman and Maxwell postulated that the formation of calcium pectates in maturing tissue could account for their resistance to enzymatic maceration by the pathogen. Natural resistance was associated with the conversion of pectin to calcium pectate during hypocotyl maturation.

Wills and Moore (1969) reported that black shank of tobacco incited by Phytophthora parasitica var. nicotianae was greatly retarded when resistant tobacco varieties were supplied with nutrient solutions containing 25-50 ppm calcium, as compared with disease development in plants receiving 250 ppm calcium. The latter concentration is the optimum for the growth of tobacco. When inoculation was delayed for 24 hours after the transfer of plants from a medium containing 250 ppm calcium to one containing 25 ppm calcium, disease was less than when there was no time lapse

between transfer and inoculation. They suggested that the expression of the low calcium effect was dependent on the composition of the medium at the time of inoculation, and that this effect may induce increased membrane permeability with a consequent decrease in disease. These conclusions do not agree with those obtained by other workers.

According to Thatcher (1939, 1942) increased permeability of the plasma membranes or host cytoplasm would make nutrients freely available to the invading parasite and result in susceptible reactions, whereas marked decreases would restrict the nutrient supply and result in resistance. Williams and Keen (1967) reported that the youngest and oldest leaves of cucumber plants are most resistant to angular leaf spot, and also exhibit the lowest inherent permeability.

Doupnik (1968) found that when the first young leaves of susceptible oat varieties were treated with victorin solutions which contained 0.1 M calcium salts, symptoms of Victoria blight failed to develop. These leaves under the same conditions released smaller quantities of electrolytes, i.e., were less permeable, than those which were treated with victorin solutions that contained no calcium salts.

Boron compounds have been used for many years to control deficiency diseases. Brenchley (1937) reported that injection with boron solutions, or the application of boron sprays to foliage, gave 100% control of corky core and drought spot of apples.

Deficiency of this element in plants, not only has induced numerous physiological diseases but has rendered deficient plants readily susceptible to infectious diseases which may not have affected non-deficient plants.

Edgington and Walker (1958) found that boron and calcium, separately and in combination with each other, exerted marked effects on the development of Fusarium wilt of tomato caused by Fusarium oxysporum f. lycopersici. When tomato plants were grown in nutrient solutions containing 100 ppm calcium, the disease index decreased significantly with increasing boron from 0.001 to 0.25 ppm. With calcium at 5 ppm, disease was very severe at both 0.001 and 10 ppm boron, and at 500 ppm calcium the index increased consistently with increase in boron from 0.001 to 0.25 and 10 ppm. They found in addition that calcium concentration in the substrate did not affect boron uptake quantitatively, and that the influence of boron on disease was dependent on the calcium supply.

III. MATERIALS AND METHODS

A. Seed Germination

Flax seeds, variety Marine, harvested in September 1967 and obtained from the Canada Department of Agriculture Research Station, Morden, Manitoba, by the courtesy of Dr. Kenaschuk, were used in experiments from January 1968 to May 1969. The seeds were stored in a cold room at 6°C.

The germination procedure was the same for all experiments. Seeds were surface sterilized in a 3% chlorine solution and soaked in sterile distilled water for six hours. They were then placed on the surface of washed silica sand contained in perforated flats lined with newsprint. The flats were placed in larger aluminium trays containing sufficient water to keep the sand moist. High humidity was maintained by covering the trays with polyethylene sheets.

As soon as the cotyledons emerged, the trays were transferred to a growth chamber maintained at 100% relative humidity and 21°C. Seedlings were provided with light from fluorescent tubes which supplied a light intensity of 700-800 ft-c. The humidity of the chamber and the level of water in the trays were lowered progressively as the hypocotyls elongated, in order that

the roots of the seedlings would elongate in the direction of the increasing water gradient. After 5 days, the seedlings were transferred to the nutrient solutions.

B. Liquid Cultures

1. Nutrient solutions

All solutions were prepared from salts of Reagent grade and the distilled water used in their preparation was obtained from a stainless steel still, to avoid contamination from metal ions.

Plants were supplied with Hoagland's solutions (Hoagland and Arnon, 1950) or modifications thereof. Boron and calcium were used at three different levels, and the nine possible combinations of the elements at these levels are given in Table 1. When the concentration of calcium supplied as $Ca(NO_3)_2$ in Hoagland's solution was decreased, the nitrate was kept constant by adding $NaNO_3$. $CaCl_2$ was added to give high levels of calcium.

The composition of the normal Hoagland's solution was as follows:

Major Elements	cc/l
KH_2PO_4	1.0
KNO_3	5.0
$Ca(NO_3)_2$	5.0
$MgSO_4$	2.0

TABLE 1. Calcium and Boron Composition of Nutrient Solutions

	Ca low		Ca normal		Ca high	
	Ca ppm	B ppm	Ca ppm	B ppm	Ca ppm	B ppm
B low	40.0	0.0	200	0.0	480	0.0
B normal	40.0	0.5	200	0.5	480	0.5
B high	40.0	2.5	200	2.5	480	2.5

Minor Elements	ppm
H_3BO_3	0.5
$MnCl_2$	0.5
$ZnSO_4.7H_2O$	0.02
$CuSO_4.5H_2O$	0.05
$H_2MoO_4.H_2O$	0.01

Iron was supplied as EDTA and was prepared by the method recommended by Hewitt (1966). It was added at the rate of 1 cc/l every two days. The pH of the nutrient solutions was adjusted to 6.2 by adding 1.00 N NaOH.

All solutions were stored in polyethylene containers in the dark at 25°C.

The solutions were aerated from a vacuum pressure pump, "Little Giant", Model No. 13152, supplied by the Gelman Instrument Company. It pumped air through a rubber tubing line to 3-way valves, from which the air was conducted by a short length of rubber tubing to plastic straws which passed through ¼" holes in the plant supports and dipped into the nutrient solutions in the containers. Plastic straws were used instead of glass tubing to avoid boron contamination of the solutions.

Solutions were aerated for 3 hours daily during the early stages of plant growth, but the period was extended as the plants matured. Mature plants were aerated

for 6 hours daily. Solutions were changed at 4-day intervals. The volume was restored to the 1-litre mark with distilled water as water was absorbed by the plants or lost by evaporation.

2. Containers

Flax plants were cultured in polyethylene containers of one liter capacity, fitted with covers of marine plywood. The translucent containers were coated with two layers of aluminium paint in order to inhibit the growth of algae in the nutrient solutions. Before every experiment the containers were washed with a hot soap solution and rinsed thoroughly in distilled water.

3. Plant supports

The apparatus used for supporting the flax plants in the nutrient solutions is illustrated in Figure 1. These were made from 3/4" Marine plywood and cut into 5 x 5" rectangles. Each support, which also provided a cover for the containers, had six holes, five of 1" diameter in which the seedlings were inserted, and one of $\frac{1}{4}$" diameter for the aeration system. Each of the larger holes was

fitted with a size 2 one-hole rubber stopper which was cut vertically through the centre and pieces of polyurethane of ½" thickness placed on the cut rubber surfaces (Fig. 2). The seedlings were placed between this rubber-polyurethane "sandwich" and inserted into the holes of the covers so that their roots dipped into the nutrient solutions.

This method of supporting the plants proved superior to other methods. Because polyurethane is flexible, it contracts as the hypocotyl expands, thus providing rigid support for seedlings as well as for mature plants. After the covers had been washed, rinsed and dried, they were impregnated with hot paraffin which, when it cooled, left a thin film of wax on the covers. This protected them from becoming soaked with the nutrient solutions.

C. Culture of Fusarium oxysporum f. Lini

F. oxysporum f. lini was isolated in pure culture from diseased plants obtained from the Plant Pathology experimental plots at Macdonald College. Stock cultures were maintained on potato dextrose agar slants in test tubes at 6°C.

For the preparation of inoculum, the organism was subcultured on P.D.A. in petri dishes for 5 days and disks taken from the advancing edge of the colony were

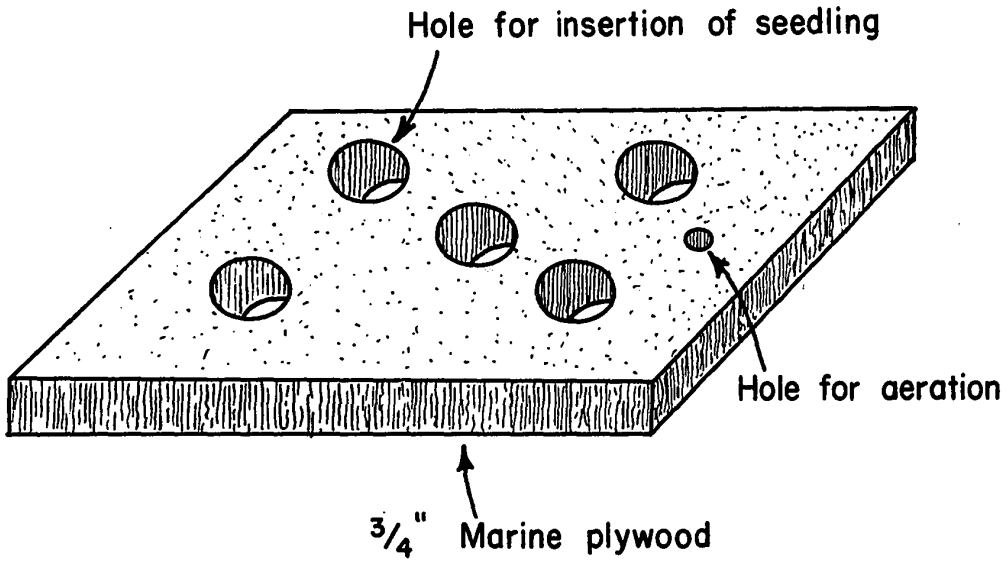


Fig. 1. Diagrammatic representation of apparatus for supporting plants.

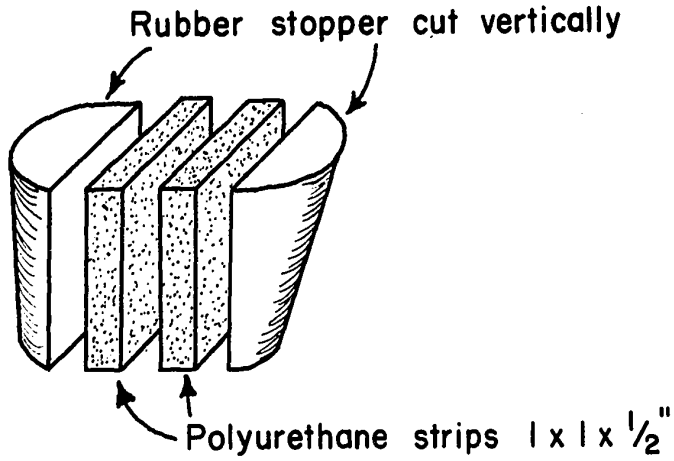


Fig. 2. Diagrammatic representation of rubber-polyurethane "sandwich".

used to seed a potato dextrose liquid medium. The medium was prepared in the following manner:

250 g of peeled, sliced potatoes were steamed for one hour in 500 ml of distilled water in an autoclave, after which the liquid was filtered through cheesecloth and the volume adjusted to 1000 ml with distilled water. 20 g of dextrose were added per liter of solution. 100 ml aliquots of the solution were poured into 500 ml erlenmeyer flasks, which were plugged with cotton wool, and autoclaved at 15 psi for 20 minutes. The cooled medium was then inoculated, and the fungus allowed to grow on a rotary shaker for 7 days at 25°C in the dark.

D. Method of Inoculation

The spore/mycelium suspension used to inoculate the flax plants in all experiments contained 3.52×10^4 spores/ml of solution.

The plants, held firmly in their support, were removed from the nutrient solutions and the roots dipped for 5 minutes in the inoculum. The plants were then replaced in their respective solutions which were not renewed until after four days. This time period allowed for adequate infection of all inoculated plants. After 4 days, the mycelium adhering to the plant roots was removed by washing the roots in a stream of tap water.

E. Disease Measurement

At the end of each experiment, the plants were removed from their supports by cutting them at the crown, just above the first secondary roots. Measurements of shoot height were taken from the cotyledons to the tips of the primary shoots. The fresh weights of roots and shoots were also recorded.

Disease intensity was evaluated by two methods:

(1) Sections taken 10 mm from the crown were examined for vascular discolouration. This method was recommended by Dimond et al (1952) and has given valid disease estimates for Fusarium wilt of tomato. Each section was rated on a scale of 0-3, where 0 = no discolouration, 3 = severe discolouration. The disease percentage was calculated from the formula,

$$\% \text{ disease} = \frac{nxv}{ZxN} \times 100$$

where n = no. of plant sections examined,

v = numerical rating for each section,

Z = highest numerical rating on scale,

N = total no. of plants examined.

(2) The number of chlorotic and necrotic leaves on each plant was determined as a fraction of the total number of leaves. The amount of disease for each plant was obtained by multiplying this fraction by 100; i.e.,

$$\% \text{ disease} = \frac{\text{no. of chlorotic and necrotic leaves}}{\text{total number of leaves/plant}} \times 100$$

An average of the disease percentage for each treatment was obtained from an assessment of the five plants grown in each container.

The effect of calcium and boron nutrition on the advance of Fusarium in flax stems was determined by cutting each plant from the crown to the apex into 5 mm sections, surface sterilizing each section in 3% chlorine solution, and plating them on P.D.A. For each treatment, the number of sections from which the fungus was reisolated, was plotted against the height attained by the fungus, to give an estimate of the amount of infection, and the invasiveness of the fungus.

F. Experimental Conditions

Preliminary experiments were conducted in a greenhouse at approximately 24°C. Because there was inadequate temperature control in the greenhouse, all subsequent experiments were conducted in controlled environment chambers. The environmental conditions were as follows:

Light intensity	2000 ft-c
Humidity	55%

Temperature	
-Preinoculation	21°C Day, 21°C Night
-Postinoculation	23°C Day, 21°C Night
Daylength	15 hours light, 9 hours darkness

The objective of the first experiment was to establish the pathogenicity of F. oxysporum f. lini to Marine flax.

Sterile greenhouse soil contained in 6" pots was inoculated with a spore and mycelium suspension of the fungus and after 7 days was seeded with surface sterilized flax seeds. Seedlings emerged after approximately 14 days, and showed the first wilt disease symptoms after 28 days. The plants were harvested after 42 days and surface sterilized sections of the crown, lower stem and upper stem were plated on P.D.A. in petri dishes.

In the second preliminary experiment, 5-day-old seedlings were transferred from the silica sand to nutrient solutions of varying calcium and boron composition. The plants were allowed to grow to maturity and the symptoms of nutrient deficiency and excess were observed throughout the growth period.

Three major experiments were conducted. For these, the experimental plots were arranged in a completely

random design. In each experiment there were five plants for each treatment and two replicates/treatment, giving a total of 72 plots, of which 36 were inoculated and 36 remained as the uninoculated controls.

In the first of these experiments, seedlings were grown in solutions with the calcium and boron regimes described in Table 1, for 14 days. Eighteen plots, two for each treatment were then inoculated and a similar group of plants used as uninoculated controls. An equivalent number of plants were inoculated on the 21st day. The plants were harvested 14 days after inoculation.

In another experiment, some plants were grown in complete solutions for 14 days, inoculated and then transferred to altered nutrient solutions. A corresponding group of plants were grown for the same time in altered solutions and then transferred to complete solutions after inoculation. This experiment was terminated on the 35th day.

In the final experiment, plants were treated in a manner similar to that described for the above experiment, except that they were inoculated on the 21st day, and the experiment was concluded after 42 days.

All experimental data was analysed by the Analysis of Variance (Snedecor, 1956) and Duncan's multiple range test (Steel and Torrie, 1960).

IV. EXPERIMENTAL RESULTS

A. The Effect of Calcium and Boron Composition of the Nutrient Solutions on Disease Development

1. Pre-inoculation symptoms

a) Boron deficiency and excess

Flax plants grown in boron deficient solutions showed the first deficiency symptoms after approximately 10 days. Foliar symptoms included rosetting and chlorosis of the apical leaves and mild chlorosis of the cotyledons and lowest leaves. The stems had short internodes and the leaves were abnormally enlarged with a purplish discolouration of the tips. The chlorosis of the apical leaves became progressively more severe, and after about 14 days, the tips of these young leaves became necrotic. At about the same time, numerous secondary branches were produced at the cotyledonary level on the primary shoots.

Root symptoms appeared earlier than foliar symptoms. After 4-5 days, the roots of all boron deficient plants became necrotic at the apices. This necrosis of the tips apparently stimulated the production of numerous lateral roots, but these soon aborted. The roots were buff-coloured with numerous stunted rootlets, as compared with the cream

coloured elongate roots of plants grown in complete nutrient solutions. A necrosis of the crown region was also observed in all boron deficient plants.

