The relation of calcium nutrition and bacterial populations in roots to development of leaf symptoms in soybeans

Antonio Garza
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THE RELATION OF CALCIUM NUTRITION AND BACTERIAL POPULATIONS IN ROOTS TO DEVELOPMENT OF LEAF SYMPTOMS IN SOYBEANS.

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THE RELATION OF CALCIUM NUTRITION AND BACTERIAL
POPULATIONS IN ROOTS TO DEVELOPMENT OF
LEAF SYMPTOMS IN SOYBEANS

by

Antonio Garza

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Major Subject: Plant Pathology

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

Head of Major Department

Signature was redacted for privacy.

Dean of Graduate College

Iowa State University
Of Science and Technology
Ames, Iowa

1964
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I. INTRODUCTION

The relation of plant nutrition to disease development has been investigated for several crops and pathogens. Nutrient concentration has affected plants and pathogens in many ways. Sometimes plants grown in high nutrient concentrations grow vigorously and may resist or escape infection by pathogens. In other circumstances, plants growing vigorously may furnish a suitable media for the development of the pathogen and susceptibility may increase. Consequently, the effect of nutrients on disease development in plants is varied, and, so far, not readily explained. The effects are probably exerted on the host and the pathogen separately, as well as on the interaction of the two.

The function of Ca (calcium) in plant metabolism and its agronomic importance has been stressed many times. Studies have shown that Ca is important for normal cell function in a variety of ways, and that the various effects may depend on some fundamental structural role. It has been suggested that Ca is involved in the structure and/or maintenance of the plasma and vascular membranes.

Recently, attention has been given to Ca nutrition and growth regulators in relation to the calcium pectate of the cell wall and its resistance to Fusarium organisms.

Howell and Bernard (1961) categorized 44 soybean varieties according to tolerance to P (phosphorus) concentrations. These
authors considered discoloration (necrosis) of the tissues to be a physiological response of soybeans to high concentrations of P. Dunleavy et al. (1964) reported the relation of P concentration to high bacterial populations.

The present investigation was conducted to study the effect of high Ca nutrition on soybeans, and the relation of high Ca to disease development and bacterial populations.
II. LITERATURE REVIEW

No previous work has been published regarding the expression of leaf symptoms in soybeans grown in high Ca nutrient solutions. Most of the work has dealt with deficiency and interaction of Ca with mineral absorption.

The relation of each one of the essential mineral elements to the composition and physiological characteristics of soybean plants was investigated by Ginsburg (1925). Pathological conditions due to the lack of any one element appeared first and most pronounced in plants grown in calcium-free solution, followed in order by N, K, and Mg. The plants grown in the incomplete solutions, with the exception of those grown in solutions lacking S, always absorbed more Ca and less N and Mg. High Ca in these plants resulted in low N and Mg content, and low Ca content resulted in high N and Mg content.

The role of major elements in plant nutrition as related to disease development has been reported in a series of papers by Walker and others (1945a, 1945b, 1946, 1948). The relation of nutrition to development of symptoms of cabbage yellows, using basal Hoagland solution at different concentrations, was reported by Walker and Hooker (1945a). Their results indicated a progressive decline in rate of disease development in susceptible plants as the salt concentration was increased. When K was omitted from the solution, the rate of disease development
increased in the susceptible plants, but when N and P were omitted the rate of disease development decreased.

An excess of K and N increased the disease index when studying cabbage clubroot disease; increasing P had little effect, but omission of K or P usually decreased the disease index. Omission of N increased the disease index (Walker and Hooker 1945b).

Fusarium wilt of tomato (*Fusarium oxysporum* f. *lycopersici* (Sacc.) Snyd. and Hans.) was studied in relation to plant nutrition. The nutrient solutions used were 0.1, 0.5, 2.0 and 3.0 times the basal Hoagland solution. Disease development in susceptible or intermediate resistant hosts grown at a temperature optimum for wilt, decreased with an increase in nutrient concentration. When nutrient concentration was increased up to 2 times, the diffusion pressure deficit in cells of uninoculated plants increased. Growth of the host alone, or the pathogen alone, was low in the 0.1 solution; reached a maximum in the 1.0 time solution; and decreased with further increase in the total salt concentration. Studies of the effect of nutrient balance upon disease development revealed that infected plants wilted more severely when they received a low concentration of K or a high concentration of N. Solutions low in N or high in K brought about a decrease in disease severity (Walker and Foster 1946).

The effects of nutrient concentration on bacterial canker disease (*Corynebacterium michiganense* (Smith) Jensen) of tomato
was studied by Walker and Kendrick (1948). The authors reported that the degree of development of the wilt phase of the disease increased with increasing concentration from 0.1 to 2.0 times the concentration of the basal solution. The production of cankers on the stems of hosts was opposite to the wilt phase, being greater at 0.1 concentration and less at 3.0 times the basal concentration. Difference in the development of the wilt phase at high and low levels of N, K and P was not significant.

The relation of plant nutrition to bacterial wilt of tomato (Pseudomonas solanacearum E. F. Smith) was reported by Gallegly and Walker (1949) in another paper. The concentration of the solutions used were 0.1, 0.5, 1.0, 2.0, and 3.0 times the concentration of the salts in the Hoagland and Snyder solution. They also used low and high N, P and K levels, as well as low P adjusted to low, medium, and high pH. Disease development during the summer months was greatest at 0.1 nutrient concentration and decreased with an increase in salt concentration. In early spring and late autumn disease development increased with an increase in salt concentration from 0.1 up to 1.0 time, but decreased sharply with further increase.

Disease development in the summer in the unbalanced solutions increased at the low K level and decreased at the high N level. When the sand substrate was held at 32°C disease development decreased at high N concentration and increased at
low N concentration. The reverse was true when the substrate was held at 24°C. The two sand temperatures had no effect on disease development in low K solutions. There were indications that the pH of the salt solution reduced disease development.

There is very little literature relating Ca utilization to soybean growth. Ohlrogge (1960) has recently summarized the mineral nutrition of soybeans and he reported that apparent abundance of Ca under field conditions may have removed the incentive for its study; however, this does not preclude the possibility of a vital role of Ca as a factor limiting the yield of soybeans.

Experiments dealing with nutrition in water culture involving different Ca concentrations and different plant species have been reported by Olsen (1942). The Ca concentration at which the plants reached their highest production of dry material varied with the species. As Ca concentration was increased, Sinapis alba L., Dianthus barbatus L., Hordeum distichon L. absorbed more Ca but less Mg and K. When the K content of the nutrient solution was low, the production of dry material by the plants decreased. When the Ca content of the solution was increased, the plants received insufficient K except when cultivated with high K solutions.

The need of Ca for proper growth and development of seedlings was noted in an early paper by True (1921) and later by Albrecht (1941). Changes in wheat and corn root tips when grown
in nutrient solutions deficient in Ca were reported by Bamford (1931). Pronounced cell injury was correlated with the ratio of Ca to all of the other components of the solution.

Sorokin and Sommer (1940) described the effects of the absence of Ca on cells and tissues of Garden pea (\textit{Pisum sativum} L.). Roots responded noticeably to small amounts of Ca. Normal mitotic cell divisions were disturbed in the absence of Ca. Polyploid nuclei, binucleate cells, constricted nuclei, and accumulation of amitotic divisions were observed. Even more important was the observation that tissues lacked resistance to infection by the microorganisms that penetrated the cells and accelerated the desintegration of the protoplasts. A detailed account of the effect of Ca deficiency in the tomato plant was reported by Kalra (1956).

Submicroscopic aspects of Ca deficiency in the shoot apex of barley (\textit{Hordeum vulgare} L.) were studied by Marinos (1962). The author presented evidence that indicated Ca is essential for the maintenance of structural integrity of the various membranous components of the protoplast, and that Ca can exert a profound influence on the metabolism of the cell, ranging from the respiratory mechanism and ion uptake to the less understood reactions that culminate in wall development and cell growth.

"Damping off" was considered associated with the physicochemical properties of the soil by Albrecht and Jenny (1931). By increasing Ca concentration the authors were able to decrease
the number of disease plants; they considered that hydrogen-ion concentration was not an important factor.

The role of Ca in producing healthy radicles of cotton was described by Presley and Leonard (1948). These workers did not describe any further symptoms of this deficiency or indicate a possible relationship with the seedling disease complex. Ranney and Bird (1958) listed CaCl\textsubscript{2} and Ca(NO\textsubscript{3})\textsubscript{2}, along with various other compounds, as being effective in helping to maintain stands of cotton in which seedling diseases were present. Treatments of Ca on seeds with varying stages of deterioration produced more healthy and vigorous seedlings than those from untreated seed (Metzer et al. 1961).