Boron toxicity symptoms were apparent after ten days in plants supplied with 2.5 ppm boron. The symptoms appeared first on the lower leaves and cotyledons as a marginal and tip necrosis, which eventually involved the entire leaf. There were no obvious symptoms of boron toxicity on the plant roots.

b) Calcium deficiency and excess

Foliar symptoms of calcium deficiency appeared approximately 14 days after the seedlings had been transferred to deficient nutrient solutions. These plants showed only a mild chlorosis of the apical and sub-apical leaves. There were no deficiency symptoms on the roots. Calcium at 480 ppm had no apparent toxic effects on flax plants.

c) Calcium-boron interaction

Symptoms of boron deficiency first appeared on plants supplied with 40 ppm calcium 10 days after they had been placed in deficient solutions. The chlorosis and purpling of the leaves decreased in severity with increase in the level of calcium in the nutrient solutions. Two days later, deficiency symptoms were also observed on plants grown at normal (200 ppm) and high (480 ppm)

calcium levels. Boron toxicity symptoms were most severe at 40 and 480 ppm calcium. At 40 ppm calcium and zero-boron, symptoms of calcium deficiency were masked by those of boron deficiency. Calcium deficiency symptoms were more pronounced at 0.5 than at 2.5 ppm boron.

Plants grown at 480 ppm calcium and 0.5 ppm boron, had no symptoms. At 0 and 2.5 ppm boron, the symptoms on plants grown at 480 ppm calcium were only those of the boron deficiency and excess, respectively.

2. Post-inoculation symptoms

a) Wilt disease symptoms

Plants were inoculated by dipping the roots in the spore/mycelium suspension of F. oxysporum f. lini produced in shake culture. Some plants showed a transient wilting of the apical and sub-apical leaves, but these recovered their rigidity about 15 minutes after the roots had been removed from the inoculum and the plants replaced in their respective nutrient solutions.

The first wilt disease symptoms were observed 4 days after inoculation. These were an initial chlorosis of the lowest leaves and cotyledons, followed by necrosis and curling of these leaves. The chlorosis and necrosis gradually progressed to the upper leaves. These symptoms

were clearly differentiated from those of nutrient deficiencies, as they were initiated on the lower leaves and progressed upward, whereas the latter began on the apical leaves and progressed downward.

Many inoculated plants were stunted and showed a hyponasty of the leaves (Fig. 3). Others were spindly and sometimes taller than uninoculated plants grown under the same conditions. The stems of some inoculated plants showed a unilateral purplish-brown discolouration of the lower regions. These symptoms of Fusarium wilt were similar to those first described by Bolley (1901). In the terminal stages of the disease, the more severely affected plants which by this time were completely necrotic, showed a marked curling of the shoot apices. The roots of all inoculated plants were light brown in colour and appeared slimy. Foliar and root symptoms of nutrient deficiency or excess were more pronounced in inoculated than in uninoculated plants.

b) Boron deficiency and excess

After approximately 21 days, the primary and secondary shoot apices of all boron deficient plants were completely necrotic. At the conclusion of the experiment, the leaves of inoculated and uninoculated plants deficient in both boron and calcium, were completely chlorotic or

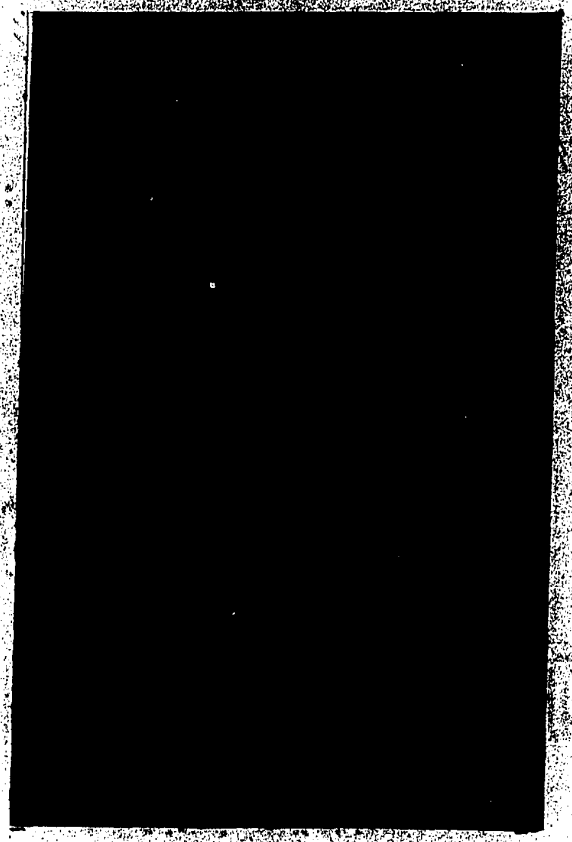


Fig. 3. Plants grown for 35 days in normal nutrient solutions and inoculated when 21 days old. Inoculated plant on the right, uninoculated on the left. Note chlorosis, necrosis and hyponasty of the leaves, and the reduced root system of the inoculated plant.

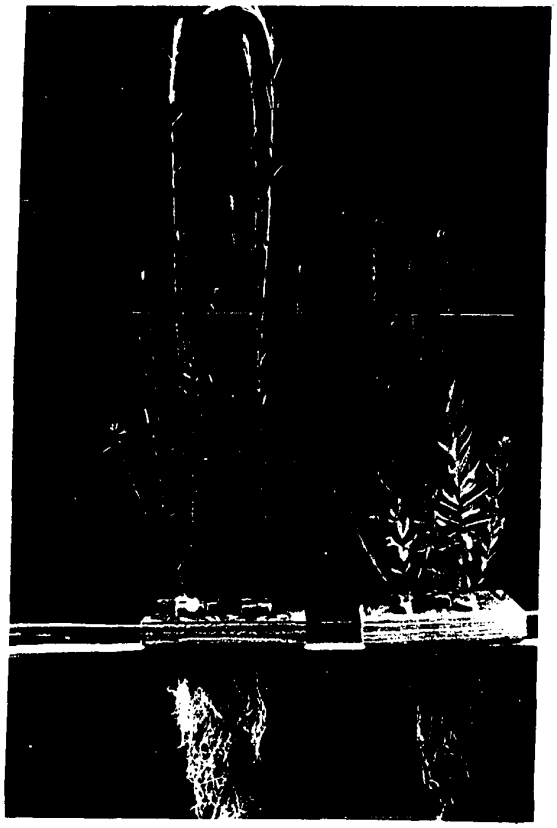


Fig. 3. Plants grown for 35 days in normal nutrient solutions and inoculated when 21 days old. Inoculated plant on the right, uninoculated on the left. Note chlorosis, necrosis and hyponasty of the leaves, and the reduced root system of the inoculated plant.

necrotic. The median leaves of uninoculated plants grown in solutions with no boron and 200 or 480 ppm calcium remained green with only a mild chlorotic mottling. The lower and subterminal leaves were chlorotic and the apical leaves were necrotic. The foliage of boron deficient inoculated plants supplied with the same concentrations of calcium was necrotic or yellow after 35 days (Fig. 4).

The necrosis and browning of the roots of boron deficient inoculated and uninoculated plants became progressively worse. When the experiment was terminated the roots appeared moribund.

Boron toxicity symptoms in uninoculated plants became more severe and involved a greater number of leaves as the plants matured. Inoculated plants also showed a chlorosis and necrosis of the lower leaves, but the wilt disease symptoms could be distinguished from those of the physiological disease by the fact that in the latter case, the necrosis was confined mostly to the margins and tips, whereas in the former, the necrosis involved the entire leaf. These differences are illustrated in Figs. 5 and 6.

c) Calcium deficiency and excess

Typical calcium deficiency symptoms first appeared on the basal shoots of inoculated plants supplied with 40 ppm calcium and 0.5 ppm boron, after approximately 28 days.

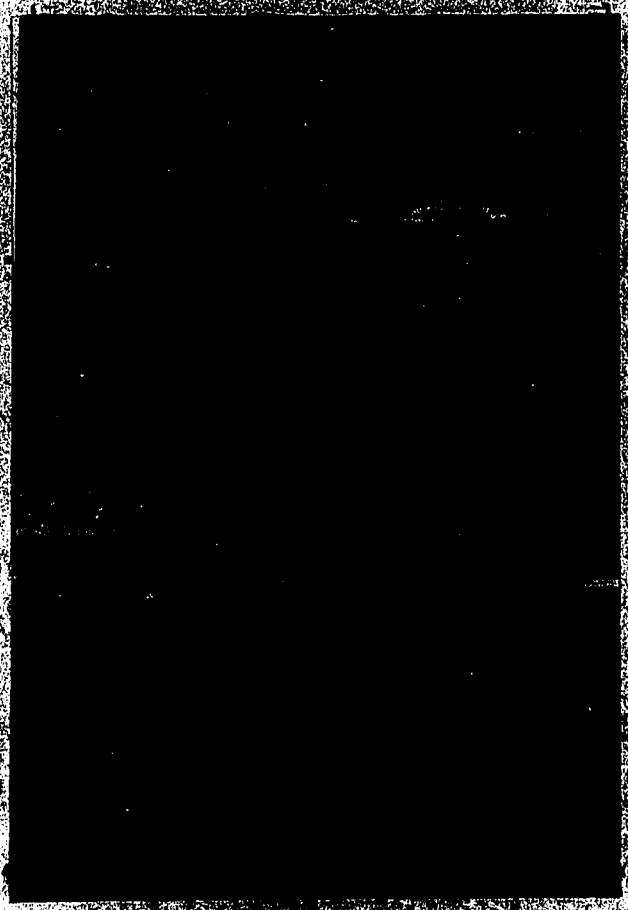


Fig. 4. Plants grown for 35 days in nutrient solutions deficient in calcium and boron and inoculated when 21 days old. Inoculated plant on the right, uninoculated on the left. Note complete chlorosis of the foliage of the inoculated plant, and only partial chlorosis of the uninoculated plant.

Fig. 4. Plants grown for 35 days in nutrient solutions deficient in calcium and boron and inoculated when 21 days old. Inoculated plant on the right, uninoculated on the left. Note complete chlorosis of the foliage of the inoculated plant, and only partial chlorosis of the uninoculated plant.

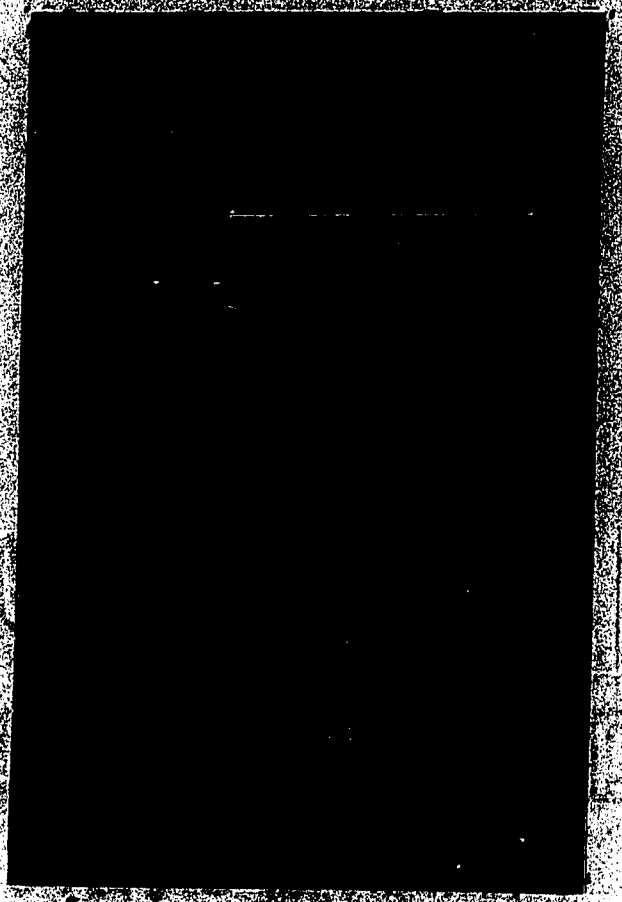


Fig. 5. Uninoculated plant grown for 35 days in nutrient solution supplied with 2.5 ppm boron and 200 ppm calcium. Note: leaf tip necrosis--boron toxicity symptom.

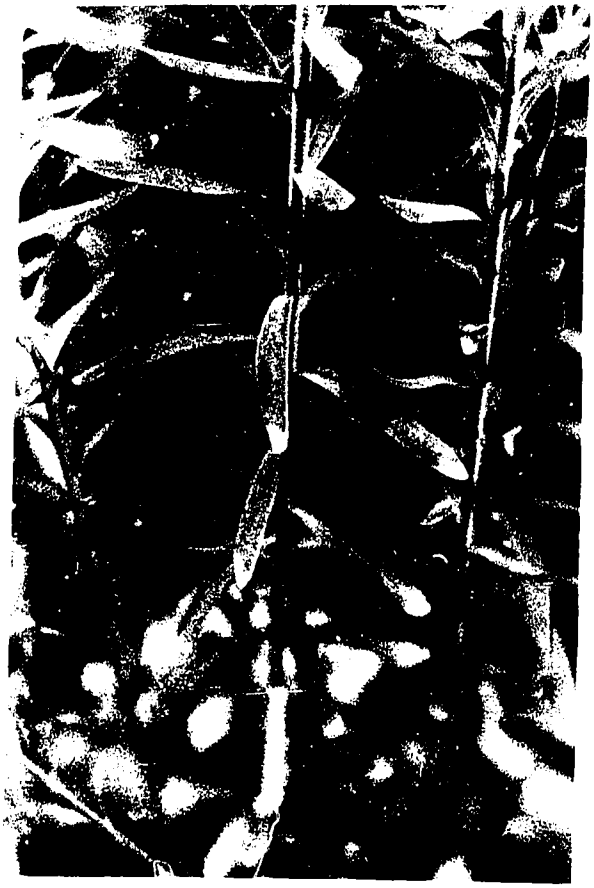


Fig. 5. Uninoculated plant grown for 35 days in nutrient solution supplied with 2.5 ppm boron and 200 ppm calcium. Note: leaf tip necrosis--boron toxicity symptom.

Fig. 6. Plant grown for 35 days in nutrient solution supplied with 2.5 ppm boron and 200 ppm calcium and inoculated when 21 days old. Note the chlorosis, necrosis and curling of the lower leaves--wilt disease symptoms, and the marginal and tip necrosis of the upper leaves--boron toxicity symptoms.

The symptoms, described as "withertop disease", involved an initial necrosis of the stem approximately four to five cm below the apex, and chlorosis of the apical leaves. After one to two days, that part of the stem above the necrotic area collapsed and eventually became necrotic. Calcium deficiency symptoms appeared about 6 days later on uninoculated plants. No withertop disease symptoms appeared on plants supplied with 2.5 ppm boron. Inoculated plants showed only the wilt disease symptoms, whereas uninoculated plants showed symptoms of boron toxicity.

Uninoculated plants grown at 200 and 480 ppm calcium, and 0.5 ppm boron, were healthy with only a slight chlorosis of the cotyledons, which was probably due to senescence of the plants. Inoculated plants supplied with 200 ppm calcium and 0.5 ppm boron showed more severe wilt disease symptoms than those supplied with 0.5 ppm boron and 480 ppm calcium.

The symptoms of calcium and boron deficiency and excess described above correspond to those reported by Laganière and Sackston (1967).

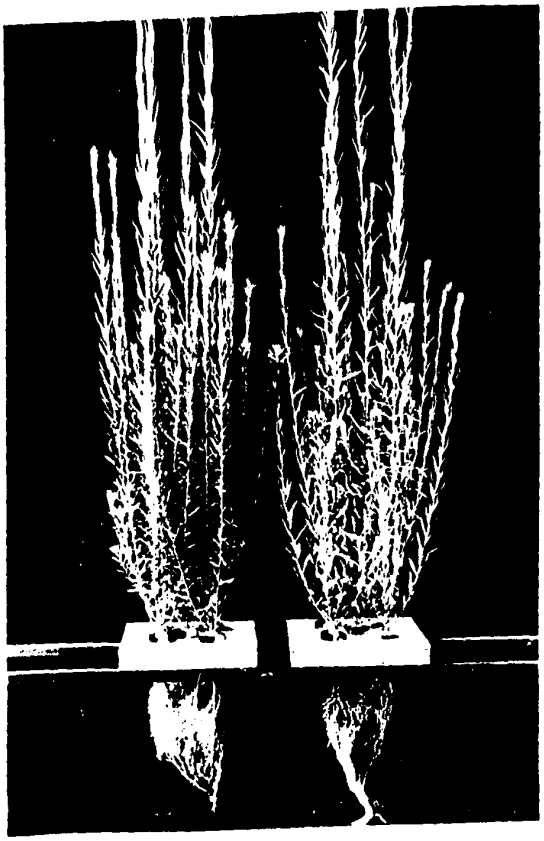
B. Relationship between Age and Nutritional Status of the Host Plant at the Time of Inoculation and Disease Development

1. Effect of calcium and boron nutrition on symptom expression

Plants grown in complete solutions for 14 and 21 days before inoculation averaged 20 cm and 27 cm in height, respectively. Plants grown in boron deficient solutions before inoculation were shorter than those subjected to any other treatments.

In all experiments, the severity of wilt disease was evaluated 14 days after inoculation. All plants inoculated when 21 days old, irrespective of the calcium and boron levels of their growth medium, were more severely diseased than those which were inoculated when 28 days old (Figs. 3 and 7, and Table 8). Flax plants grown in "altered" solutions throughout the experimental period showed similar symptoms of boron deficiency and excess, regardless of their age at the time of inoculation. The term "altered solution" will be used to describe those solutions which contain concentrations of calcium and boron other than the "normal" concentrations, i.e., 0.5 ppm boron and 200 ppm calcium, respectively. Calcium deficiency symptoms were, however, more severe in plants inoculated when 28 days old.

Fig. 7. Plants grown for 42 days in normal nutrient solutions and inoculated when 28 days old. Inoculated plant on the right, uninoculated on the left.



[illegible] grown for 42 days in normal nutrient [illegible] and inoculated when 28 days old. [illegible] plant on the right, uninoculated [illegible] left.

Uninoculated plants grown for 14 and 21 days in normal solutions, and then transferred to boron deficient solutions, showed none of the boron deficiency symptoms described previously, when they were harvested at five and six weeks of age, respectively. At 200 and 480 ppm calcium, and zero boron, there was only a slight chlorosis of the apical leaves and some necrosis of the roots. At 40 ppm calcium and all levels of boron, inoculated plants showed withertop in addition to typical wilt disease symptoms. With 2.5 ppm boron and 40 ppm calcium, uninoculated plants showed no withertop though these symptoms appeared on plants supplied with 0 or 0.5 ppm boron (Figs. 8, 9 and 10). These results differ from those of Laganière and Sackston (1967) who observed withertop on calcium deficient plants at 0.5 and 2.5 ppm boron after 31 days of exposure to the deficiency.

Symptoms of calcium and boron deficiency on uninoculated plants grown in altered solutions for 14 and 21 days and then transferred to normal solutions were similar to those described above.

When boron deficient plants were transferred to complete solutions, they recovered somewhat from the deficiency. New shoots were proliferated at the cotyledonary level and subterminally. Buds were produced at

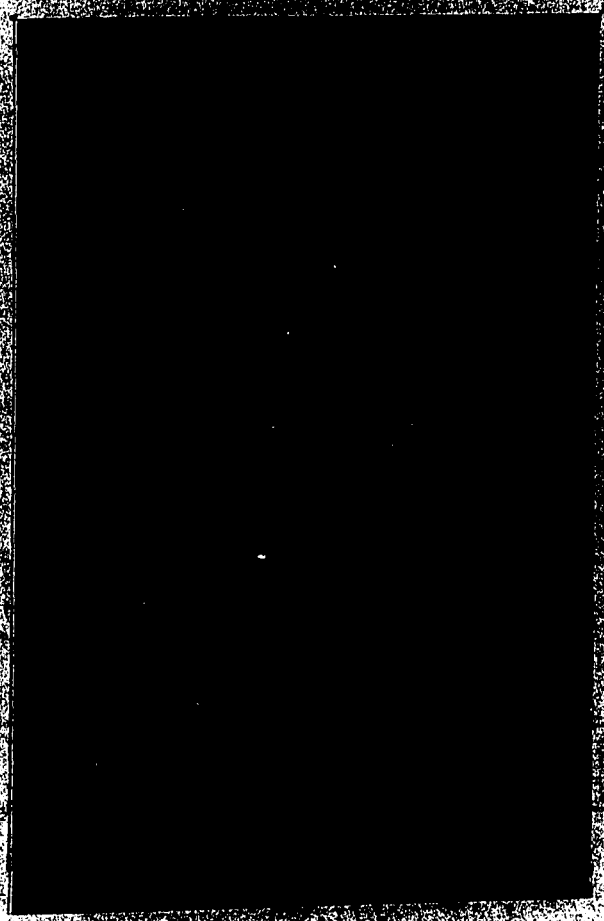
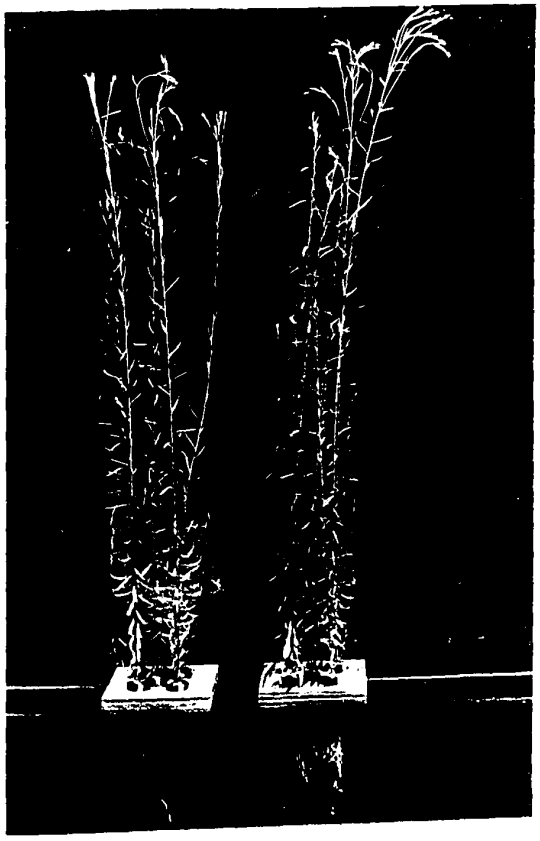
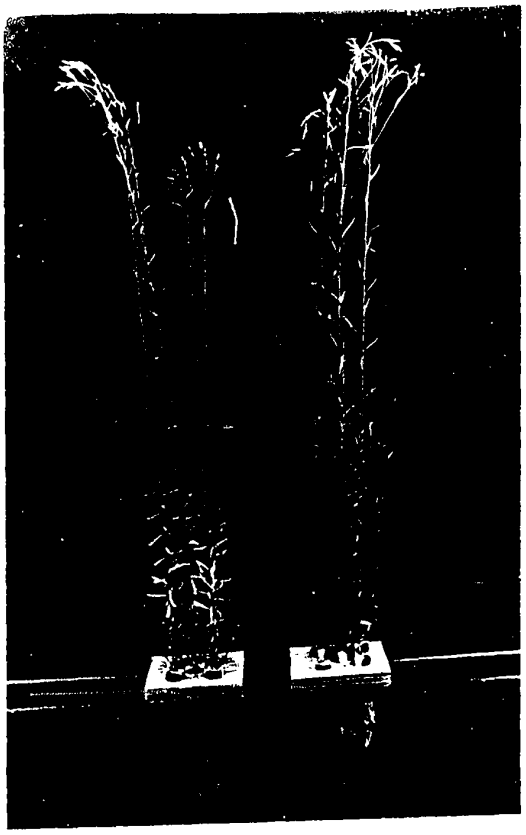
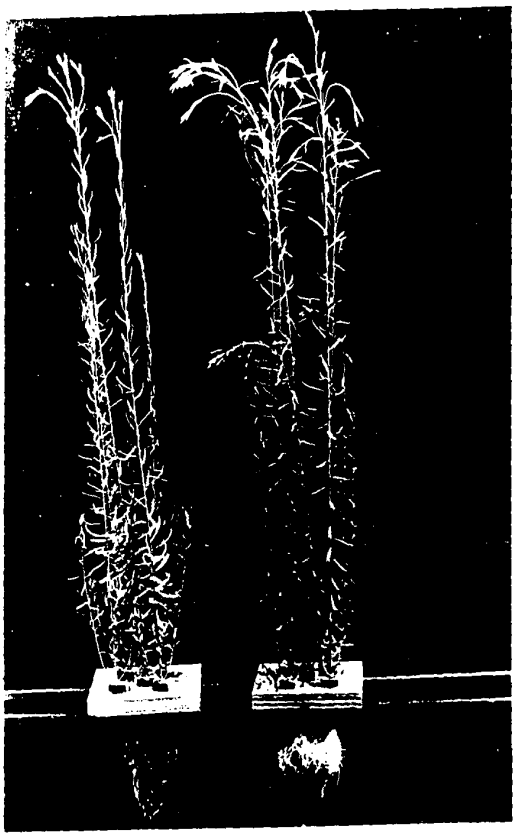


Fig. 8. Plants grown in complete nutrient solutions for 21 days before inoculation, and transferred to solutions containing 40 ppm calcium and 0 boron after inoculation. Inoculated plant on the left, uninoculated on the right. Note withertop symptoms on both the inoculated and the uninoculated plants.



. . Plants grown in complete nutrient solution for 21 days before inoculation, and transferred to solution containing 10 ppm calcium and 0 boron after inoculation. Inoculated plant on the left, uninoculated on the right. Note stimulation of growth of both the inoculated and the uninoculated plant.





Plants grown in complete nutrient solutions for 21 days before inoculation and transferred to solutions containing 40 ppm calcium and 2.5 ppm boron. Inoculated plant on the left, uninoculated plant on the right. Note that wilted symptoms appear only on the inoculated plant.

almost every node along the primary shoot, but these did not elongate. New roots were also formed, having a normal white colour and of normal length. Recovery was most rapid for plants which had been subjected to the low boron, low calcium treatment. There were no withertop symptoms on plants which had been grown in calcium deficient solutions. They showed only a slight chlorosis of the apical leaves and chlorosis or necrosis of the tips of sub-apical leaves, but there was no collapse of the stems.

The necrosis resulting from boron toxicity was irreversible, and there was no recovery when plants grown at 2.5 ppm boron were transferred to normal solutions.

Plants grown for 21 days in deficient solutions did not recover as rapidly as those exposed to the deficiency for only 14 days.

Plants grown in solutions deficient in calcium and boron and inoculated when 21 or 28 days old, did not recover after exposure to normal solutions, although some spindly new shoots were produced subterminally on boron deficient plants. Fourteen days after inoculation these plants were almost completely chlorotic with some necrosis and curling of the lowest leaves (Fig. 11). There were no withertop symptoms on inoculated plants grown at 40 ppm calcium and 0.5 or 2.5 ppm boron.

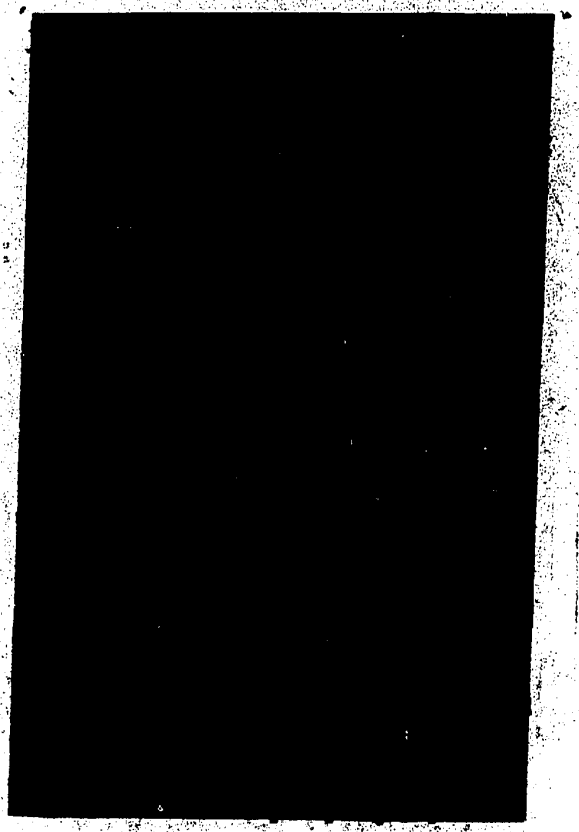


Fig. 11. Plants grown in solutions containing 40 ppm calcium and 0 boron for 21 days before inoculation and transferred to normal solutions after inoculation. Inoculated plant on the left, uninoculated on the right.

[illegible] grown in solutions containing 40 ppm cadmium for 8 days for 21 days before inoculation [illegible] to normal solutions after [illegible] inoculated [illegible] [illegible].

When plants grown for 21 days in complete solutions were inoculated and transferred to altered solutions, symptoms of calcium deficiency appeared before those of boron deficiency.

Laganière and Sackston (1967) observed a fasciation of the stems, leaves and flowers of flax plants which had been exposed to boron deficiency for 28 days and then returned to normal nutrient solutions. In these experiments flax plants were observed to produce more than one enlarged leaf at the nodes. This is not an unusual phenomenon in Linum spp. (Hayward, 1967).

2. The effect of pre-inoculation and post-inoculation nutrition of the host on disease development

When flax plants were grown continuously in solutions with a deficiency of calcium or boron the height and weight of inoculated and uninoculated plants were significantly reduced as compared to plants grown in normal solutions (Tables 2 and 3). All plants supplied with 40 ppm calcium and no boron were smaller and inoculated plants had a higher disease index than those exposed to any other treatment. Height and weight of inoculated and uninoculated boron deficient plants increased significantly as the calcium level of the solutions was increased from 40 to 480 ppm.

Uninoculated plants grown in normal solutions showed the highest gain in shoot weight. Inoculated plants supplied with 480 ppm calcium and 0.5 or 2.5 ppm boron were larger than those grown at other calcium and boron levels (Tables 2 and 3, and Figs. 12 and 13).

When plants were grown in complete solutions for 14 days and transferred to altered solutions, gain in shoot weight was greatest for uninoculated plants supplied with 200 ppm calcium and 2.5 ppm boron. For inoculated plants, shoot weight was highest and the disease index was lowest when plants received 480 ppm calcium and 0.5 ppm boron (Tables 4 and 8). Disease was most severe at 40 ppm calcium and 2.5 ppm boron (Fig. 14 and Table 8).

Calcium and boron had a less significant effect on plant height and weight when plants were exposed to complete nutrient solutions for 21 days before inoculation (Table 5). The inoculation effect was highly significant. Calcium and boron effects were significant at the 5% level. Disease was least severe at 2.5 ppm boron and 480 ppm calcium. Shoot weight was least reduced at 0.5 ppm boron and 480 ppm calcium (Fig. 15).

When plants were grown in altered solutions for 14 days and transferred to complete solutions, shoot heights of inoculated and uninoculated plants were significantly

reduced by calcium and boron deficiency (Table 6). Disease was most severe at 40 ppm calcium and zero boron; the severity decreased significantly with increase in the calcium level from 40 to 480 ppm. Disease was least severe at 0.5 ppm boron and 480 ppm calcium (Table 8).

Gain in shoot weight was greatest at 40 ppm calcium and 0.5 ppm boron for plants which had been grown in altered solutions for 14 or 21 days before inoculation and transferred to complete solutions after inoculation (Figs. 16 and 17).

C. The Effect of Calcium and Boron Nutrition of Flax on the Advance of *Fusarium* in the Stems

Flax plants which had been grown for 14 and 21 days in nutrient solutions of varying calcium and boron composition, were inoculated with *F. oxysporum* f. *lini* as described previously. The plants were collected 14 days after inoculation, and the amount of infection, and the rate of invasion of the stems, were assessed. Serial sections 5 mm long, taken from the crown to the apex of all inoculated plants, were surface sterilized and plated on P.D.A. in petri dishes. For each treatment, ten sections were plated, and the number of reisolations obtained from these sections was used as a measure of infection for that

TABLE 2. Response of Flax Plants Grown in Nutrient Solutions of Varying Calcium and Boron Composition for 35 Days and Inoculated with Fusarium when 21 Days Old (Average of 10 Plants in each Case).

Treatment	Shoot Heights (cm)		Fresh Wts of Roots (g)		Fresh Wts of Shoots (g)	
	Inoc.	Uninoc.	Inoc.	Uninoc.	Inoc.	Uninoc.
CaL BL	18.1	20.2	3.35	4.53	11.91	20.46
CaL BN	23.4	33.3	6.51	7.99	13.94	30.01
CaL BH	27.8	35.5	5.89	8.82	16.00	32.12
CaN BL	20.0	21.3	2.78	4.27	14.80	22.50
CaN BN	28.6	41.8	5.59	10.32	16.73	36.77
CaN BH	24.8	40.0	5.40	9.42	14.24	32.38
CaH BL	27.3	23.4	4.34	4.70	17.02	24.00
CaH BN	32.3	37.2	5.02	10.34	18.27	35.08
CaH BH	26.7	34.7	5.48	7.08	15.58	30.59
LSD 5%	6.5		6.67		10.95	
LSD 1%	8.9		9.14		15.00	

TABLE 3. Response of Flax Plants Grown in Nutrient Solutions of Varying Calcium and Boron Composition for 42 Days and Inoculated with Fusarium when 28 Days Old (Average of 10 Plants in each Case).

	Shoot Heights (cm)		Fresh Wts of Roots (g)		Fresh Wts of Shoots (g)	
Treatment	Inoc.	Uninoc.	Inoc.	Uninoc.	Inoc.	Uninoc.
CaL Bl	20.3	18.9	7.65	5.84	21.74	21.44
CaL BN	52.5	58.9	14.15	17.24	35.99	57.33
GaL BH	54.5	53.7	11.48	14.89	33.79	45.89
CaN BL	18.0	18.0	5.70	5.30	20.33	24.06
CaN BN	63.8	67.3	11.39	16.76	42.74	62.66
CaN BH	52.4	64.9	11.24	16.79	35.04	56.49
CaH BL	25.3	23.2	5.85	7.62	23.16	29.08
CaH BN	50.6	63.0	8.78	18.24	35.17	61.12
CaH BH	60.9	54.1	13.23	13.25	44.10	47.26
LSD 5%	12.01		7.40		11.88	
LSD 1%	17.5		10.14		16.28	

TABLE 4. Response of Flax Plants Grown in Complete Solutions for 14 Days Before Inoculation with Fusarium and Transferred to Altered Solutions after Inoculation (Average of 10 Plants in each Case).