Horsfall et al. (1954) worked on the relation of the Ca and hydrogen-ion of the soil and tubers to development of potato scab. Scab was increased when Ca content of the tubers was increased. The Ca content of the tuber was governed by the content of replaceable Ca in the soil, which was in turn governed by hydrogen-ion concentration and the amount of Ca applied to the soil.

Blossom-end rot of tomato was related to the Ca nutrition of the plant. Field and greenhouse experiments by Evans and Troxler (1953) demonstrated that high Ca fertilization, CaCl\textsubscript{2} sprays, and Ca(C\textsubscript{6}H\textsubscript{11}O\textsubscript{7})\textsubscript{2}. H\textsubscript{2}O (calcium gluconate) injections into tomato fruit, either prevented, or markedly reduced, the incidence of the disease.
Ca deficiency, as related to the infection of *Nicotiana glutinosa* L. by tobacco mosaic virus, was studied by Chessin and Scott (1955). Differences in local lesion counts between controls and Ca deficient plants were highly significant. Part of the work indicated that Ca deficiency affected intrinsic cell susceptibility, rather than mechanical resistance to virus entry.

Pectic enzymes produced by *Fusarium oxysporium f. lycopersici* (Sacc) Snyd. and Hans. were reported by Gothoskar et al. (1955) to be important factors in causing the characteristic wilt and vascular browning associated with Fusarium wilt of tomato. Ca and B (boron) nutrition on the development of Fusarium wilt of tomato were studied by Edgington and Walker (1958). The authors reported that an increase in Ca in the nutrient definitely retarded the development of wilt. Response of growth regulators and Ca nutrition as related to the Ca bonding of the pectic substances of the plant cell wall has been studied by Corden and Edgington (1960), and Edgington, Corden and Dimond (1961). The nature of the pectic substance in the host was apparently correlated with the resistance of the pectins to hydrolysis by fungal pectolytic enzymes and with resistance of the host to Fusarium wilt.

Polyvalent cations often exert a striking influence on the absorption of monovalent ions. The accumulation of K and Br (bromine) was demonstrated by Viets (1944) to be accelerated by
the alkaline earth metals and Al (aluminum). Uptake of K was either depressed or stimulated by Ca, depending upon the concentration of the former (Overstreet et al. 1952). The deleterious effect of low pH on the growth of lettuce and tomato could be lessened by increasing the Ca content of the nutrient solution according to Arnon and Johnson (1942). The work of Fawzy et al. (1954) indicated that pH was a factor in the behavior of other polyvalent cations in addition to Ca. The stimulating effect increased markedly as the pH of the solution decreased. Varying the Ca content of the nutrient solution, Wadleigh and Bower (1950) were able to influence the relative Na (sodium) and K content of red kidney bean plants (Phaseolus vulgaris L.). Kahn and Hanson (1957) suggested that Ca increased the affinity between K and a postulated carrier, but decreased the velocity of the metabolic phase of K uptake in a second independent reaction.

The effect of Ca on the absorption of several monovalent cations over a wide range of pH was recently reported by Jacobson et al. (1960). They concluded that the absorption of K, Rb (rubidium) and Cs (cesium) is enhanced at low pH by Ca, and only slightly by Na. The absorption of Li (lithium) was repressed almost completely by Ca at all pH values.

The influence of Ca on root mitochondria was studied by Florell (1956). An external Ca concentration of $10^{-4}$ mole per liter increased the amount of mitochondria by 54% and their protein content by 29%. Neither respiratory activity of the
mitochondria nor the amount of other cytoplasmic material was affected by Ca. The author suggested that Ca favored the formation of mitochondria by means of its general influence on the organization of the cytoplasm.

Uptake of Ca and Cl by the roots of *Zea mays* L. was a non-metabolic process in the meristematic portion of the root tip, according to Handley and Overstreet (1961). The uptake of these ions into vacuolated portions of the roots was strongly temperature-dependent and, hence, largely metabolic.

Growth rates as influenced by CaCl$_2$, SrCl$_2$ (strontium chloride), MgCl$_2$ (magnesium chloride), and KCl (potassium chloride) were studied by Cooil and Bonner (1957) using *Avena* coleoptiles. Elongation rates were inhibited greatly by the Ca and Sr salts, and to a lesser extent by the Mg salt. Growth inhibition was reversed slowly by Ca free solutions containing indoleacetic acid. However, the reversal was much more rapid when K salts were added. If both K and Ca were present in the medium, the coleoptiles grew normally. Coleoptiles also increased their internal osmotic pressure greatly in the presence of CaCl$_2$ and sucrose above sections in sucrose alone. Wall elasticity was decreased and turgor pressure was increased when immersed in CaCl$_2$ solutions.

Root elongation is a two step process consisting of plastic stretching followed by cell wall formation in which the stretching is amplified by indoleacetic acid but inhibited by coumarin, according to Burstrom (1957).
Physiological experiments have indicated that different strains of *Rhizobium japonicum* (Kirchner) Buchanan are correlated positively with nitrogen-fixing efficiencies and nitrate reductase activities of *Rhizobium* cells from nodules of inoculated plants (Cheniae and Evans 1957). When soybean plants were inoculated with pure cultures of Rhizobia, and grown aseptically in the absence of a source of combined N, they produced nodules with high nitrate reductase activity. The nitrate reductase activity of *Rhizobium* cells from nodules was studied by Cheniae and Evans (1960) in relation to factors known to influence N₂-fixation by nodules. A definite pattern of enzyme activity, as affected by the age of plant, was found. It consisted of an increase of the specific activity of nitrate reductase until an optimum was reached, and then a decline of activity when the nodules approached senescence. The authors reported that the application of NO₃ salts and, in some cases, NH₄ salts to soybean plants resulted in a decreased activity of nitrate reductase in nodules.

Further investigations have been conducted on the properties of an enzyme complex isolated from soybean-nodule bacteroids by Lowe and Evans (1961). The authors reported that the system not only will catalyze the reduction of nitrate to nitrite by use of DPNH or succinate as electron donors, but also will catalyze the transfer of electrons from succinate to oxygen. This process is inhibited by nitrate, suggesting that both oxygen and nitrate compete for electrons from a common source.
Numerous accounts have been published of the action of antibiotics in controlling bacterial plant diseases. Crown gall (*Agrobacterium tumefaciens* (Smith and Townsend) Conn.) Brown and Boyle (1944); halo blight of beans (*Pseudomonas phaseolicola* Dowson) Zaumeyer et al. (1953); bacterial wilt of corn (*Bacterium stewartii* E. F. Smith) Lansing and Lockwood (1957); black-rot of crucifers (*Xanthomonas campestris* (Pammel) Dowson) Klisiewicz and Pound (1960).

Knowledge of the movement of antibiotics in higher plants has been reviewed by Crowdy and Pramer (1955). Apparently a number of antibiotics were absorbed and translocated; others were not. The authors reported that neutral and acidic substances were more consistent in the results than the basic and amphoteric antibiotics.

The ease with which streptomycin is absorbed and translocated from foliar sprays appears to depend on the nature of the plant treated. Significant movement of the antibiotic in sprayed foliage has been reported for beans (*Phaseolus sp.*) Crossan and Krupka (1955); maize (*Zea mays* L.) Sabet (1956); broccoli (*Brassica oleracea* var. *italica* L.) Natti (1957) and Coleus sp. Dowler and Goodman (1958).

The effect of variation in external concentration, pH, temperature, competitive ions, respiratory inhibitors, and glucose on the rate of streptomycin uptake by cells of the alga *Nitella clavata* Kutzing (Pramer, 1956) have been reported. The
author pointed out that the process is an active transport involving an expenditure of energy by the cell.

Knowledge of the mode of action of antibiotics in plant disease control is obscure. Pramer (1959) suggested that antibiotics may control plant diseases by (1) acting directly on the pathogen; (2) neutralizing toxins secreted by the pathogen; (3) acting on the host; (4) being transformed within the plant to a substance having greater or different activity; or (5) a combination of two or more of these factors.

There is evidence that some antibiotics are degraded by plants; Prescott et al. (1956) and Gray (1958) reported that streptomycin amine and streptomycin oxime were converted in plants to compounds of an unknown nature that demonstrated increased activity.
III. MATERIALS AND METHODS

Two soybean (Glycine max (L.) Merr.) varieties, Ford and Chief, were used in all experiments and they were grown in sand-nutrient cultures and water cultures. They were chosen because they presented a different reaction when grown in a complete nutrient solution containing low or high levels of Ca. Ford is a selection from the cross Lincoln x Richland and backcrossed to Lincoln. Chief is a selection from a cross between Illini and Illinois type 95.