	Shoot Heights (cm)		Fresh Wts of Roots (g)		Fresh Wts of Shoots (g)	
Treatment	Inoc.	Uninoc.	Inoc.	Uninoc.	Inoc.	Uninoc.
CaL BL	45.2	46.9	3.59	3.27	25.99	28.80
CaL BN	57.3	58.5	5.38	4.74	25.97	25.94
CaL BH	45.5	58.1	5.31	5.16	21.77	32.63
CaN BL	56.5	48.5	3.99	3.57	28.78	34.85
CaN BN	65.0	62.4	4.17	8.01	29.21	38.64
CaN BH	63.3	69.9	4.52	6.42	30.60	40.38
CaH BL	60.7	49.2	3.15	2.91	28.90	29.61
CaH BN	60.8	66.5	6.06	4.85	28.28	35.84
CaH BH	67.0	62.5	4.06	5.34	28.10	34.67
LSD 5%	10.0		5.39		10.10	
LSD 1%	13.7		7.38		13.84	

TABLE 5. Response of Flax Plants Grown in Complete Solutions for 21 Days Before Inoculation with Fusarium and Transferred to Altered Solutions after Inoculation (Average of 10 Plants in each Case)

	Shoot Heights (cm)		Fresh Wts of Roots (g)		Fresh Wts of Shoots (g)	
Treatment	Inoc.	Uninoc.	Inoc.	Uninoc.	Inoc.	Uninoc.
CaL BL	66.0	64.3	10.62	11.50	32.85	43.98
CaL BN	71.1	69.6	9.88	15.15	31.32	43.42
CaL BH	53.0	71.4	6.80	11.73	21.85	42.11
CaN BL	68.8	50.7	8.33	7.74	32.11	36.33
CaN BN	73.2	64.3	9.58	11.57	34.23	43.48
CaN BH	70.9	69.0	10.76	12.98	35.27	42.00
CaH BL	65.7	61.7	7.67	9.68	30.13	37.68
CaH BN	69.8	69.1	9.68	12.12	37.68	42.52
CaH BH	55.5	66.9	10.93	10.23	32.31	39.56
LSD 5%	12.4		4.25		7.51	
LSD 1%	17.00		5.83		10.29	

TABLE 6. Response of Flax Plants Grown in Altered Solutions for 14 Days before Inoculation with *Fusarium* and Transferred to Complete Solutions after Inoculation (Average of 10 Plants in each Case)

	Shoot Heights (cm)		Fresh Wts of Roots (g)		Fresh Wts of Shoots (g)	
Treatment	Inoc.	Uninoc.	Inoc.	Uninoc.	Inoc.	Uninoc.
CaL BL	13.7	17.4	2.86	8.45	8.97	26.45
CaL BN	66.6	72.3	6.27	6.68	32.63	36.62
CaL BH	67.3	66.7	6.26	9.85	29.58	36.13
CaN BL	41.8	36.8	3.55	8.95	17.94	31.90
CaN BN	67.0	67.7	5.45	6.18	26.38	31.93
CaN BH	54.9	64.5	5.87	5.85	24.55	28.59
CaH BL	53.7	63.9	4.21	9.48	21.96	33.47
CaH BN	62.0	62.8	5.87	5.68	28.26	28.90
CaH BH	62.3	67.5	5.08	8.90	23.55	35.10
LSD 5%	11.0		3.30		15.02	
LSD 1%	15.1		4.52		20.58	

TABLE 7. Response of Flax Plants Grown in Altered Solution for 21 Days before Inoculation with Fusarium and Transferred to Complete Solutions after Inoculation (Average of 10 Plants in each Case)

Treatment	Shoot Heights (cm)		Fresh Wts of Roots (g)		Fresh Wts of Shoots (g)	
	Inoc.	Uninoc.	Inoc.	Uninoc.	Inoc.	Uninoc.
CaL BL	15.1	13.9	3.58	8.13	11.59	17.54
CaL BN	71.6	71.6	10.25	11.76	37.05	49.19
CaL BH	70.6	74.5	9.59	13.81	34.79	48.63
CaN BL	18.4	15.8	4.44	8.16	14.56	20.50
CaN BN	71.0	75.3	9.52	12.36	28.26	48.03
CaN BH	59.4	77.0	8.96	9.49	30.92	42.74
CaH BL	26.5	38.3	4.67	6.98	19.92	26.98
CaH BN	66.9	40.6	10.35	10.31	34.71	44.86
CaH BH	59.7	75.0	7.70	10.07	28.50	49.95
LSD 5%	3.9		3.79		12.88	
LSD 1%	5.4		5.19		17.64	

TABLE 8. Development of Fusarium Wilt in Flax Plants Grown in Nutrient Solutions of Varying Calcium and Boron Composition and Inoculated when 21 and 28 Days Old

Disease Percent												
Plants Inoculated at 21 Days							Plants Inoculated at 28 Days					
Ca & B supplied before inoculation				Ca & B supplied after inoculation				Ca & B supplied before inoculation			Ca & B supplied after inoculation		
B ppm	Ca ppm			B ppm	Ca ppm			B ppm	Ca ppm		B ppm	Ca ppm	
0	40	72	a*	2.5	40	77	a	0	40	62 a	2.5	40	45 a
0	200	69	ab	0	40	72	ab	0	480	58 ab	0	40	43 ab
0	480	50	c	0	200	51	c	0	200	42 bc	2.5	480	40 ab
2.5	480	48	c	0.5	40	48	cd	2.5	40	32 bcd	0	480	35 ab
2.5	40	44	c	0.5	200	43	de	2.5	200	31 bcd	0.5	40	35 ab
0.5	200	43	c	0	480	39	de	0.5	200	30 bcd	0.5	480	35 ab
0.5	40	40	c	2.5	200	38	def	0.5	40	29 bcd	0.5	200	34 ab
2.5	200	38	c	2.5	480	36	ef	0.5	480	27 bcd	0	200	32 ab
0.5	480	34	c	0.5	480	32	g	2.5	480	24 d	2.5	200	24 c

*All means within a column not followed by the same letter(s) are significantly different from each other at the 5% level according to Duncan's new multiple range test.

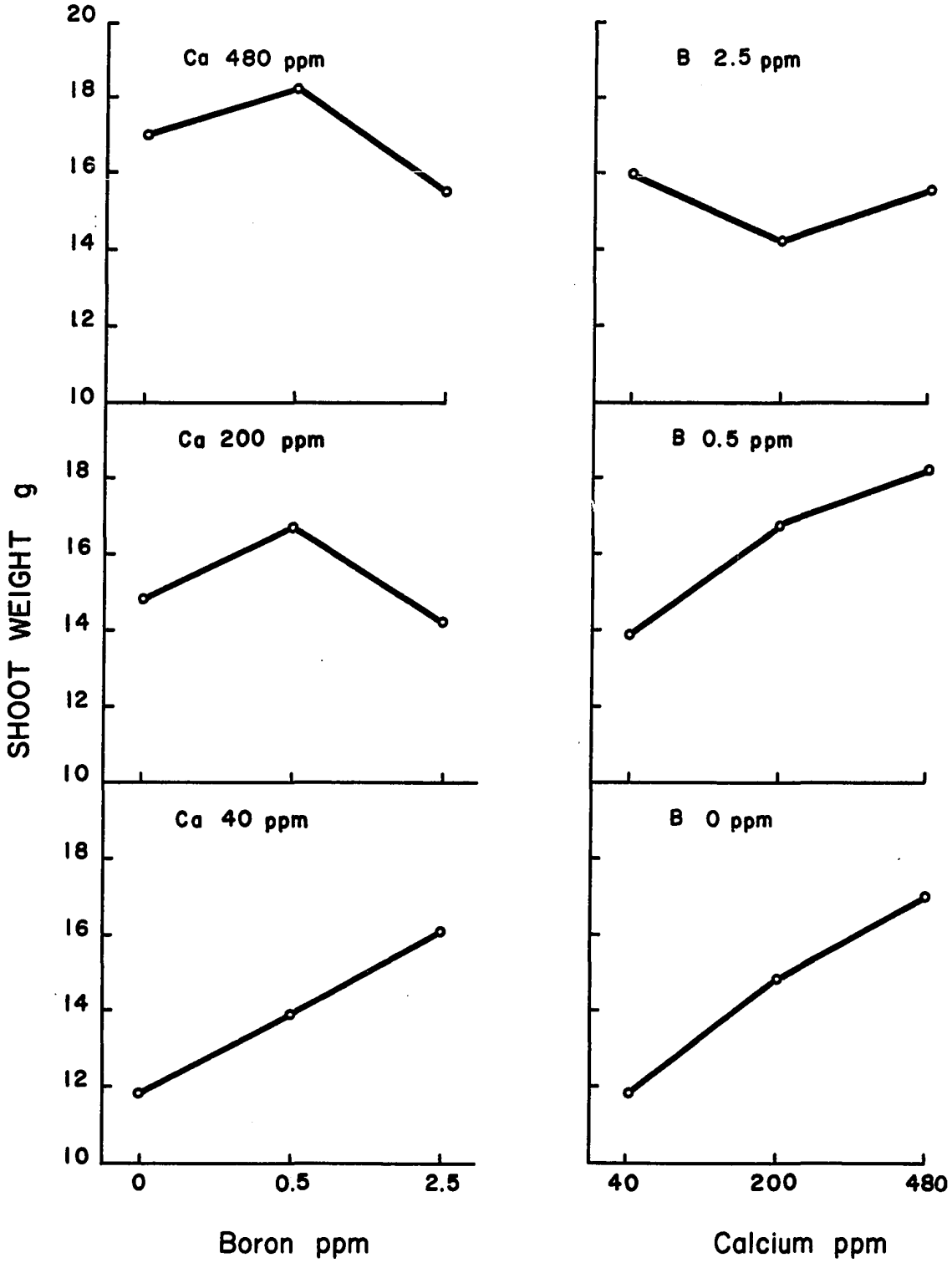


Fig. 12. Shoot weights for plants grown in solutions of various calcium and boron composition for 35 days and inoculated with Fusarium when 21 days old.

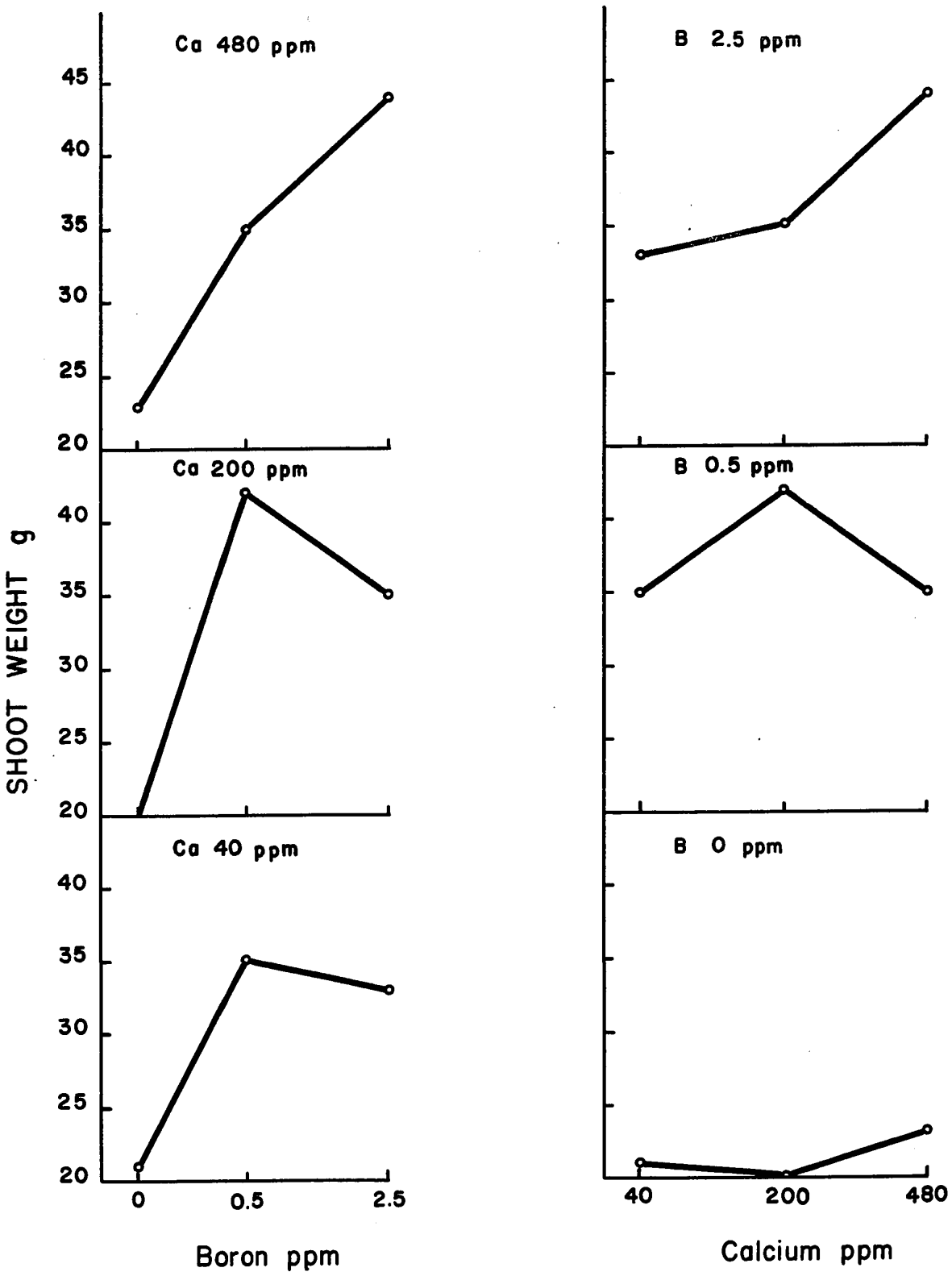


Fig. 13. Shoot weights for plants grown in solutions of various calcium and boron composition for 42 days and inoculated with Fusarium when 28 days old.

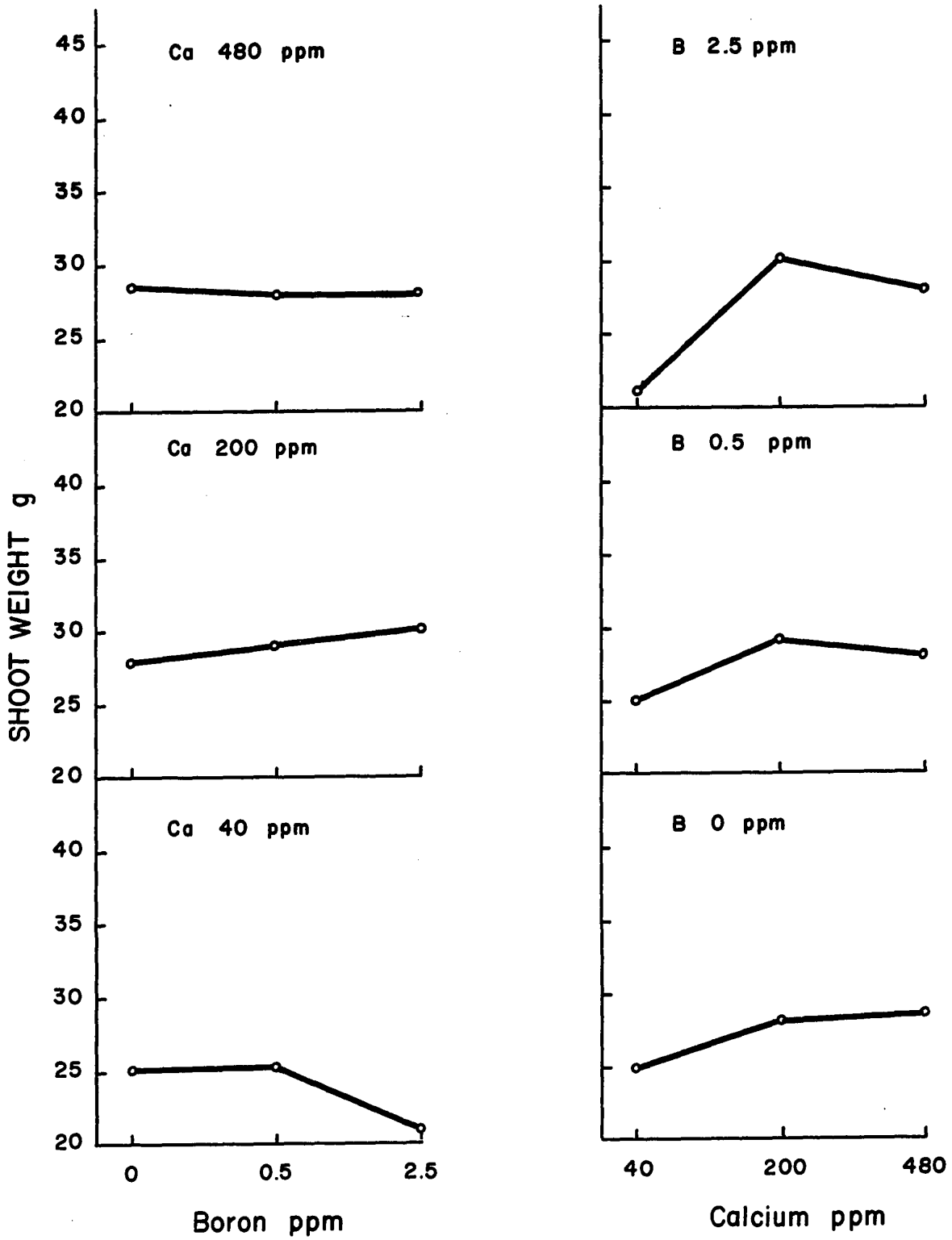


Fig. 14. Shoot weights for plants grown in complete solutions for 14 days before inoculation with Fusarium and transferred to altered solutions after inoculation.