A. Nutrient Solution

The nutrient solution used in these experiments was a modification of the solution used by Moser (1942). It contained the same chemicals suggested by Moser to give a pH range of 6.0 to 6.5. The concentration of the salts and the ppm of the elements are presented in Table 1.

Table 1. Molar concentration and ppm of elements in the solution

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>Mols/l.</th>
<th>Element</th>
<th>ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>KH$_2$PO$_4$</td>
<td>0.00197</td>
<td>N</td>
<td>110.3</td>
</tr>
<tr>
<td>K$_2$HPO$_4$</td>
<td>0.00099</td>
<td>P</td>
<td>91.6</td>
</tr>
<tr>
<td>KNO$_3$</td>
<td>0.00709</td>
<td>K</td>
<td>431.5</td>
</tr>
<tr>
<td>(NH$_4$)$_2$SO$_4$</td>
<td>0.00039</td>
<td>Mg</td>
<td>64.0</td>
</tr>
<tr>
<td>MgSO$_4$</td>
<td>0.00263</td>
<td>Ca</td>
<td>40.1 to 320.8</td>
</tr>
<tr>
<td>CaCl$_2$</td>
<td>--------</td>
<td>Cl</td>
<td>71.0 to 568.0</td>
</tr>
</tbody>
</table>
A stock solution of minor elements, as recommended by Hoagland (1950), was added to each liter of nutrient solution at the rate of 1 ml/l. The minor element solution contained the following chemicals and ppm of elements:

- 2.86 g boric acid
- 1.81 g manganese chloride
- 0.22 g zinc sulfate
- 0.08 g copper sulfate
- 0.02 g molybdenic acid

Iron was added to the solution by using iron chelate (21.5 g/l commercial Versenol contains 9% metallic iron, 12.9% iron as Fe₂O₃, and 57.04% iron as NaFe EDTA) added at the rate of 1 ml/l. of solution when needed.

The solution was modified by using different concentrations of CaCl₂, Ca(NO₃)₂, P or NaCl to meet the needs of each experiment described here.

**B. Sand Culture**

Quartz sand washed with HCl and rinsed thoroughly with distilled water, was used in one experiment with 40, 80, 160 and 320 ppm of CaCl₂ concentrations. Ten seeds of Ford or Chief were sown in each 1 gal glazed pot, and irrigated with distilled water for 6 days. Plants were thinned after emergence to leave the five best seedlings. Nutrient solution was added in the amount of 200 ml daily until the end of the experiment. Each treatment was replicated four times for each variety.
C. Water Culture

Seeds of Ford and Chief were sown in sterile vermiculite and irrigated with tap water. Individual seedlings were rinsed in distilled water and transplanted to nutrient solution when they were approximately 5 cm long and the cotyledons started to expand.

The seedlings were suspended in a wooden frame 21 cm in diameter with 8 mm hardware cloth on top. The hardware cloth was covered with aluminum foil and punctured to introduce a 2 cm plastic tubing. Seedlings were introduced through the plastic tube and supported by the cotyledons. Nutrient solution level in the pot was maintained by adding alternately, every other day, distilled water or nutrient solution.

Compressed air was supplied to nutrient solutions by air lines connecting to air stones immersed in the nutrient solutions. Supplemental light (500 watts bulbs) was used to provide the plants with 15 hrs of light, daily, during the experiment. Greenhouse temperatures ranged from 70 to 75°F at night and from 80 to 85°F during the day.

Notes on plant characters were taken at the end of each experiment. Plant height was determined by measuring the length of the plant from the cotyledonary node to the top growing point. Leaf length and width was recorded from the blade of each one of the leaflets of the first trifoliate leaves.
Root length was measured from the cotyledonary node to tip of the tap root.

Dry weight of tops (aerial parts of the plant) and roots were obtained by weighing the respective plant part after drying for 72 hrs in an oven at 100° C.

Notes on leaf symptoms were taken by rating the unifoliolate, first trifoliate and second trifoliate leaves, from 1 to 5 according to the following description:

1 = Healthy leaf
2 = Vein necrosis, light flecking or both
3 = Approximately half of the leaf chlorotic or necrotic
4 = Approximately three-fourths of the leaf chlorotic or necrotic
5 = Entire leaf chlorotic or necrotic.

At the completion of the experiment, bacterial populations were determined from the nutrient solutions and the roots of the plants by the dilution plate technique. Bacteria were obtained from roots by crushing a uniform section of the primary root in sterile, distilled water, and putting 0.05 ml in petri plates with beef lactose agar medium. Numbers of colonies were obtained per sq. cm. Populations were calculated in thousands per ml of sample. Standard bacteriological techniques were used in identification of bacteria (Breed et al. 1957).

The pH of the nutrient solutions was determined when first prepared, and at the end of the experiments.

Determination of percentage of N, P and K was made by di-
gesting 0.50 g oven-dry samples on a hot plate in the presence of H₂SO₄ in a volumetric flask for 1 hr beyond the time the solution became colorless. After cooling, the solution was brought to volume by addition of ammonia-free water. The N was determined by the micro-Kjeldahl method. A boric acid mixed indicator solution was added to an aliquot of distillate. The ammonium in this solution was titrated against a standard H₂SO₄ solution. The P was determined on an aliquot in a colorimeter in the presence of added vanadomolybdate solution. The K was determined on an aliquot of the solution with a flame photometer using Li as an internal standard. All results were reported as percentages of the total N, P and K in the plant part in an oven dry basis.

Experiments were conducted and modified as listed below:

A. Quartz sand with 40, 80, 160 and 320 ppm CaCl₂
B. Aerated and non-aerated water culture with 40 and 320 ppm CaCl₂
C. Non-aerated water culture with 40 and 320 ppm of Ca(NO₃)₂
D. Aerated water culture with 40 and 320 ppm CaCl₂, but only 31 ppm of P
E. Aerated water culture with 10 ppm CaCl₂ but 71 and 568 ppm Cl
F. Aerated water culture with 320 ppm CaCl₂ with the varieties mixed in the same pot in indicated proportions
G. Aerated and non-aerated water culture with 320 ppm CaCl₂ and *Rhizobium japonicum* treatments.

H. Aerated and non-aerated water culture with 320 ppm CaCl₂ and streptomycin treatments.

I. Identification of bacteria.

Special methods of treatments used in some of these experiments are described with the results of each experiment.
IV. RESULTS

A. Effect of Four Ca Concentrations on Plant Height and Disease Development

The effect of 40, 80, 160 and 320 ppm of CaCl$_2$ on plant height and disease development was studied for Ford and Chief soybeans. Plants were grown for 26 days in quartz sand.

1. Plant height

Ford was taller than Chief, and the difference was greater when plants were grown in 160 ppm Ca concentration. Maximum height of Ford occurred in plants grown in 80 ppm Ca, and reduced slightly when grown in 160 and 320 ppm Ca concentration (Table 2). Height of Chief plants did not vary significantly.

2. Disease development

The disease ratings of Ford leaves increased as the Ca concentration was increased. Disease rating for the unifoliate and first trifoliate were highest at 320 ppm Ca concentration (Table 3). Development of leaf symptoms in Ford indicated a relation of the Ca concentration of the nutrient solution to disease development. Chief developed no symptoms.

B. Effect of 40 and 320 ppm CaCl$_2$ on Ford and Chief Plant Growth and Disease Development, when Grown in Aerated and Non-aerated Nutrient Solutions

1. Plant height

Ford was significantly taller than Chief when grown in
Table 2. Mean height of Ford and Chief soybeans grown in quartz sand at four Ca concentrations after 26 days

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Mean height (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>40</td>
</tr>
<tr>
<td>Ford</td>
<td>33.0</td>
</tr>
<tr>
<td>Chief</td>
<td>30.9</td>
</tr>
</tbody>
</table>

*a Mean of 20 plants

Table 3. Mean disease leaf rating of Ford soybeans grown in quartz sand at four Ca concentrations after 26 days

<table>
<thead>
<tr>
<th>Leaf</th>
<th>Mean rating</th>
<th>Ca concentration (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>40</td>
<td>80</td>
</tr>
<tr>
<td>Unifoliate</td>
<td>1.8</td>
<td>4.0</td>
</tr>
<tr>
<td>First trifoliate</td>
<td>1.3</td>
<td>2.0</td>
</tr>
<tr>
<td>Second trifoliate</td>
<td>1.5</td>
<td>1.7</td>
</tr>
</tbody>
</table>

*a Mean of 20 plants

*Rating 1 to 5
1 = Healthy leaf
2 = Vein necrosis, light flecking or both
3 = Approx. 1/2 leaf chlorotic or necrotic
4 = Approx. 3/4 leaf chlorotic or necrotic
5 = Entire leaf chlorotic or necrotic
aerated solutions containing 40 ppm Ca, but in solutions containing 320 ppm, Chief was taller than Ford. Ford grown in aerated solutions containing 320 ppm Ca was 10.6 cm shorter than when grown in non-aerated solutions. Difference between aerated and non-aerated solutions for the other treatments was not significant (Table 4).