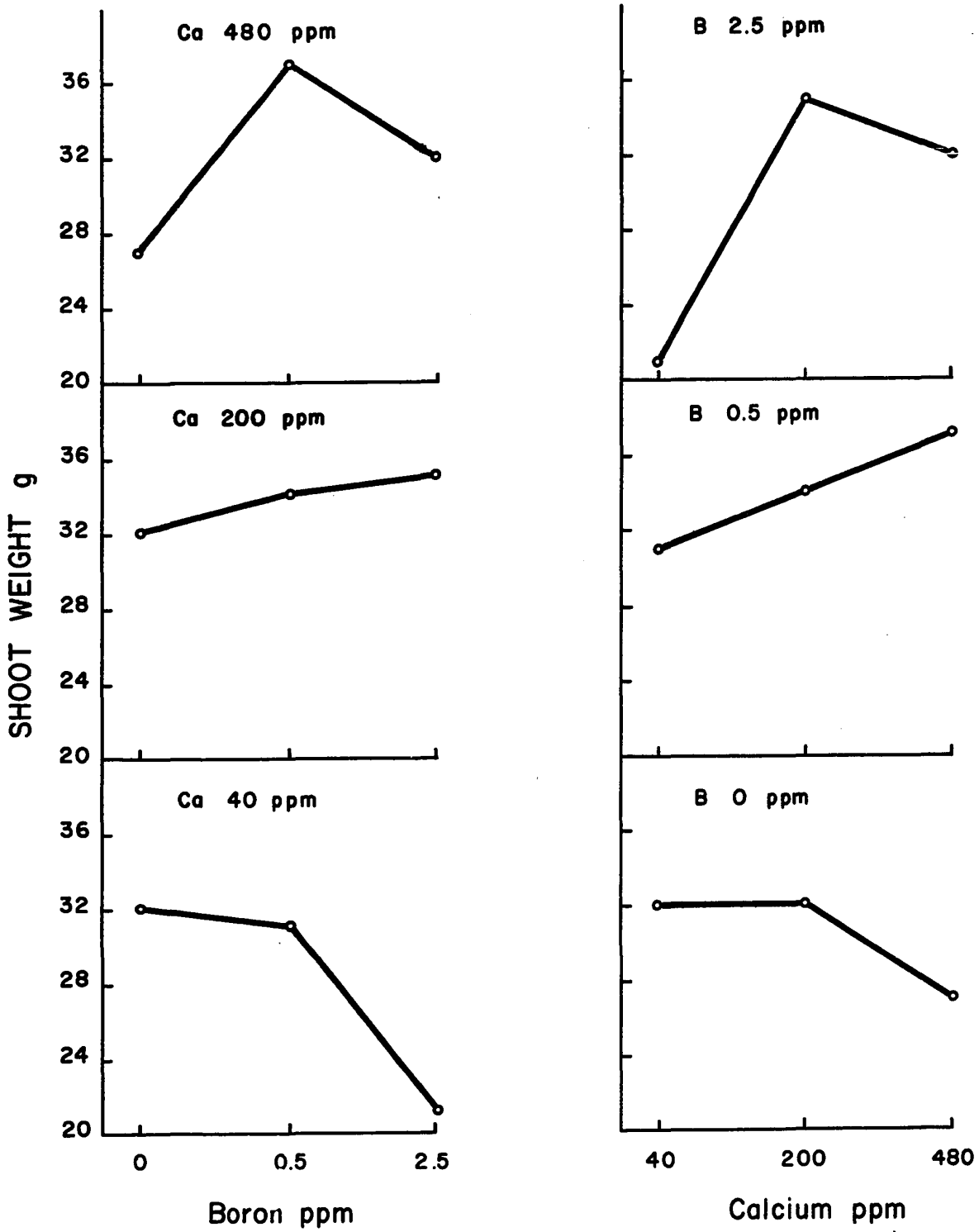


Fig. 15. Shoot weights for plants grown in complete solutions for 21 days before inoculation with Fusarium and transferred to altered solutions after inoculation.

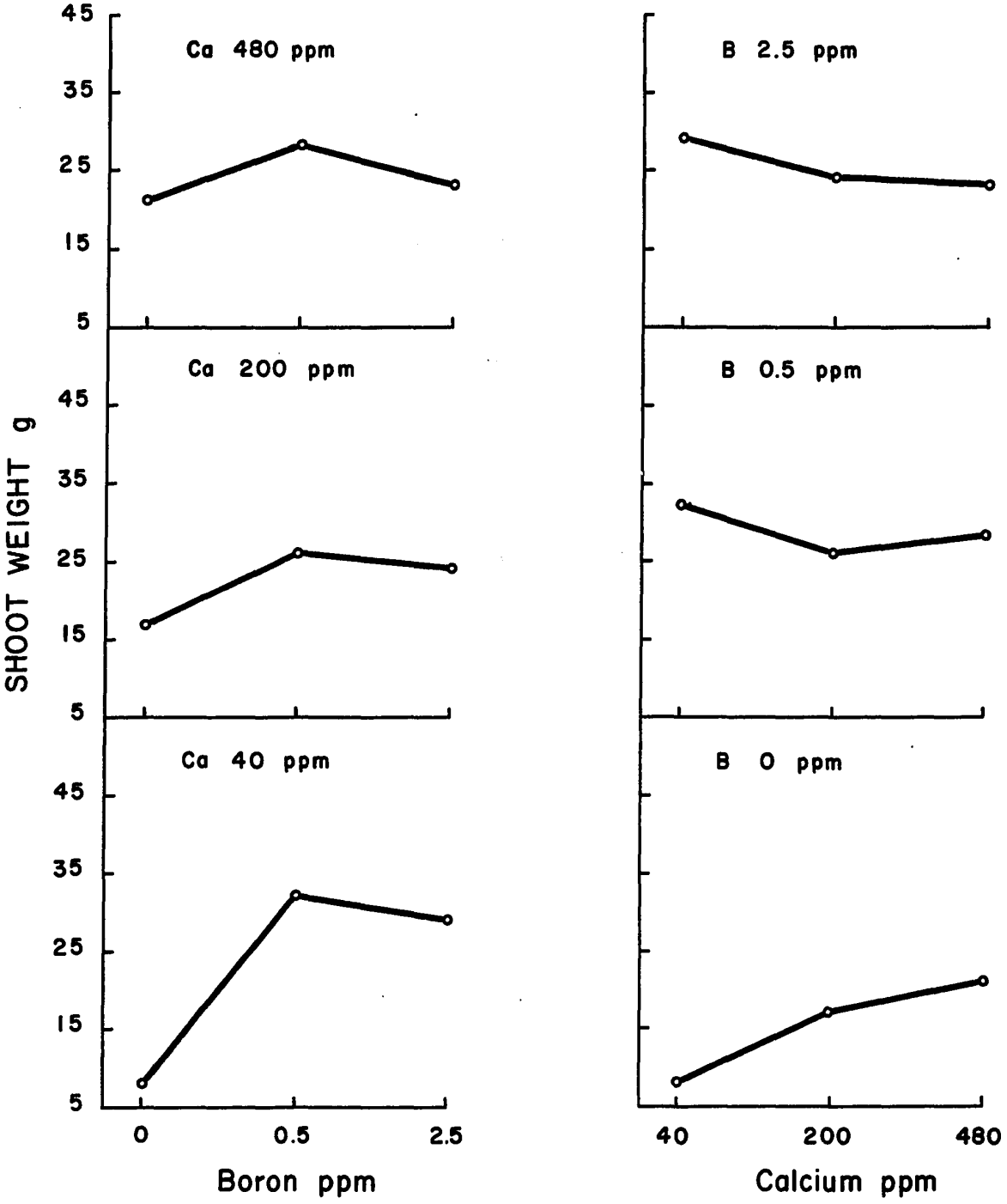


Fig. 16. Shoot weights for plants grown in altered solutions for 14 days before inoculation with Fusarium and transferred to complete solutions after inoculation.

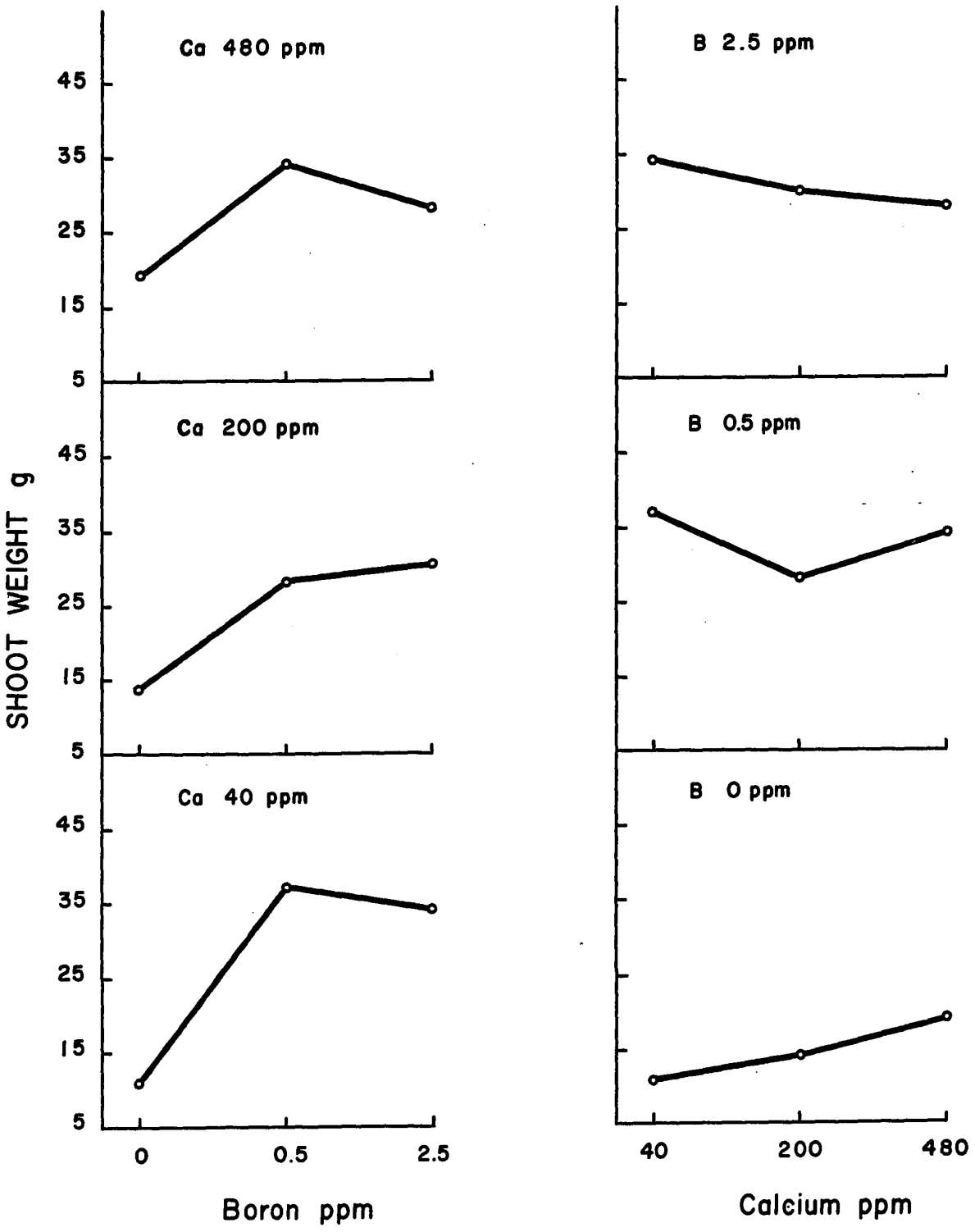


Fig. 17. Shoot weights for plants grown in altered solutions for 21 days before inoculation with Fusarium and transferred to complete solutions after inoculation.

treatment. The height at which the pathogen was reisolated was used as an estimate of its invasiveness.

For plants inoculated when 21 days old and exposed to boron deficient conditions throughout the growth period, advance of the pathogen in the plant stems was unrestricted at all concentration of calcium (Fig. 18). At 40 and 480 ppm calcium, the pathogen was reisolated at a height of 120 mm, and 130 mm at 200 ppm calcium. The amount of infection was also very high at these concentrations of calcium and boron. Advance of Fusarium was least restricted at 0.5 ppm boron and 200 ppm calcium. When the mineral element supply of the susceptible host was adequate, the pathogen was in a most favourable environment for growth and reproduction. A concentration of 2.5 ppm boron and 40 and 480 ppm calcium retarded the growth of Fusarium.

Plants inoculated when 28 days old were least colonized by F. oxysporum f. lini at zero boron and 40 ppm calcium (Fig. 19). With the boron level at 0.5 and 2.5 ppm, progress of the fungus was greatest at 40 ppm calcium. At high boron levels, growth was most restricted, and although there was 100% infection, Fusarium only attained a height of 40 mm in the plant, as compared with 150 mm for plants supplied with normal calcium and boron levels.

When plants were grown in complete solutions for 14 and 21 days before inoculation, advance of Fusarium was most restricted at 480 ppm calcium and 0.5 ppm boron (Figs. 20 and 21). For plants inoculated when 21 days old, growth was unrestricted at low calcium levels, irrespective of the boron content of the plant tissues, and at normal boron and calcium levels. For plants inoculated when 28 days old, advance of the fungus was greatest at 200 ppm calcium and zero boron, though there was only 50% infection.

Disease was most severe and the pathogen least restricted at 40 ppm calcium and 2.5 ppm boron. Disease was least severe and the ascent of the pathogen most restricted at 480 ppm calcium and 0.5 ppm boron.

When the calcium and boron deficiency occurred before inoculation, flax stems were least colonized by Fusarium at 40 ppm calcium and zero boron, and 40 ppm calcium and 0.5 ppm boron for plants inoculated when 21 and 28 days old, respectively. The pathogen was least restricted at zero boron and 480 ppm calcium (Figs. 22 and 23).

Fig. 18. Colonization of flax plants by F. oxysporum f. lini. Plants were grown in nutrient solutions of various calcium and boron composition and inoculated when 21 days old.