When data for height of the two varieties grown in each concentration were combined, plants grown in solutions containing 40 ppm Ca were significantly taller than plants grown in solutions containing 320 ppm Ca in both aerated and non-aerated solutions. When data for plant height of each variety at the two concentrations were combined, Ford was significantly taller than Chief in non-aerated solutions, but not in aerated solutions. Combined data of concentrations and varieties showed that height of plants grown in non-aerated solutions was greater than the height of plants in the aerated solutions (Table 4).

2. **Leaf length and width**

The leaf length of Ford in aerated solutions containing 40 ppm Ca was significantly greater than the leaf length of Chief, while in solutions containing 320 ppm Ca Chief had longer leaves than Ford. This difference occurred also in non-aerated solutions containing 320 ppm Ca. Plants grown in non-aerated solutions had longer leaves than plants grown in aerated solutions (Table 5). Combining the data for leaf length of the two varieties grown in each concentration, plants grown in 40 ppm Ca
Table 4. Mean height of Ford (F) and Chief (C) soybeans grown in aerated and non-aerated nutrient solutions at two Ca concentrations with combined data for varieties and concentrations

<table>
<thead>
<tr>
<th>Solution</th>
<th>Ca concentration (ppm)</th>
<th>Mean plant height (cm)</th>
<th>Variety</th>
<th>Combined varieties Concentration variety</th>
<th>Combined concentrations concentration and varieties</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>40</td>
<td>320</td>
<td>F</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>Aerated</td>
<td>52.8</td>
<td>42.0</td>
<td>36.0</td>
<td>43.8</td>
<td>47.4 39.9 44.4 42.9 43.7</td>
</tr>
<tr>
<td>Non-aerated</td>
<td>53.3</td>
<td>44.6</td>
<td>46.6</td>
<td>43.5</td>
<td>49.0 45.1 50.0 44.0 47.0</td>
</tr>
</tbody>
</table>

*Analysis of variance and F test calculated

*Mean of 20 plants
solutions had longer leaves than plants grown in solutions containing 320 ppm. Combining the data for the same variety at the two concentrations, Chief had longer leaves than Ford when grown in aerated solutions, but this difference did not occur in non-aerated solutions. Leaf length of all plants grown in non-aerated solutions was greater than in aerated solutions (Table 5).

Leaves of Ford were narrower than Chief when grown in solutions containing 320 ppm Ca, but no difference existed when grown in solutions containing 40 ppm Ca. When leaf width data of the two varieties were combined, plants grown in solutions containing 40 ppm Ca had wider leaves than plants grown in solutions containing 320 ppm Ca. When leaf width data for each variety at the two concentrations were combined, no difference existed between Ford and Chief. The leaves of all plants grown in non-aerated solutions were wider than those of all plants in aerated.

3. **Root length**

Roots of Ford plants were longer than Chief while grown in aerated solutions containing 40 ppm Ca, but in solutions containing 320 ppm Ca Chief had longer roots than Ford (Table 6).
Table 5. Mean leaf length and width of Ford (F) and Chief (C) soybeans grown in aerated and non-aerated nutrient solutions at two Ca concentrations with combined data for varieties and concentrations

<table>
<thead>
<tr>
<th>Ca concentration (ppm)</th>
<th>Mean(^b) of combined varieties</th>
<th>Mean(^b) of combined concentrations</th>
<th>Mean(^b) of combined concentrations and varieties</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>40</td>
<td>320</td>
<td></td>
</tr>
<tr>
<td>Variety</td>
<td>F C F C</td>
<td>F C</td>
<td></td>
</tr>
<tr>
<td>Aerated</td>
<td>6.4 6.1 5.2 6.2</td>
<td>6.3 5.7 5.8 6.2</td>
<td>6.0</td>
</tr>
<tr>
<td>Non-aerated</td>
<td>6.9 6.8 6.0 6.5</td>
<td>6.8 6.2 6.5 6.6</td>
<td>6.5</td>
</tr>
</tbody>
</table>

Leaf length (cm)

Leaf width (cm)

\(^a\)Analysis of variance and F test calculated

\(^b\)Mean of 60 leaves
Table 6. Mean root length of Ford (F) and Chief (C) soybeans grown in aerated and non-aerated nutrient solutions at two Ca concentrations with combined data for varieties and concentrations

<table>
<thead>
<tr>
<th>Solution</th>
<th>Mean^b root length (cm)</th>
<th>Mean^b of combined concentration ppm</th>
<th>Mean^b of combined concentrations and varieties</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ca concentration (ppm)</td>
<td>Variety</td>
<td>Variety</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>F</td>
<td>C</td>
</tr>
<tr>
<td>Aerated</td>
<td>55.8</td>
<td>49.5</td>
<td>38.1</td>
</tr>
<tr>
<td>Non-aerated</td>
<td>33.6</td>
<td>36.0</td>
<td>30.7</td>
</tr>
</tbody>
</table>

^aAnalysis of variance and F test calculated

^bMean of 20 plants
Under non-aerated conditions no significant differences were observed. Root length of all the treatments in aerated solutions was significantly greater than the non-aerated solutions. Combining the data for root length of the two varieties at each concentration, no significant difference between plants grown in solutions containing 40 and 320 ppm Ca was obtained. When data for root length of each variety at the two concentrations were combined, Chief had longer roots than Ford in aerated solutions, but no difference existed between varieties grown in non-aerated solutions. Root length of all plants was greater in aerated solutions than in non-aerated solutions (Table 6).

4. **Dry weight of tops and roots**

   The dry weight of the tops of Ford plants grown in solutions containing 40 ppm Ca was greater than Chief in both aerated and non-aerated solutions, but in solutions containing 320 ppm Ca, Chief tops were greater than Ford. The dry weight of Ford tops grown in solutions containing 40 ppm Ca was higher than the weight of Ford grown in solutions containing 320 ppm Ca (Table 7). Combined dry weight data for the tops of the two varieties grown in each concentration were higher at 40 ppm Ca than at 320 ppm Ca. Combining dry weight data for the same variety at the two concentrations, no significant differences between Ford and Chief were found. Combined data for top dry weights for all plants grown in non-aerated solutions
<table>
<thead>
<tr>
<th>Solution</th>
<th>Variety</th>
<th>Ca concentration (ppm)</th>
<th>Mean(^b) of combined varieties</th>
<th>Mean(^b) of combined concentrations</th>
<th>Mean(^b) of combined concentrations and varieties</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>C</td>
<td>40</td>
<td>320</td>
<td></td>
</tr>
<tr>
<td>Aerated</td>
<td>3.404</td>
<td>2.138</td>
<td>1.553</td>
<td>2.855</td>
<td>2.771 2.204 2.478 2.497 2.487</td>
</tr>
<tr>
<td>Non-aerated</td>
<td>4.048</td>
<td>2.837</td>
<td>2.571</td>
<td>2.756</td>
<td>3.442 2.664 3.310 2.796 3.053</td>
</tr>
</tbody>
</table>

**Dry weight tops\(^c\), (g)**

<table>
<thead>
<tr>
<th>Solution</th>
<th>Variety</th>
<th>Ca concentration (ppm)</th>
<th>Mean(^b) of combined varieties</th>
<th>Mean(^b) of combined concentrations</th>
<th>Mean(^b) of combined concentrations and varieties</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>C</td>
<td>40</td>
<td>320</td>
<td></td>
</tr>
<tr>
<td>Aerated</td>
<td>0.685</td>
<td>0.448</td>
<td>0.330</td>
<td>0.549</td>
<td>0.566 0.439 0.507 0.498 0.503</td>
</tr>
<tr>
<td>Non-aerated</td>
<td>0.731</td>
<td>0.527</td>
<td>0.463</td>
<td>0.499</td>
<td>0.629 0.481 0.597 0.513 0.555</td>
</tr>
</tbody>
</table>

\(^a\)Analysis of variance and \(F\) test calculated

\(^b\)Mean of four replications

\(^c\)Weight of five plants
were greater than in aerated solutions (Table 7).