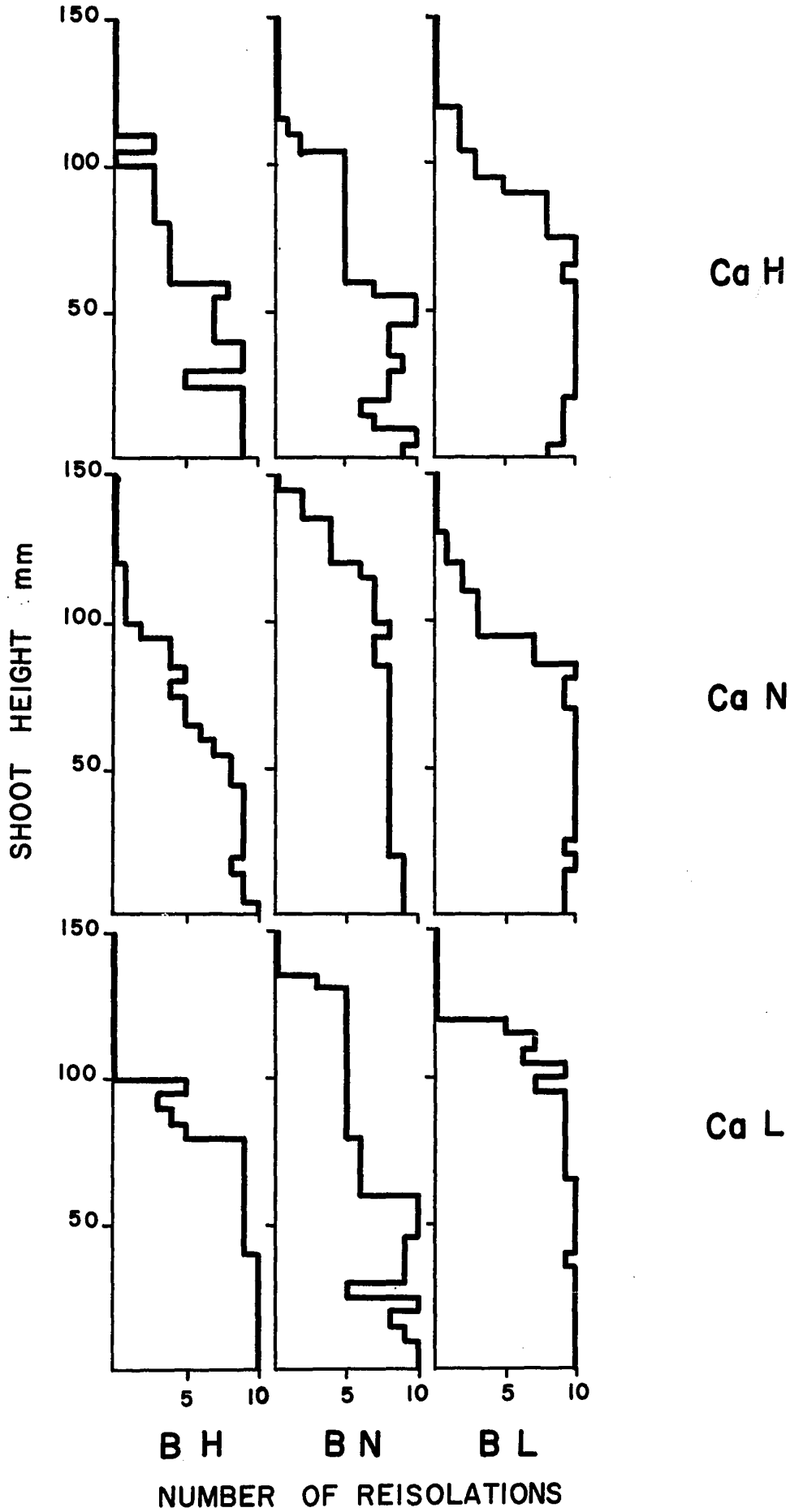

150
100
50
150
100
50
150
100
50
SHOOT HEIGHT mm
Ca H
Ca N
Ca L
5
10
5
10
5
10
B H
B N
B L
NUMBER OF REISOLATIONS

Fig. 18.

Fig. 19. Colonization of flax plants by F. oxysporum f. lini. Plants were grown in nutrient solutions of various calcium and boron composition and inoculated when 28 days old.

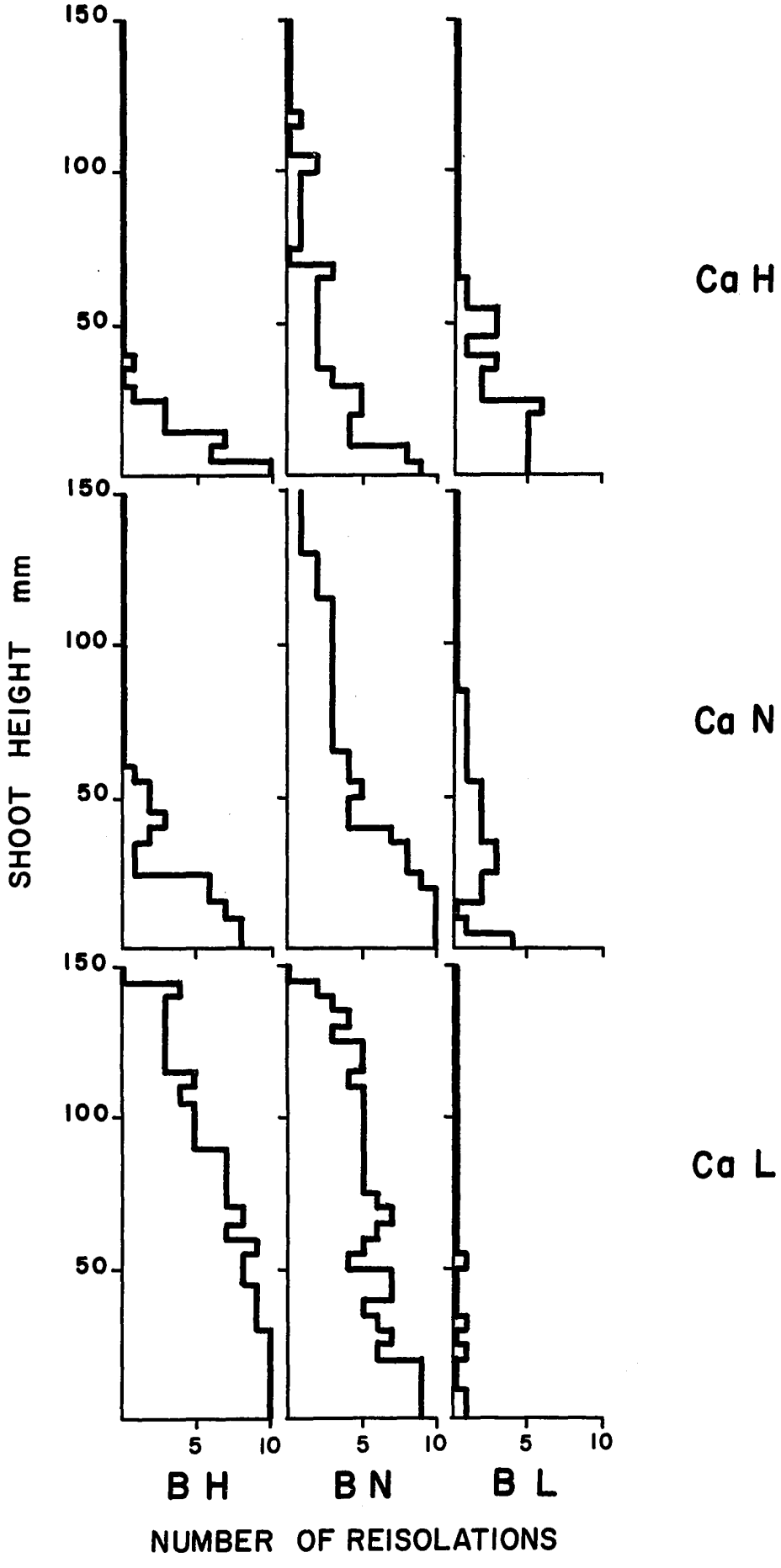

150
100
50
Ca H
Ca N
Ca L
SHOOT HEIGHT mm
5
10
B H
B N
B L
NUMBER OF REISOLATIONS

Fig. 19.

Fig. 20. Colonization of flax plants by F. oxysporum f. lini. Plants were grown in complete solutions for 14 days before inoculation and transferred to altered solutions after inoculation.

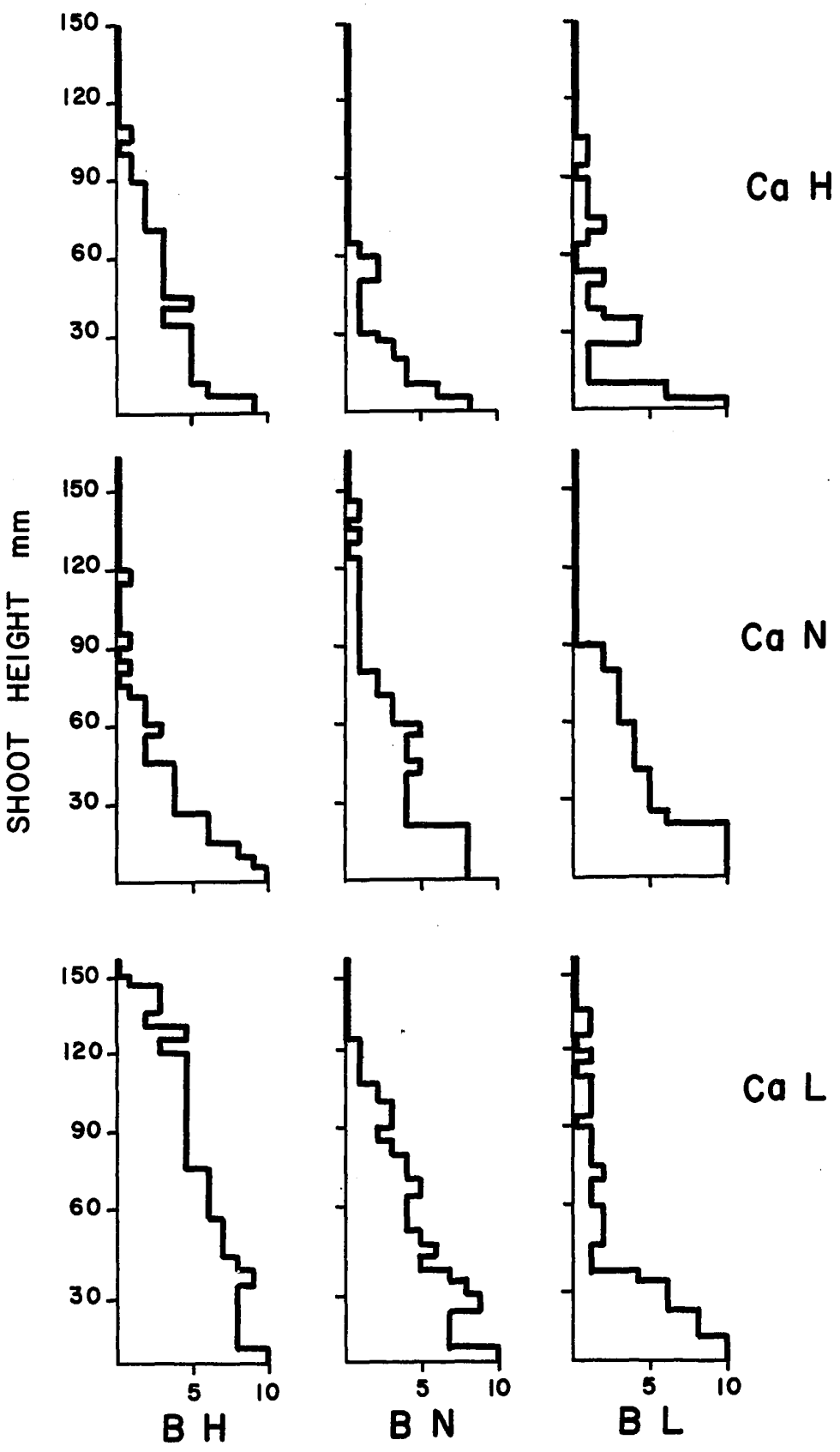

150
120
90
60
30
Ca H
Ca N
Ca L
SHOOT HEIGHT mm
5
10
B H
B N
B L
NUMBER OF REISOLATIONS

Fig. 20.

Fig. 21. Colonization of flax plants by F. oxysporum f. lini. Plants were grown in complete solutions for 21 days before inoculation and transferred to altered solutions after inoculation.

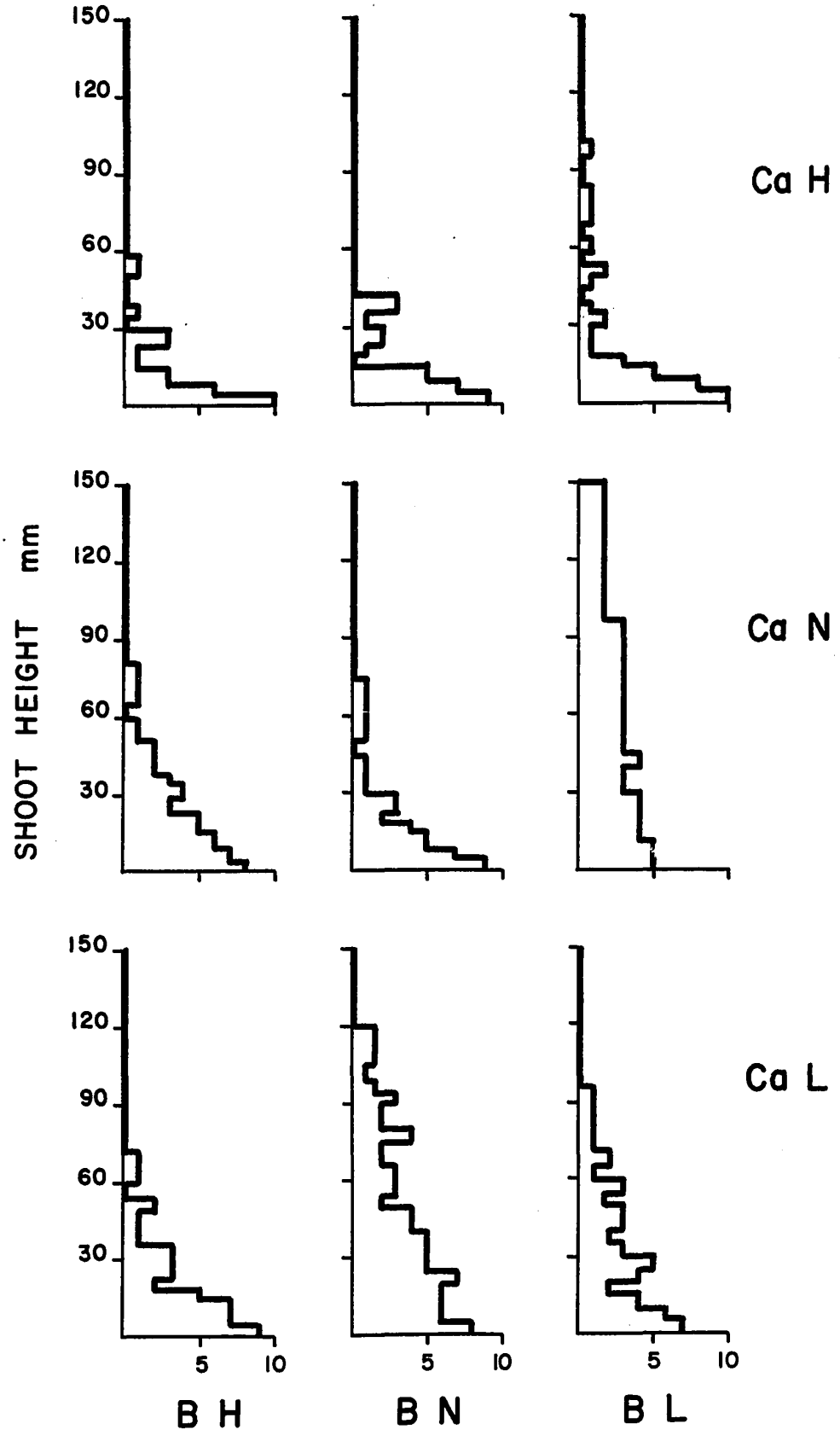

150
120
90
60
30
SHOOT HEIGHT mm
Ca H
Ca N
Ca L
5
10
B H
B N
B L
NUMBER OF REISOLATIONS

Fig. 21.

Fig. 22. Colonization of flax plants by F. oxysporum f. lini. Plants were grown in altered solutions for 14 days before inoculation and transferred to complete solutions after inoculation.

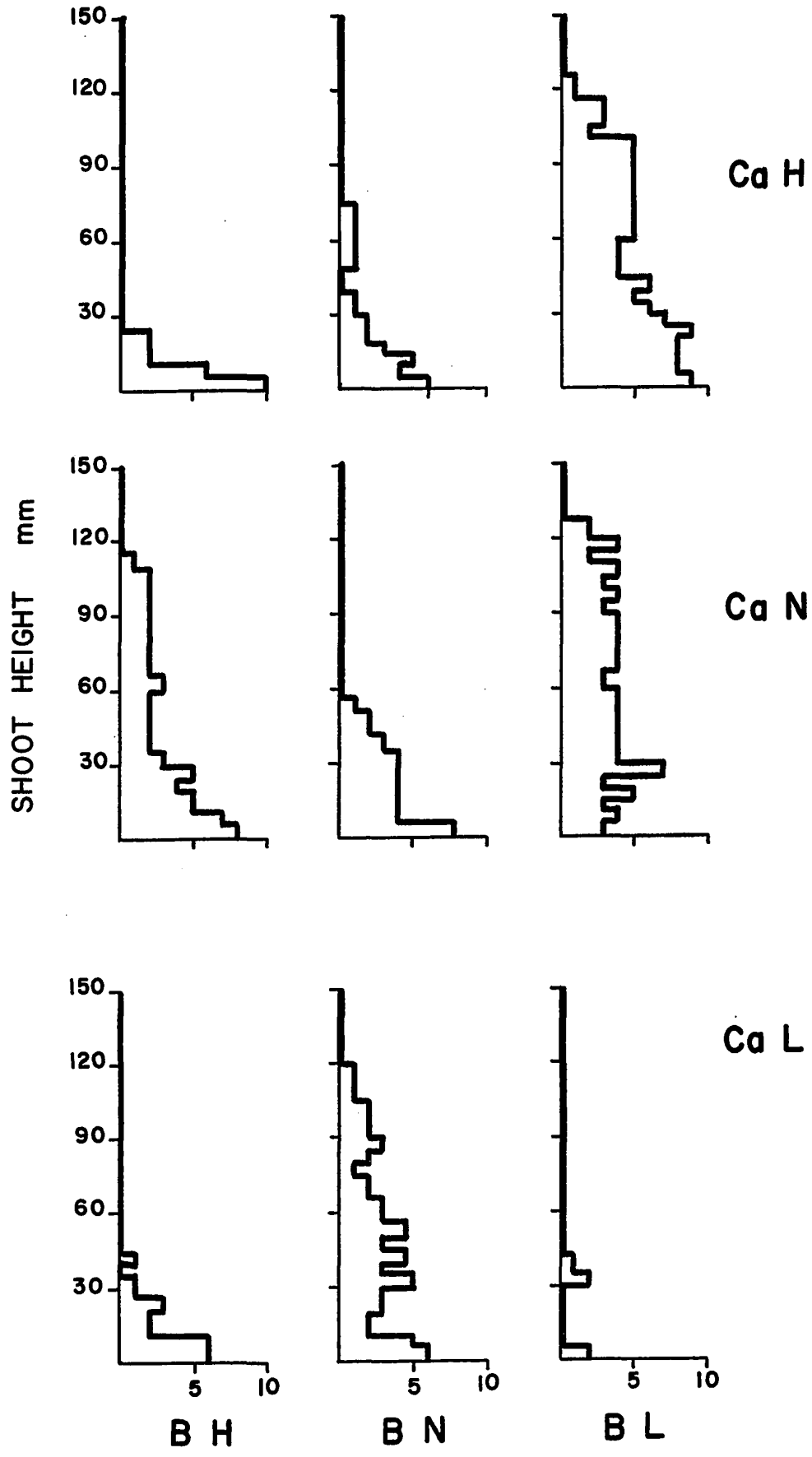

150
120
90
60
30
SHOOT HEIGHT mm
Ca H
Ca N
Ca L
5
10
B H
B N
B L
NUMBER OF REISOLATIONS

Fig. 22.