No significant differences in the dry weights of the roots in all treatments were observed.

5. Disease rating

A definite effect on the development of leaf symptoms in Ford was observed in relation to the high Ca concentration. The leaves of plants grown in solutions containing 40 ppm Ca did not develop symptoms, or if they did, they were slight. Plants grown in 320 ppm Ca solutions had more severe symptoms and several leaves were completely dead when rated. Aeration also increased the symptoms (Table 8). No symptoms occurred in Chief.

6. Content of N, P and K

The N content of tops of Chief plants was significantly higher than Ford. Differences between high and low concentrations of Ca or aerated and non-aerated solutions were not significant. The P content in tops of Ford was higher than Chief. Plants grown in aerated solutions had higher P content than plants grown in non-aerated solutions (Table 9). Ford plants grown in solutions containing 320 ppm Ca had more P content than plants grown in 40 ppm Ca solutions. These differences were not significant in Chief. Ford and Chief plants did not differ in K content of tops, but plants grown in solutions containing 40 ppm Ca had higher K content than plants grown
Table 8. Mean disease ratings of Ford (F) and Chief (C) soybeans grown in aerated and non-aerated nutrient solutions at two Ca concentrations, and combined data for aerated and non-aerated solutions

<table>
<thead>
<tr>
<th>Leaf</th>
<th>Variety</th>
<th>Ca concentration (ppm)</th>
<th>Combined aerated and non-aerated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>40</td>
<td>320</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>C</td>
<td>Solution\textsuperscript{b}</td>
</tr>
<tr>
<td></td>
<td>a</td>
<td>na</td>
<td>a</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>C</td>
<td>F</td>
</tr>
<tr>
<td>Unifoliate\textsuperscript{c}</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.6</td>
<td>1.9</td>
<td>1.0</td>
</tr>
<tr>
<td>First trifoliate\textsuperscript{d}</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.8</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Second trifoliate\textsuperscript{d}</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Rating 1 to 5: 1 = Healthy leaf  
2 = Vein necrosis, light flecking or both  
3 = Approx. 1/2 leaf chlorotic or necrotic  
4 = Approx. 3/4 leaf chlorotic or necrotic  
5 = Entire leaf chlorotic or necrotic

\textsuperscript{b} Solution: a = aerated, na = non-aerated

\textsuperscript{c} Mean of 40 leaves

\textsuperscript{d} Mean of 60 leaves
in solutions containing 320 ppm Ca. Plants grown in aerated and non-aerated solutions did not differ significantly in K content (Table 9).

The N content of the roots was not significantly different for any treatment. Ford roots had higher P content than Chief roots, but no significant difference in P content of plants grown in high and low Ca, or between plants grown in aerated and non-aerated solutions, existed. The K content of the roots was significantly higher in plants grown in non-aerated solutions than in aerated solutions. Differences between varieties or concentrations were not significant (Table 9).

<table>
<thead>
<tr>
<th>Table 9. Percentage of N, P and K in tops and roots of Ford and Chief soybeans grown in aerated and non-aerated solutions at two Ca concentrations a</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tops</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>Percentage N</strong></td>
</tr>
<tr>
<td>Ford</td>
</tr>
<tr>
<td>Chief</td>
</tr>
<tr>
<td><strong>Percentage P</strong></td>
</tr>
<tr>
<td>Ford</td>
</tr>
<tr>
<td>Chief</td>
</tr>
<tr>
<td><strong>Percentage K</strong></td>
</tr>
<tr>
<td>Ford</td>
</tr>
<tr>
<td>Chief</td>
</tr>
</tbody>
</table>

^aAnalysis of variance and F test calculated

^bSolution: a = aerated; n = non-aerated
7. Bacterial populations

Samples from non-aerated solutions containing 40 ppm of Ca in which Ford was grown had higher bacterial populations than samples in which Chief was grown, but samples from aerated solutions had no significant differences in bacterial populations. Non-aerated solutions containing 320 ppm Ca in which Chief was grown contained higher bacterial populations than comparable solutions in which Ford was grown (Table 10). This difference was not as great when plants grown in aerated solutions were compared. When bacterial populations of all samples from solutions containing 40 ppm Ca were compared with bacterial populations of all samples from solutions containing 320 ppm Ca, the latter contained higher bacterial populations than the former. Comparing all samples from non-aerated solutions in which Ford and Chief were grown, bacterial populations were higher in samples in which Chief was grown. Combined populations from all aerated solutions contained higher bacterial populations than the non-aerated solutions (Table 10).

Samples taken from the roots of Ford plants grown in non-aerated solutions containing 40 and 320 ppm of Ca had higher bacterial populations than the non-aerated Chief plants. When data from the roots of all plants grown in solutions containing 40 ppm Ca were compared with data from all plants grown in 320 ppm Ca, aerated solutions containing 40 ppm had higher bacterial populations than aerated 320 ppm solutions. Combined root data from each variety grown in the solutions containing
Table 10. Mean bacterial populations of nutrient solutions and roots of Ford (F) and Chief (C) soybeans grown in aerated and non-aerated nutrient solutions at two Ca concentrations and combined data for varieties and concentrations

<table>
<thead>
<tr>
<th>Ca concentration (ppm)</th>
<th>Mean of combined Concentration (ppm)</th>
<th>Mean of combined Concentration (ppm)</th>
<th>Mean of combined Concentration (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Variety</td>
<td>Variety</td>
<td>Variety</td>
</tr>
<tr>
<td></td>
<td>F C F C</td>
<td>40 320 F C</td>
<td>40 320 F C</td>
</tr>
<tr>
<td>Nutrient solutions</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aerated</td>
<td>12.0 9.7 11.9 13.1 10.9 12.5 11.3 11.4 11.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-aerated</td>
<td>3.3 1.8 4.2 10.1 2.6 7.2 3.8 6.0 4.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Roots</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aerated</td>
<td>11.1 11.7 9.7 7.6 11.4 8.6 10.4 9.7 10.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-aerated</td>
<td>6.3 4.9 6.7 3.4 5.6 5.1 6.5 4.1 5.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Analysis of variance and F test calculated  
\(^b\) Mean of 16 samples

40 and 320 ppm Ca indicated higher bacterial populations in Ford roots than in Chief roots when grown in non-aerated solutions. In aerated solutions Ford and Chief roots were not different. Combined data from roots of all plants grown in aerated solutions had higher bacterial populations than in non-aerated solutions (Table 10).
C. Effect of 40 and 320 ppm Ca(NO$_3$)$_2$ on Plant Growth and Disease Development of Ford and Chief Soybeans, when Grown in Non-aerated Nutrient Solutions

Ford was taller than Chief when grown either in solutions containing 40 or 320 ppm Ca, but the concentration did not markedly affect the height of either variety. Leaf length of Ford was greater when grown in solutions containing 40 ppm Ca than in solutions containing 320 ppm Ca (Table 11). Ford leaf width was also greater for plants grown in solutions containing 40 ppm Ca than in solutions containing 320 ppm Ca. Leaf width of Chief was the same both in 40 and 320 ppm Ca solutions.

Ford plant roots grown in solutions containing 40 ppm were longer than those in solutions containing 320 ppm Ca, but Chief had slightly longer roots in solutions containing 320 ppm Ca than in solutions containing 40 ppm Ca. Ford top dry weight was higher in solutions containing 40 ppm Ca than in solutions containing 320 ppm. Chief had no difference in top dry weight. Root dry weight of Ford was higher in solutions containing 40 ppm Ca than in solutions containing 320 ppm. Disease leaf symptoms were present in Ford, and increased when plants were grown in solutions containing 320 ppm Ca (Table 11). No symptoms appeared in Chief at either concentration.