Fig. 23. Colonization of flax plants by F. oxysporum f. lini. Plants were grown in altered solutions for 21 days before inoculation and transferred to complete solutions after inoculation.

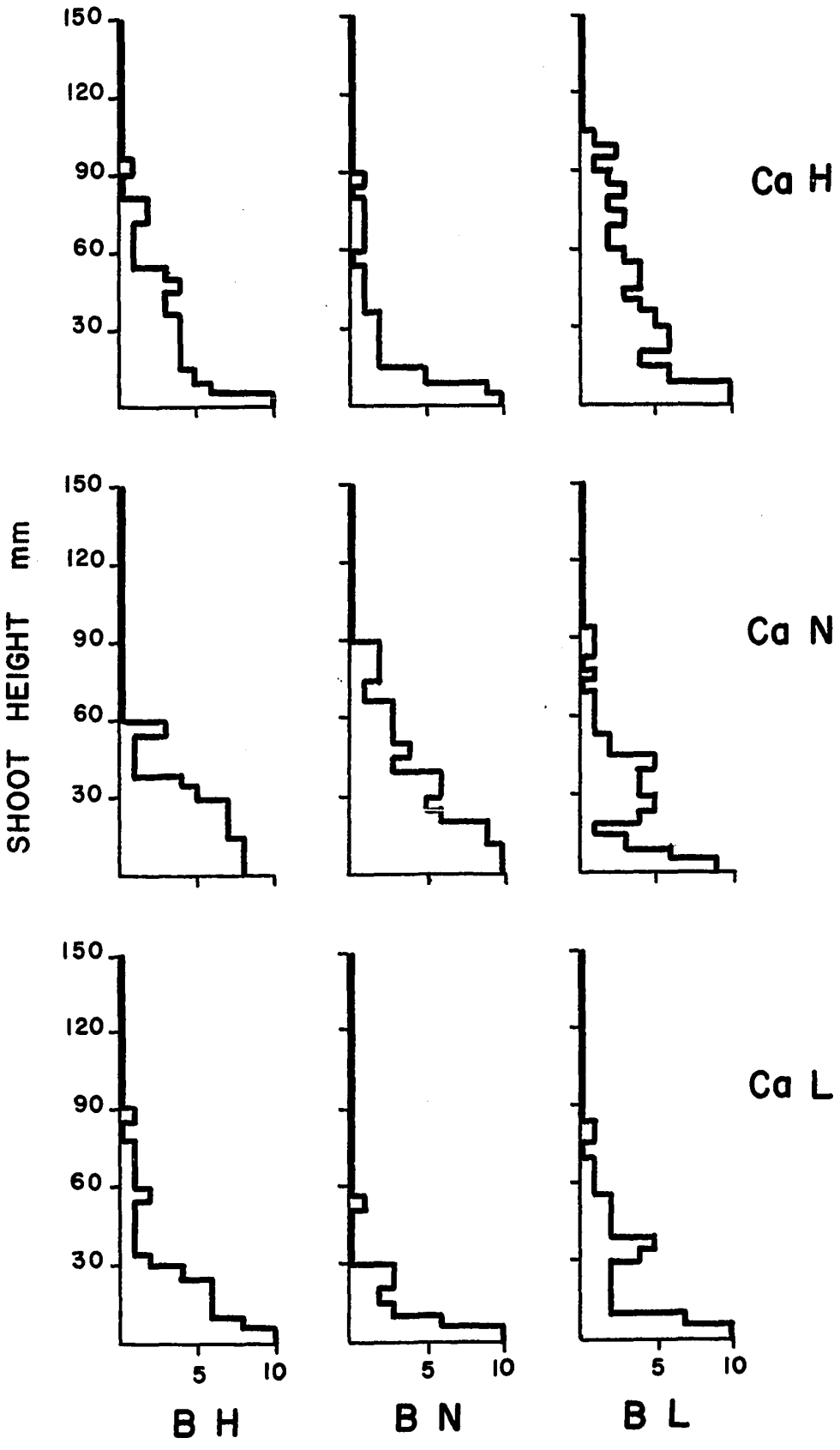

150
120
90
60
30
Ca H
Ca N
Ca L
SHOOT HEIGHT mm
5
10
B H
B N
B L
NUMBER OF REISOLATIONS

Fig. 23.

V. DISCUSSION

Wilt disease was most severe in flax plants inoculated with F. oxysporum f. lini, which were grown in nutrient solutions deficient in boron throughout the experiment, or prior to inoculation. When plants were exposed to the boron deficiency only after inoculation, wilt was less severe. The disease decreased in severity with increase in calcium levels from 40 to 200 to 480 ppm.

Boron deficient flax plants inoculated when 21 days old were readily colonized by Fusarium, regardless of the level of calcium supplied to the plants. When boron deficient plants were inoculated at 28 days, the pathogen failed to infect and successfully parasitize its host.

The role of boron in plants is still not clear, and the literature contains few references to its effect on infectious plant diseases. A boron deficiency in a plant impairs its health and vigour and it is not surprising that boron deficient flax plants were more susceptible to disease than non-deficient plants.

According to Gauch and Dugger (1954) boron is required by plants for the translocation of sugars, as a sugar-borate complex, from the leaves where they are

produced to other parts of the plant. A deficiency of boron in the plant leads to an accumulation of carbohydrates in the leaves, and as a consequence of this, a depletion of carbohydrates in the roots and young leaves.

Waggoner and Dimond (1957) observed that Fusarium wilt of tomato caused by F. oxysporum f. lycopersici was increased when the roots of the plants were treated with 2,4-dichlorophenoxy-acetic acid (2,4-D) and that disease was reduced by treatment with maleic hydrazide. 2,4-D usually reduces and maleic hydrazide usually increases the sugar content of plant tissues treated with these substances (Wood, 1968). A carbohydrate deficiency was induced in tomato plants treated with 2,4-D, and infection with F. oxysporum f. lycopersici was increased, whereas plants treated with maleic hydrazide were carbohydrate sufficient and less readily infected.

The nature and quantity of the carbohydrates required by different pathogens and their hosts are very diverse, and conditions which apply to any given host-parasite relationship may be inapplicable to another. If the nutrition of F. oxysporum f. lini is similar to that of F. oxysporum f. lycopersici, and if the carbohydrate deficiency induced by boron deficiency and 2,4-D treatment are comparable, failure of F. oxysporum f.

lini to parasitize the roots of boron deficient plants may be due to the absence of carbohydrates essential for the growth of the pathogen.

The response of tomato plants to boron seems to be determined by the direct and intimate relationship between boron and calcium in plant metabolism (Brennan and Shive, 1948). The effect of high boron levels on the development of flax wilt were influenced by the levels of calcium supplied to the plants. For this reason the high level boron effects will be discussed when the calcium effects are considered.

The role of calcium in plant diseases has been investigated intensively, and the literature contains many explanations of the mechanisms of calcium metabolism in plants. Although boron deficiency had a more significant effect on the development of Fusarium wilt of flax than did calcium, the calcium effects will be discussed at greater length as these are better understood.

When flax plants were grown continuously in altered solutions and the boron supply to these plants was adequate, disease severity decreased as the calcium level of the solutions was increased from 40 to 480 ppm. If the calcium deficiency occurred only after inoculation, wilt disease was most severe at 40 ppm calcium and 2.5 ppm boron. Edgington and Walker (1958) observed similar

effects of calcium at these boron levels in tomato plants infected with F. oxysporum f. lycopersici.

When the calcium deficiency occurred before inoculation only, wilt disease was least severe at 40 ppm calcium and 0.5 ppm boron. Although this effect of calcium was not statistically significant, it is interesting and may be important. Corden (1965) and Wills and Moore (1969) have reported that a low level of calcium supplied before inoculation only, reduced the severity of tomato wilt and tobacco black shank, respectively.

Keyworth and Dimond (1952) suggested that treatments which damaged tomato plants generally reduced the severity of Fusarium wilt disease, probably by increasing the mechanical resistance of the plants to invasion by the pathogen. Corden (1965) found that when calcium deficiency occurred in tomato plants before and after inoculation, wilt disease was increased. This appeared to be an exception to Keyworth and Dimond's hypothesis. However, when the calcium deficiency occurred before inoculation only, disease was reduced as expected from the hypothesis. This pre-inoculation effect was observed only when the calcium deficient plants were returned to normal calcium nutrition after inoculation.

The roots of calcium deficient flax plants were sparsely colonized by F. oxysporum f. lini, and the advance

of the fungus in the host stems was restricted at 40 ppm calcium and 0.5 ppm boron. This effect was observed only when plants were exposed to the calcium deficiency before inoculation and were returned to normal nutrition after inoculation. Corden (1965) obtained similar retardation of growth of F. oxysporum f. lycopersici when low and normal calcium levels were supplied to the tomato plants before and after inoculation, respectively. He suggested that an increase in the level of calcium supplied to the plants after infection, interfered with pathogenesis by limiting the growth of the fungus in the host stems. Calcium reduced Fusarium wilt by inhibiting the activity of fungal polygalacturonase which normally functions to provide a carbon source for the pathogen.

In in vitro experiments Corden found that calcium only slowed polygalacturonase activity but did not block it completely. After a 24-hour incubation period, pectate hydrolysis at all calcium levels increased greatly.

If the hypothesis of Keyworth and Dimond is valid, and the explanations proposed by Corden are applicable to Fusarium wilt of flax, the increased resistance of flax plants at low calcium levels, to Fusarium wilt, and the retardation of the growth of F. oxysporum f. lini in the flax stems, may be due to the effects of root damage on resistance and of calcium on the enzyme activity of the pathogen.

High calcium levels supplied continuously or after inoculation only, significantly reduced the severity of wilt disease when the boron supply to the plants was adequate or excessive.

Corden and Edgington (1960) and Bateman and Maxwell (1965) observed an increase in severity of tomato wilt and seedling blight of beans, respectively, when these plants were deficient in calcium. The pectic materials which form part of the cell walls and middle lamella of plants are readily hydrolysed by fungal enzymes. If these pectic compounds are bonded to calcium ions, cell wall materials are relatively resistant to maceration by fungal pectic enzymes. In the absence of sufficient calcium, calcium pectates are not formed and the pectic materials remain susceptible to the enzymes. Bateman and Maxwell showed that older bean seedlings were more resistant to seedling blight than younger seedlings and that the natural resistance of bean hypocotyls to Rhizoctonia solani was associated with the conversion of pectin to calcium pectates during hypocotyl maturation. High calcium levels supplied after inoculation may reduce wilt in flax by comparable mechanisms.

The "chronic wilt" symptoms observed in Fusarium wilt of flax may result from damage to the conducting elements of the plant by fungal metabolites, or by

obstruction of these elements by fungal materials and/or metabolic products of the infected plant, or by the action of fungal toxins (Wood, 1968). If toxins or toxic fungal metabolites are involved in the flax wilt syndrome, the effect of high and low levels of calcium on disease may be explained by the effect of calcium on the metabolism of the diseased plant.

Wheeler and Hanchey (1968) suggested that in Victoria blight of oats, caused by Helminthosporium victoriae, toxic cationic molecules may react with phosphoryl groups on the cell membrane surface, and cause lethal effects by the oxidation of sulfhydryl groups. Calcium ions which are bound to the sulfhydryl groups may protect them by interfering with the binding of the toxic cations to these groups. The absence of calcium at the membrane sites in calcium deficient plants may account for alterations in membrane permeability, due to destruction by toxins, with a consequent increase in disease intensity. Calcium sufficient plants show a greater resistance to disease probably as a result of decreased membrane permeability.

The effect of calcium on disease development may therefore be threefold:

(1) A deficiency may increase membrane permeability and increase the susceptibility of a plant to

disease, whereas an excess may increase resistance by decreasing the permeability of the plasma membranes.

(2) In adequate or excessive amounts, calcium may, through bonding of the pectic substances, decrease the susceptibility of the host tissues to degradation by fungal polygalacturonase. Conversely, when calcium is deficient, the amount of calcium bonding is reduced and host tissues are readily hydrolysed.

(3) If high levels of calcium inhibit fungal polygalacturonase, the pathogen is deprived of an important carbon source--galacturonic acid, and its invasiveness is consequently reduced.

In plants receiving normal calcium nutrition, although growth of the pathogen and disease were initially slowed, severe disease symptoms eventually developed. Because excess calcium has only a temporary effect (Corden, 1965), it is not likely to be useful in controlling Fusarium wilt.

When flax plants were grown in complete solutions before inoculation and transferred to altered solutions after inoculation, wilt disease symptoms were most severe at 40 ppm calcium and 2.5 ppm boron, and least severe at 480 ppm calcium and 2.5 ppm boron. At high boron levels toxicity symptoms were most severe at 40 ppm calcium and

decreased in severity as the calcium concentration was increased from 40 to 480 ppm. Brennan and Shive (1948) and Laganière and Sackston (1967) observed similar effects of calcium on boron toxicity, in tomato and flax plants, respectively.

High boron and calcium levels in the host plant tissues also inhibited the advance of Fusarium in flax stems. Bowen and Gauch (1966) reported that in in vitro experiments a concentration of 250 mg/l boron was toxic to N. crassa and inhibited its growth and reproduction. If flax plants which were grown in nutrient solutions containing 2.5 ppm boron, accumulated this element to much higher concentrations within the tissues, the decreased colonization of the host tissues and the reduced disease severity observed at high calcium and high boron levels may be due to the effect of high calcium concentrations on pathogenesis, and of high boron levels on the growth of the pathogen.

According to Yarwood's definition of predisposition (Yarwood, 1959), a boron deficiency before inoculation predisposed flax plants to Fusarium wilt. Low calcium treatment before inoculation followed by normal calcium nutrition, reduced disease. The increased wilt observed when plants were grown continuously in calcium

deficient solutions, or were supplied with low calcium after inoculation only, is not the result of predispositon but rather of a stimulation of pathogenesis.

Flax plants inoculated when 21 days old, irrespective of their nutritional status at the time of inoculation, showed more severe disease symptoms than plants which were inoculated when 28 days old (Tables 2 - 7). Because the anatomical and physiological changes which occur in a plant as it matures are so diverse, it is not possible to offer any valid explanations as to the cause of the increase in resistance without further detailed study of the subject.

In preliminary experiments, the severity of flax wilt was evaluated by the extent of vascular discolouration of the infected stems. Most of the fungi causing vascular wilts produce a browning of the walls of the translocating elements of the xylem. This is perhaps one of the least variable symptoms of vascular wilts (Winstead and Walker, 1953), and because of this, is regarded as a fairly reliable method of assessing disease severity (Dimond et al, 1952). This method of assessing disease proved to be unsuitable, as it was impossible to differentiate between the browning resulting from nutritional deficiencies or excess, and that due to the action of the pathogen. In subsequent experiments, disease was

estimated by the amount of chlorosis and necrosis of the leaves. Leaves were counted from the base upwards. This gave a valid estimate of the wilt disease, as the basal chlorosis and necrosis, except in a few cases, was due to the infection and not to the nutritional effects.

In assessing disease severity for the various calcium and boron treatments, differences in shoot weight were considered to be a more valid criterion for disease evaluation than differences in shoot height, because the latter showed too great variability among treatments. In many cases, inoculated plants within the same treatment were taller than uninoculated plants, and inoculated plants showed a marked hyponasty of the leaves.

Dimond and Waggoner (Wood, 1968) found that plants infected with Fusarium and Verticillium spp. contained an excess of growth promoting substances relative to uninfected plants, and that one of the more reliable symptoms of the vascular wilt diseases is the epinasty of the lower leaves of infected plants. These growth regulators were believed to be produced by the pathogen, as many fungi when supplied with tryptophane, produced growth regulating substances in vitro. Matta and Gentile (Wood, 1968) observed that the IAA concentration in banana plants infected with F. oxysporum f. cubense, was 12 µg/

100 g as compared with 1 µg/100 g in healthy plants.

The observed increase in height may be attributed to the increased concentration of growth promoting substances in plants infected with F. oxysporum f. lini. In this connection it is significant that the severity of the vascular wilts may be modified by treating plants with growth regulating substances in appropriate concentrations (Corden and Dimond, 1959).

The symptoms of boron and calcium deficiency and excess described were in most cases similar to those obtained by Laganière and Sackston (1967). They observed an extraordinary fasciation of flax plants and other deficiency and toxicity symptoms which were more or less pronounced than those observed in these experiments. Discrepancies were probably due to differences in the duration of exposure to the various nutrient treatments, and the environmental conditions used. Their experiments were carried out in the greenhouse with variable conditions whereas these were performed in controlled environment chambers.

The experimental data include only macroscopic observations of the effects of calcium and boron nutrition of flax on the development of Fusarium wilt. Additional experiments to determine the microscopic effects of these elements on pathogenesis should be conducted.

VI. SUMMARY

Marine flax plants were grown in nutrient solutions supplied with all combinations of high, normal and low levels of boron and calcium and inoculated with Fusarium oxysporum f. lini after 14 and 21 days exposure to the various nutrient treatments.

The fungus was pathogenic to flax plants and was reisolated from the roots, lower and upper stems 14 days after inoculation. The height to which the fungus invaded the stems varied according to the calcium and boron levels supplied to the host plants.

Older plants appeared less susceptible to Fusarium wilt. Plants inoculated 14 days after exposure to the various nutrient regimes were more susceptible to disease and showed more severe disease symptoms than those inoculated at 21 days.

The symptoms associated with nutrient deficiency or excess were more pronounced in inoculated than in uninoculated plants.

Inoculated plants exposed to the low boron treatment for the entire growth period were more severely diseased than those subjected to any other treatment. At the zero boron level, disease severity decreased as the calcium level was increased from 40 to 480 ppm.

Plants grown in boron deficient solutions before inoculation and then transferred to normal solutions after inoculation were also severely diseased. When the boron deficiency occurred after inoculation, however, the disease was less severe than when the deficiency occurred before inoculation only, or before and after inoculation.

When plants grown continuously in nutrient solutions of various calcium and boron composition were inoculated 14 days after exposure to the nutrient treatments (21 days old), advance of Fusarium was unrestricted in plants supplied with 0 boron and 40 or 480 ppm calcium.

When the inoculation was made after 21 days (28 days old), Fusarium failed to infect and successfully parasitize the roots of plants supplied with 0 boron and 40 ppm calcium. In solutions with 0 boron and 200 or 480 ppm calcium, the percentage infection and the extent of invasion of the host stems was increased.

At high boron levels and normal or high calcium levels the advance of Fusarium in the host stems was reduced.

When plants were grown continuously in altered solutions and inoculated after 14 days (when 21 days old), disease was severe at low calcium and low boron levels but decreased in severity with increasing boron levels from 0 to 2.5 ppm. If calcium deficient plants were inoculated when 28 days old, wilt disease was most severe at 0 and 2.5 ppm boron.

Plants grown continuously in solutions of various calcium and boron composition and inoculated when 21 or 28 days old were least severely diseased when they received 480 ppm calcium and 0.5 ppm boron.

When plants were grown in normal solutions for 14 or 21 days before inoculation and transferred to altered solutions after inoculation, plants supplied with 40 ppm calcium and 2.5 ppm boron were more severely diseased than those subjected to any other treatment. The least severely diseased plants were those supplied with 480 ppm calcium and 0.5 ppm boron.

When altered solutions were supplied to the plants for 21 or 28 days before inoculation and the plants were transferred to normal solutions after inoculation, wilt disease had least effect on the weight of shoots at 40 ppm calcium and 0.5 ppm boron.

Withertop symptoms were observed on inoculated plants kept at 40 ppm calcium and all levels of boron prior to inoculation or throughout the experiment. Withertop symptoms were observed on uninoculated plants maintained continuously in solutions at 40 ppm calcium and all levels of boron. When plants were transferred to normal solutions after 14 or 21 days, withertop developed in plants which had been maintained at 40 ppm calcium and 0 or 0.5 ppm boron, but not those with 2.5 ppm boron.

Low calcium levels prior to inoculation only, decreased wilt severity; low calcium levels before and after inoculation, or only after inoculation, increased wilt severity.

Plants supplied with 480 ppm calcium and 0.5 or 2.5 ppm boron before and after inoculation, or after inoculation only, were least severely diseased.

When plants were exposed to a calcium deficiency continuously or after inoculation only, advance of Fusarium was unrestricted at 40 ppm calcium and 0 and 0.5 ppm boron.

At 480 ppm calcium advance of the pathogen was restricted at normal and high levels of boron.

When the calcium deficiency occurred before inoculation and plants were supplied with normal calcium levels after inoculation advance of the fungus was reduced at 40 ppm calcium and 0.5 ppm boron.

There was some correlation between the invasiveness of the pathogen and the severity of disease. At 480 ppm calcium and 0.5 and 2.5 ppm boron, disease was least severe and Fusarium was most restricted.

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APPENDIX

APPENDIX TABLE 1. Analysis of Variance for Plants Grown in Nutrient Solutions of Various Calcium and Boron Composition for 35 Days and Inoculated when 21 Days Old

(i) Shoot Height

Source	df	SS.	MS.	F.
B	2	881.8	440.9	45.26 **
Ca	2	100.3	50.1	5.14 *
Ca x B	4	105.6	26.4	2.71
Inoc.	1	378.3	378.3	38.83 **
B x Inoc.	2	213.4	106.7	10.95 **
Ca x Inoc.	2	71.8	35.9	3.68 *
B x Ca x Inoc.	4	11.0	2.07	0.212
Error	18	175.4	9.74	
Total:	35	1937.6		

(ii) Root Weight

Source	df	SS.	MS.	F.
B	2	91.9	45.9	4.56 *
Ca	2	0.31	0.15	
B x Ca	4	5.17	1.29	
Inoc.	1	59.3	59.3	5.89 *
Inoc. x B	2	11.4	5.7	
Inoc. x Ca	2	3.7	1.8	
Ca x B x Inoc.	4	9.43	2.3	
Error	18	181.21	10.06	
Total:	35	237.6		

* Significant at 5% level.
** Significant at 1% level.

(iii) Shoot Weight

Source	df	SS.	MS.	F.
B	2	291.0	145.5	53.49 **
Ca	2	48.6	24.3	0.89
B x Ca	4	50.9	12.7	0.46
Inoc.	1	1747.3	1747.3	64.23 **
B x Inoc.	2	174.9	87.4	3.21
Ca x Inoc.	2	9.0	4.5	0.16
B x Ca x Inoc.	4	6.3	1.5	0.55
Error	18	489.8	27.2	
Total:	35	2817.8		

APPENDIX TABLE 2. Analysis of Variance for Plants Grown in Nutrient Solutions of Various Calcium and Boron Composition for 42 Days and Inoculated when 28 Days Old

(i) Shoot Height

Source	df	SS	MS	F
B	2	11,250.5	5,625.20	151.33 **
Ca	2	115.7	57.80	1.55
B x Ca	4	244.7	61.1	1.64
Inoc.	1	61.8	61.8	1.66
B x Inoc.	2	33.0	16.0	0.43
Ca x Inoc.	2	116.3	58.1	1.56
Ca x B x Inoc.	4	206.1	51.5	1.38
Error	18	669.2	37.17	
Total:	35	12,697.3		

(ii) Root Weight

Source	df	SS	MS	F
B	2	470.86	235.43	18.94 **
Ca	2	3.90	1.95	0.156
B x Ca	4	12.21	3.05	0.24
Inoc.	1	77.78	77.78	6.25 *
B x Inoc.	2	56.15	28.15	2.26
Ca x Inoc.	2	8.49	4.24	0.34
B x Ca x Inoc.	4	34.33	8.58	0.69
Error	18	223.89	12.43	
Total:	35	887.61		

(iii) Shoot Weight

Source	df	SS	MS	F
B	2	4,426.55	2,213.27	69.14 **
Ca	2	127.34	63.67	1.98
B x Ca	4	79.26	19.81	0.61
Inoc.	1	1,450.47	1,450.47	45.31 **
B x Inoc.	2	578.56	289.28	9.03
Ca x Inoc.	2	32.63	16.31	0.50
B x Ca x Inoc.	4	170.07	42.51	1.32
Error	18	576.31	32.01	
Total:	35	6,864.88		

APPENDIX TABLE 3. Analysis of Variance for Plants Grown in Complete Nutrient Solutions for 14 Days before Inoculation and Transferred to Altered Solutions after Inoculation

(i) Shoot Height

Source	df	SS	MS	F
B	2	849.4	424.7	18.70 **
Ca	2	653.0	326.5	14.38 **
B x Ca	4	118.6	29.6	1.30
Inoc.	1	0.1	0.1	
B x Inoc.	2	223.2	111.6	4.91 *
Ca x Inoc.	2	115.8	57.9	2.55
B x Ca x Inoc.	4	123.3	30.8	1.35
Error	18	409.7	22.7	
Total:	35	2,493.1		

(ii) Shoot Weight

Source	df	SS	MS	F
B	2	24.53	12.26	0.52
Ca	2	246.03	123.01	5.31 *
B x Ca	4	19.33	4.83	
Inoc.	1	360.55	360.55	15.58 **
B x Inoc.	2	52.73	26.36	1.13
Ca x Inoc.	2	19.98	9.99	
B x Ca x Inoc.	4	25.76	6.44	
Error	18	416.58	23.14	
Total:	35	1,165.49		

(iii) Root Weight

Source	df	SS	MS	F
B	2	304.93	152.46	2.311
Ca	2	33.55	16.77	0.254
B x Ca	4	11.74	2.93	
Inoc.	1	18.04	18.04	
B x Inoc.	2	28.94	14.47	
Ca x Inoc.	2	80.76	40.38	
B x Ca x Inoc.	4	94.69	23.67	
Error	18	1,187.11	65.95	
Total:	35	1,759.76		

(iv) Percent Infection

Source	df	SS	MS	F
B	2	257.3	128.6	6.46 **
Ca	2	389.8	194.9	9.79 **
B x Ca	4	121.1	30.2	1.51
Inoc.	1	233.6	233.6	11.73 **
B x Inoc.	2	100.5	50.2	2.52
Ca x Inoc.	2	18.7	9.3	0.46
B x Ca x Inoc.	4	35.7	8.9	0.44
Error	18			
Total:	35	1,156.7		

APPENDIX TABLE 4. Analysis of Variance for Plants Grown in Altered Solutions for 14 Days before Inoculation and Transferred to Complete Solutions after Inoculation

(i) Shoot Height

Source	df	SS	MS	F
B	2	5,967.6	2,983.8	85.49 **
Ca	2	780.3	390.1	11.17 **
B x Ca	4	3,189.5	797.3	22.84 **
Inoc.	1	102.3	102.3	2.93
B x Inoc.	2	9.0	4.5	0.128
Ca x Inoc.	2	20.7	10.3	0.295
B x Ca x Inoc.	4	165.6	41.4	1.18
Error	18	628.2	34.9	
Total:	35	10,863.2		

(ii) Shoot Weight

Source	df	SS	MS	F
B	2	371.77	185.88	14.53 **
Ca	2	20.10	10.05	
B x Ca	4	361.28	90.32	7.06 **
Inoc.	1	629.59	629.59	49.22 **
B x Inoc.	2	183.39	91.69	7.16 **
Ca x Inoc.	2	4.41	2.20	
B x Ca x Inoc.	4	55.43	13.85	1.082
Error	18	230.30	12.79	
Total:	35	1,856.27		

(iii) Root Weight

Source	df	SS	MS	F
B	2	5.85	2.92	0.710
Ca	2	3.67	1.83	0.445
B x Ca	4	10.04	2.51	0.610
Inoc.	1	67.16	67.16	16.340 **
B x Inoc.	2	39.37	19.68	4.788 *
Ca x Inoc.	2	2.29	1.14	0.277
B x Ca x Inoc.	4	7.51	1.87	0.454
Error	18	74.11	4.11	
Total:	35	210.00		

(iv) Percent Infection

Source	df	SS	MS	F
B	2	6.7	3.3	0.84
Ca	2	14.4	7.2	1.84
B x Ca	4	12.4	3.1	0.79
Inoc.	1	736.7	736.7	188.8 **
B x Inoc.	2	393.5	196.7	50.43 **
Ca x Inoc.	2	9.2	4.6	1.04
B x Ca x Inoc.	4	55.7	13.9	3.56 *
Error	18	70.6	3.9	
Total:	35	1,299.2		

APPENDIX TABLE 5. Analysis of Variance for Plants Grown in Complete Solutions for 21 Days before Inoculation and Transferred to Altered Solutions after Inoculation

(i) Shoot Height

Source	df	SS	MS	F
B	2	272.3	136.1	4.94 *
Ca	2	1.7	0.8	
B x Ca	4	185.8	46.4	1.684
Inoc.	1	16.2	16.2	0.58
B x Inoc.	2	390.0	195.0	7.07 **
Ca x Inoc.	2	339.7	169.8	6.163 **
B x Ca x Inoc.	4	81.9	20.4	0.74
Error	18	496.0	27.55	
Total:	35	1,783.6		

(ii) Shoot Weight

Source	df	SS	MS	F
B	2	149.75	74.87	1.493
Ca	2	33.16	16.58	0.33
Ca x B	4	255.72	63.93	1.274
Inoc.	1	679.55	679.55	13.54 **
B x Inoc.	2	42.48	21.24	0.423
Ca x Inoc.	2	147.68	73.84	1.472
B x Ca x Inoc.	4	42.40	10.60	0.211
Error	18	902.93	50.16	
Total:	35	2,253.67		

(iii) Root Weight

Source	df	SS	MS	F	
B	2	155.70	77.85	23.88	**
Ca	2	8.35	4.17	1.27	
B x Ca	4	12.19	3.04	0.93	
Inoc.	1	53.81	53.81	16.50	**
B x Inoc.	2	6.55	3.27	1.00	
Ca x Inoc.	2	5.33	2.66	0.81	
B x Ca x Inoc.	4	8.86	2.21	0.67	
Error	18	58.80	3.26		
Total:	35	309.59			

(iv) Percent Infection

Source	df	SS	MS	F	
B	2	110.9	55.4	2.082	
Ca	2	2.0	1.0		
B x Ca	4	27.4	6.8	0.025	
Inoc.	1	711.2	711.2	26.73	**
B x Inoc.	2	190.42	95.1	3.37	*
Ca x Inoc.	2	16.4	8.2	0.30	
B x Ca x Inoc.	4	28.6	7.1		
Error	18	478.8	26.6		
Total:	35	1,565.5			

APPENDIX TABLE 6. Analysis of Variance for Plants Grown in Altered Solutions for 21 Days before Inoculation and Transferred to Complete Solutions after Inoculation

(i) Shoot Height

Source	df	SS	MS	F	
B	2	1,987.2	993.6	275.24	**
Ca	2	19.8	9.9	2.74	
B x Ca	4	62.5	15.6	4.32	*
Inoc.	1	43.9	43.9	12.16	**
B x Inoc.	2	14.3	7.1	1.96	
Ca x Inoc.	2	24.4	12.2	3.37	
B x Ca x Inoc.	4	8.7	2.1	0.58	
Error	18	65.1	3.61		
Total:	35	2,225.9			

(ii) Shoot Weight

Source	df	SS	MS	F	
B	2	3,621.86	1,810.93	48.18	**
Ca	2	66.85	33.42	0.88	
B x Ca	4	216.65	54.16	1.44	
Inoc.	1	1,286.90	1,286.90	34.24	**
B x Inoc.	2	146.88	73.44	1.95	
Ca x Inoc.	2	8.01	4.00	0.01	
B x Ca x Inoc.	4	71.00	17.75	0.31	
Error	18	676.45	37.58		
Total:	35	6,094.60			

(iii) Root Weight

Source	df	SS	MS	F	
B	2	41.67	20.83	2.719	
Ca	2	11.74	5.87	0.766	
B x Ca	4	43.60	10.90	1.422	
Inoc.	1	33.91	33.91	4.426	*
B x Inoc.	2	11.90	5.95	0.912	
Ca x Inoc.	2	13.97	6.98	0.776	
B x Ca x Inoc.	4	9.68	2.47		
Error	18	137.94			
Total:	35	294.73			

(iv) Percent Infection

Source	df	SS	MS	F	
B	2	48.8	24.4	4.70	*
Ca	2	15.6	7.8	1.52	
B x Ca	4	7.8	1.9	0.37	
Inoc.	1	605.8	605.8	118.78	**
B x Inoc.	2	32.4	16.2	3.17	
Ca x Inoc.	2	27.2	13.6	2.66	
B x Ca x Inoc.	4	47.5	11.8	2.31	
Error	18	93.5	5.1		
Total:	35	878.6			