Bacterial populations in samples from nutrient solutions containing 40 ppm Ca in which Ford was grown were higher than
Table 11. Mean plant height, leaf length, leaf width, root length, dry weight and disease rating of Ford and Chief soybeans grown in non-aerated nutrient solution containing 40 and 320 ppm Ca(NO$_3$)$_2$

<table>
<thead>
<tr>
<th>Ca concentration (ppm)</th>
<th>Variety</th>
<th>Mean plant characters$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean plant height (cm)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>Ford</td>
<td>63.3</td>
</tr>
<tr>
<td></td>
<td>Chief</td>
<td>57.9</td>
</tr>
<tr>
<td>320</td>
<td>Ford</td>
<td>63.8</td>
</tr>
<tr>
<td></td>
<td>Chief</td>
<td>59.2</td>
</tr>
</tbody>
</table>

$^a$Mean of 15 plants

$^b$Rating 1 to 5:  
1 = Healthy leaf  
2 = Vein necrosis, light flecking or both  
3 = Approx. 1/2 leaf chlorotic or necrotic  
4 = Approx. 3/4 leaf chlorotic or necrotic  
5 = Entire leaf chlorotic or necrotic

$^c$Leaf:  
U = Unifoliate  
F = First trifoliate  
S = Second trifoliate
those from solutions in which Chief was grown. Nutrient solutions in which Chief plants were grown containing 320 ppm Ca had higher bacterial populations than solutions in which Ford plants were grown. When populations of bacteria in solutions containing 320 ppm Ca containing the two varieties were combined, nutrient solutions containing 320 ppm Ca had higher bacterial populations than solutions containing 40 ppm Ca. When populations of the two concentrations were combined, nutrient solutions in which Chief was grown contained higher bacterial populations than those in which Ford was grown (Table 12).

Bacterial populations in Ford roots were slightly different from Chief in solutions containing 40 ppm Ca, but in solutions containing 320 ppm Ca, Ford roots had higher bacterial populations than Chief. Combined bacterial populations in roots of the two varieties grown within each solution were higher in the roots of plants grown at 320 ppm Ca concentration than at 40 ppm. When bacterial populations within each variety in the two concentrations were combined, samples from Ford roots had higher populations than samples from Chief roots (Table 12).

D. Effect of Low P and 40 and 320 ppm CaCl₂ on Plant Growth and Disease Development in Ford and Chief Soybeans, when Grown in Aerated Nutrient Solutions

In this experiment the P content of the nutrient solutions was reduced from 91 ppm to 31 ppm. Concentrations of CaCl₂ were maintained at 40 and 320 ppm.

Ford was taller than Chief when grown in solutions con-
Table 12. Mean bacterial populations in nutrient solution and roots of Ford (F) and Chief (C) soybeans grown in non-aerated nutrient solutions containing 40 and 320 ppm Ca(NO$_3$)$_2$, and combined data for varieties and concentrations

<table>
<thead>
<tr>
<th>Ca concentration (ppm)</th>
<th>Variety</th>
<th>Mean of combined varieties</th>
<th>Concentration (ppm)</th>
<th>Variety</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>F</td>
<td>40</td>
<td>F</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>C</td>
<td>320</td>
<td>C</td>
</tr>
<tr>
<td>Nutrient solutions</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.5</td>
<td>2.4</td>
<td>3.6</td>
<td>15.4</td>
</tr>
<tr>
<td></td>
<td>4.0</td>
<td>9.5</td>
<td>4.6</td>
<td>8.9</td>
</tr>
<tr>
<td>Roots</td>
<td>4.4</td>
<td>2.9</td>
<td>15.9</td>
<td>7.7</td>
</tr>
<tr>
<td></td>
<td>3.7</td>
<td>11.9</td>
<td>10.2</td>
<td>5.4</td>
</tr>
</tbody>
</table>

$^a$Mean of nine samples

...
Table 13. Mean plant height, leaf length, leaf width, root length, dry weights and disease rating of Ford and Chief soybeans grown in aerated nutrient solution containing 40 and 320 ppm CaCl₂ and P content reduced from 91 to 31 ppm.

<table>
<thead>
<tr>
<th>Concentration (ppm)</th>
<th>Varieties</th>
<th>Mean plant characters</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Plant ht (cm)</td>
<td>Leaf length (cm)</td>
<td>Leaf width (cm)</td>
<td>Root length (cm)</td>
<td>Leaf Dry weight (g)</td>
<td>Root Dry weight (g)</td>
<td>Disease rating unifoli-iate leaves</td>
</tr>
<tr>
<td>40</td>
<td>Ford</td>
<td>49.9</td>
<td>7.8</td>
<td>5.6</td>
<td>60.6</td>
<td>8.51</td>
<td>1.69</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>Chief</td>
<td>47.1</td>
<td>7.3</td>
<td>5.0</td>
<td>60.6</td>
<td>6.00</td>
<td>1.15</td>
<td>1.0</td>
</tr>
<tr>
<td>320</td>
<td>Ford</td>
<td>53.2</td>
<td>7.4</td>
<td>5.6</td>
<td>65.3</td>
<td>6.77</td>
<td>1.26</td>
<td>2.8</td>
</tr>
<tr>
<td></td>
<td>Chief</td>
<td>45.0</td>
<td>6.6</td>
<td>4.8</td>
<td>71.8</td>
<td>5.28</td>
<td>0.96</td>
<td>1.0</td>
</tr>
</tbody>
</table>

aAnalysis of variance and F test calculated
bMean of 20 plants
cWeight of five plants

Dry weight of Ford tops was significantly greater than Chief at both concentrations. Dry weight of Ford roots was significantly greater than Chief at both concentrations. The dry weight of Ford and Chief roots grown in solutions containing 40 ppm Ca was greater than the weight of the roots of both varieties grown in solutions containing 320 ppm Ca. When Ford was grown in solutions containing 320 ppm Ca, disease ratings were high, but symptoms were not so pronounced as when nutrient
solutions containing higher P concentration were used (Table 13).

When the nutrient solutions were assayed for bacterial populations, all solutions in which Ford was grown contained higher populations than solutions in which Chief was grown. No difference between combined varieties in solutions containing 40 and 320 ppm Ca was observed. There was no difference between treatments when roots were assayed for bacterial populations, (Table 14).

E. Effect of 10 ppm Ca and 71 and 568 ppm Cl on Plant Growth and Disease Development in Ford and Chief Soybeans, when Grown in Aerated Nutrient Solutions

In an effort to determine if Ca was largely involved in the expression of leaf symptoms, the concentration of Ca in the nutrient solution was lowered to 10 ppm for all treatments; Cl (NaCl) was maintained at the same concentration as in the previous experiments (71 and 568 ppm).

Plant height was not significantly different among plants grown in solutions containing 71 or 568 ppm Cl. Leaves of plants grown in 71 ppm Cl solutions were longer than leaves from plants grown in 568 ppm Cl solutions (Table 15). No significant differences were observed for leaf width. Root length did not vary significantly within these treatments. Dry weight of Ford tops and roots was significantly greater than of Chief
Table 14. Mean bacterial populations in nutrient solutions and roots of Ford (F) and Chief (C) soybeans grown in aerated nutrient solutions containing 40 and 320 ppm CaCl₂, P content reduced from 91 to 31 ppm, and combined data for varieties and concentrations

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Nutrient solutions</th>
<th>Roots</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>7.9</td>
<td>2.0</td>
</tr>
<tr>
<td>C</td>
<td>4.3</td>
<td>2.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ca concentration (ppm)</th>
<th>Mean of combined varieties</th>
<th>Mean of combined concentration (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td></td>
<td>F C</td>
</tr>
<tr>
<td>320</td>
<td></td>
<td>F C</td>
</tr>
</tbody>
</table>

- Analysis of variance and F test calculated
- Mean of 16 samples

in both solutions containing 71 ppm Cl and 568 ppm Cl. Ford leaves became completely chlorotic, but did not show the usual characteristic necrotic symptoms at any Cl concentration (Table 15). The leaves of Chief did not show any symptoms.

Bacterial populations in samples from the nutrient solutions were greater in solutions containing 568 ppm Cl than in solutions containing 71 ppm Cl. No significant difference in bacterial population between varieties was observed. Bacterial populations in samples from roots of Chief were higher than from roots of Ford at both concentrations, as well as when combined concentrations were compared, but this difference was not significant when varieties were combined (Table 16).
Table 15. Mean plant height, leaf length, leaf width, root length and dry weights of Ford (F) and Chief (C) soybeans grown in aerated solutions containing 10 ppm CaCl$_2$ and 71 and 568 ppm Cl$^-$

<table>
<thead>
<tr>
<th>Cl concentration (ppm)</th>
<th>Variety</th>
<th>Mean plant characters$^b$</th>
<th>Mean plant characters$^b$</th>
<th>Mean plant characters$^b$</th>
<th>Mean plant characters$^b$</th>
<th>Mean plant characters$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Plant height (cm)</td>
<td>Leaf length (cm)</td>
<td>Leaf width (cm)</td>
<td>Root length (cm)</td>
<td>Dry weight tops (g)</td>
</tr>
<tr>
<td>71</td>
<td>Ford</td>
<td>48.7</td>
<td>7.7</td>
<td>5.5</td>
<td>51.4</td>
<td>5.70</td>
</tr>
<tr>
<td></td>
<td>Chief</td>
<td>45.1</td>
<td>7.5</td>
<td>5.1</td>
<td>61.6</td>
<td>4.33</td>
</tr>
<tr>
<td>568</td>
<td>Ford</td>
<td>47.0</td>
<td>7.2</td>
<td>5.2</td>
<td>55.1</td>
<td>4.76</td>
</tr>
<tr>
<td></td>
<td>Chief</td>
<td>44.9</td>
<td>7.1</td>
<td>5.1</td>
<td>54.7</td>
<td>3.97</td>
</tr>
</tbody>
</table>

$^a$Analysis of variance and F test calculated

$^b$Mean of 20 plants

$^c$Weight of 4 plants
Table 16. Mean bacterial populations in nutrient solutions and roots of Ford (F) and Chief (C) soybeans grown in aerated nutrient solutions containing 10 ppm Ca and 71 and 568 ppm Cl, and combined data for varieties and concentrations

<table>
<thead>
<tr>
<th>Ca concentration (ppm)</th>
<th>Nutrient solutions</th>
<th>Roots</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>C</td>
</tr>
<tr>
<td>71</td>
<td>6.2</td>
<td>6.0</td>
</tr>
<tr>
<td>568</td>
<td>F</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>5.7</td>
<td>9.6</td>
</tr>
</tbody>
</table>

^aAnalysis of variance and F test calculated

bMean of 16 samples

F. Interaction of Ford and Chief Grown together in the Same Nutrient Solution

The number of plants of Ford and Chief grown together in the same pot was varied from one to five. Pots containing six control plants of each variety were included. All solutions were aerated and contained 320 ppm Ca.

The average height of one Ford plant grown with five Chief plants was 40.6 cm; two Ford plants grown with four Chief plants had 47.7 cm average height. A greater number of Ford plants grown with fewer Chief plants did not vary in plant height in relation to the control pots. The average height of Ford
plants when grown alone was 50.0 cm. The average plant height of one Chief plant grown together with five Ford plants was 52.0 cm. Increasing the number of Chief plants in the pots did not affect height of Chief in relation to the control pots. The average height of Chief control plants was only 49.4 cm (Table 17 and Figure 1).

Leaf length and leaf width did not show any specific trend in relation to the number of plants of each variety grown together. Root length of Ford plants increased as the number of Ford plants in the pot was increased. When the number of Chief plants was increased, the root length of Chief plants decreased (Figure 2).

Dry weight of Ford tops increased as the number of Ford plants in the pot was increased; however, erratic results were obtained from Chief (Figure 3). An increase in the dry weight of Ford roots was obtained as the number of Ford plants was increased. Chief showed a tremendous reduction in root dry weight when one Chief plant was grown with five Ford plants (Figure 4).

Disease symptoms in the unifoliate leaves of Ford had the same rating in all treatments; however, ratings of the first trifoliates were reduced as the number of Ford plants was increased in the pots. The second trifoliates showed the same trend except for the treatment with one Ford plant. In this case, the symptoms on the Ford leaves were not so severe as
Table 17. Mean plant height, leaf length, leaf width, root length and dry weights of tops and roots of Ford and Chief soybeans grown together (six plants per pot) in aerated nutrient solutions containing 320 ppm CaCl₂

<table>
<thead>
<tr>
<th>Plant character</th>
<th>Variety</th>
<th>Number of plants of indicated variety in each pot</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant height (cm)</td>
<td>Ford</td>
<td>40.6</td>
<td>47.7</td>
<td>49.5</td>
<td>48.9</td>
<td>51.7</td>
<td>50.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chief</td>
<td>52.0</td>
<td>48.5</td>
<td>49.6</td>
<td>50.6</td>
<td>48.5</td>
<td>49.4</td>
<td></td>
</tr>
<tr>
<td>Leaf length (cm)</td>
<td>Ford</td>
<td>---</td>
<td>5.7</td>
<td>5.4</td>
<td>5.7</td>
<td>5.8</td>
<td>5.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chief</td>
<td>6.1</td>
<td>6.4</td>
<td>5.9</td>
<td>6.1</td>
<td>6.2</td>
<td>6.2</td>
<td></td>
</tr>
<tr>
<td>Leaf width (cm)</td>
<td>Ford</td>
<td>---</td>
<td>4.2</td>
<td>4.0</td>
<td>4.2</td>
<td>4.3</td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chief</td>
<td>4.2</td>
<td>4.4</td>
<td>4.2</td>
<td>4.3</td>
<td>4.3</td>
<td>4.3</td>
<td></td>
</tr>
<tr>
<td>Root length (cm)</td>
<td>Ford</td>
<td>41.5</td>
<td>48.8</td>
<td>46.4</td>
<td>50.0</td>
<td>52.9</td>
<td>49.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chief</td>
<td>59.5</td>
<td>53.4</td>
<td>50.7</td>
<td>50.3</td>
<td>50.6</td>
<td>48.4</td>
<td></td>
</tr>
<tr>
<td>Dry weight tops (g)</td>
<td>Ford</td>
<td>0.331</td>
<td>0.563</td>
<td>0.555</td>
<td>0.574</td>
<td>0.667</td>
<td>0.565</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chief</td>
<td>0.953</td>
<td>1.099</td>
<td>1.054</td>
<td>1.044</td>
<td>1.066</td>
<td>1.041</td>
<td></td>
</tr>
<tr>
<td>Dry weight roots (g)</td>
<td>Ford</td>
<td>0.067</td>
<td>0.088</td>
<td>0.096</td>
<td>0.110</td>
<td>0.106</td>
<td>0.107</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chief</td>
<td>0.155</td>
<td>0.179</td>
<td>0.176</td>
<td>0.177</td>
<td>0.175</td>
<td>0.180</td>
<td></td>
</tr>
</tbody>
</table>

a Mean of 4 to 24 plants

b Weight per plant
Figure 1. Influence of the number of plants of Ford and Chief soybeans grown together on plant height. Plants were grown in aerated solutions containing 320 ppm Ca.
Figure 2. Influence of the number of plants of Ford and Chief soybeans grown together on root length. Plants were grown in aerated solutions containing 320 ppm Ca.
Figure 3. Influence of the number of plants of Ford and Chief soybeans grown together on dry weight of tops. Plants were grown in solutions containing 320 ppm Ca.
Figure 4. Influence of the number of plants of Ford and Chief soybeans grown together on dry weight of roots. Plants were grown in solutions containing 320 ppm Ca.
in the other treatments (Table 18 and Figure 5).

Table 18. Mean disease rating of leaves of Ford soybeans grown in aerated nutrient solutions containing 320 ppm CaCl₂ with different numbers of Chief plants

<table>
<thead>
<tr>
<th>Leaf</th>
<th>Mean disease rating</th>
<th>Number of Chief plants</th>
<th>Number of Ford plants</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Unifoliate</td>
<td>3.0</td>
<td>3.0</td>
<td>2.9</td>
</tr>
<tr>
<td>1st trifoliate</td>
<td>4.5</td>
<td>4.2</td>
<td>3.7</td>
</tr>
<tr>
<td>2nd trifoliate</td>
<td>2.0</td>
<td>3.4</td>
<td>2.7</td>
</tr>
</tbody>
</table>

aMean of 8 to 82 leaves

bRating 1 to 5
1 = Healthy leaf
2 = Vein necrosis, light flecking or both
3 = Approx. 1/2 leaf chlorotic or necrotic
4 = Approx. 3/4 leaf chlorotic or necrotic
5 = Entire leaf chlorotic or necrotic

G. Effect of Rhizobium japonicum on Plant Growth and Disease Development of Ford and Chief Soybeans when Grown in 320 ppm CaCl₂ Aerated and Non-aerated Nutrient Solutions

A suspension of Rhizobium japonicum (Kirchner) Buchanan was added to nutrient solutions containing 320 ppm Ca. Both aerated and non-aerated solutions were used. The Rhizobium suspension was obtained by grinding nodules (1 g per liter)
Figure 5. Influence of the number of plants of Ford and Chief soybeans grown together on disease rating on Ford. Plants were grown in solutions containing 320 ppm Ca.
from soybean roots. The suspension was added at the rate of 25 ml per day and 250 ml per week. A previous experiment indicated that the addition of Rhizobium could retard development of leaf symptoms in Ford plants.

Plant height was considerably increased by the addition of Rhizobium when compared to the control plants. Only slight differences in plant height between rates of application were obtained. Leaf length was greater in plants grown in aerated solutions with 25 ml daily added Rhizobium than in the aerated control. Leaf width was also favored by the addition of Rhizobium. Root length was greater in the aerated control than in any of the Rhizobium treatments. Plants grown in solutions with Rhizobium had heavier top and root dry weights than control plants. The dry weight of plants grown in non-aerated solutions was always greater than the weight of plants grown in aerated solutions (Table 19).

Disease symptoms on Ford unifoliate leaves in the control treatments had an average rating of 3.7 in non-aerated solutions and 3.3 in aerated solutions; the addition of Rhizobium did not alleviate the symptoms. Ratings of the first trifoliate leaves of plants grown in non-aerated solutions with daily or weekly applications of Rhizobium were considerably lower than the ratings of the same leaves in the non-aerated controls. These differences were not as great in aerated solutions. The second trifoliate leaves of plants grown in solutions with
Table 19. Mean plant height, leaf length, leaf width, root length, and dry weight of Ford soybeans grown in aerated and non-aerated solutions containing 320 ppm CaCl₂ and R. japonicum

<table>
<thead>
<tr>
<th>Rhizobium treatment</th>
<th>Solution</th>
<th>Mean plant characters⁵</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Plant height (cm)</td>
<td>Leaf length (cm)</td>
<td>Leaf width (cm)</td>
<td>Root length (cm)</td>
<td>Dry weight tops (g)</td>
</tr>
<tr>
<td>25 ml daily</td>
<td>na</td>
<td>47.9</td>
<td>6.3</td>
<td>4.8</td>
<td>45.0</td>
<td>7.33</td>
</tr>
<tr>
<td></td>
<td>a</td>
<td>49.3</td>
<td>6.4</td>
<td>4.9</td>
<td>58.7</td>
<td>6.65</td>
</tr>
<tr>
<td>250 ml weekly</td>
<td>na</td>
<td>46.9</td>
<td>6.3</td>
<td>4.7</td>
<td>43.1</td>
<td>7.44</td>
</tr>
<tr>
<td></td>
<td>a</td>
<td>45.6</td>
<td>5.8</td>
<td>4.4</td>
<td>54.2</td>
<td>5.02</td>
</tr>
<tr>
<td>Control</td>
<td>na</td>
<td>43.5</td>
<td>5.9</td>
<td>4.5</td>
<td>42.6</td>
<td>3.04</td>
</tr>
<tr>
<td></td>
<td>a</td>
<td>35.2</td>
<td>5.3</td>
<td>4.0</td>
<td>67.5</td>
<td>2.82</td>
</tr>
</tbody>
</table>

⁵Mean of 24 plants

Mean of 24 plants

Solution  a = aerated,  na = non-aerated

Rhizobium were healthier than the second trifoliate leaves of the controls (Table 20). Aeration apparently inhibited the beneficial effect of the Rhizobium treatments.

H. Effect of Streptomycin on Plant Growth and Disease Development of Ford and Chief Soybeans, when Grown in Aerated and Non-aerated Nutrient Solutions Containing 320 ppm CaCl₂

Suspensions of 1 percent Agri-mycin 100 (15 percent streptomycin and 1.5 percent terramycin) and 0.5 percent Agri-
### Table 20. Mean disease rating of leaves of Ford soybeans grown in aerated and non-aerated nutrient solutions containing 320 ppm CaCl$_2$ and *R. japonicum*

<table>
<thead>
<tr>
<th>Leaf</th>
<th>25 ml daily</th>
<th>250 ml weekly</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>na</td>
<td>a</td>
<td>na</td>
</tr>
<tr>
<td>Unifoliate</td>
<td>3.5</td>
<td>3.3</td>
<td>2.5</td>
</tr>
<tr>
<td>1st trifoliate</td>
<td>1.6</td>
<td>3.2</td>
<td>1.2</td>
</tr>
<tr>
<td>2nd trifoliate</td>
<td>1.0</td>
<td>1.1</td>
<td>1.0</td>
</tr>
</tbody>
</table>

*a Mean of 48 and 72 leaves

*b Rating 1 to 5
1 = Healthy leaf
2 = Vein necrosis, light flecking or both
3 = Approx. 1/2 leaf chlorotic or necrotic
4 = Approx. 3/4 leaf chlorotic or necrotic
5 = Entire leaf chlorotic or necrotic

*c Solution: n = non-aerated
a = aerated

Strep (37 percent streptomycin sulfate) were added to the nutrient solutions in which Ford and Chief were grown. The antibiotics were added at the rate of 2 ml per day and plants were observed for 3 weeks.

Plants grown in solutions with Agri-mycin were stunted and greener than the plants grown in Agri-Strep or control solutions. The average height of Ford plants in Agri-mycin was 13.4 cm in non-aerated solutions and 14.0 cm in the aerated
solutions. Chief plants were only 8.8 cm in non-aerated solutions and 9.3 cm in aerated solutions. The average height of the plants in the control solutions ranged from 23.8 to 25.3 cm in aerated and non-aerated solutions. Plants grown in Agri-Strep solutions were slightly reduced in height in comparison to the control plants.

The leaves of plants treated with Agri-mycin were also considerably shorter. Leaf width was the same as control plants. The roots of plants grown in solutions with Agri-mycin were considerably shorter than the controls, but the root length of plants grown with Agri-Strep was slightly greater than the controls. Dry weight of tops of plants grown in solutions with Agri-mycin was lighter than that of the control plants. Dry weight of roots of Ford plants was greater in the Agri-mycin solutions than in the controls, but dry weight of Chief roots was higher in the controls than in the Agri-mycin treatments. Dry weight of roots of Ford and Chief grown in Agri-Strep solutions was higher than the weight of the control plants (Table 21).

Disease ratings of the unifoliate leaves of Ford plants treated with antibiotics were not different from the controls. All the ratings were between 2.0 and 3.0; however, symptoms in plants treated with Agri-mycin appeared one week later than in the Agri-Strep and control treatments. Symptoms in the first
Table 21. Mean plant height, leaf length, leaf width, root length, dry weight and disease ratings of Ford and Chief soybeans grown in aerated and non-aerated solutions containing 320 ppm CaCl₂ and streptomycin

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Variety</th>
<th>Solution</th>
<th>Plant height (cm)</th>
<th>Leaf length (cm)</th>
<th>Leaf width (cm)</th>
<th>Root length (cm)</th>
<th>Dry weight tops (g)</th>
<th>Dry weight roots (g)</th>
<th>Disease rating</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ford</td>
<td>na</td>
<td>13.4</td>
<td>4.6</td>
<td>3.5</td>
<td>32.4</td>
<td>1.851</td>
<td>0.623</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ford</td>
<td>a</td>
<td>14.0</td>
<td>4.7</td>
<td>3.5</td>
<td>44.6</td>
<td>1.772</td>
<td>0.528</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chief</td>
<td>na</td>
<td>8.8</td>
<td>4.6</td>
<td>3.3</td>
<td>33.5</td>
<td>1.533</td>
<td>0.548</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chief</td>
<td>a</td>
<td>9.3</td>
<td>4.8</td>
<td>3.4</td>
<td>47.5</td>
<td>1.885</td>
<td>0.615</td>
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<td></td>
<td>Ford</td>
<td>na</td>
<td>24.0</td>
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<td>3.8</td>
<td>47.3</td>
<td>2.760</td>
<td>0.631</td>
</tr>
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<td></td>
<td></td>
<td>Ford</td>
<td>a</td>
<td>23.4</td>
<td>5.3</td>
<td>3.9</td>
<td>58.7</td>
<td>2.291</td>
<td>0.518</td>
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<td></td>
<td>Chief</td>
<td>na</td>
<td>19.2</td>
<td>6.0</td>
<td>4.5</td>
<td>54.4</td>
<td>3.721</td>
<td>0.829</td>
</tr>
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<td></td>
<td></td>
<td>Chief</td>
<td>a</td>
<td>20.0</td>
<td>6.2</td>
<td>4.4</td>
<td>68.4</td>
<td>3.673</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>na</td>
<td>24.3</td>
<td>5.2</td>
<td>3.8</td>
<td>47.2</td>
<td>2.344</td>
<td>0.502</td>
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<td></td>
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<td>Control</td>
<td>a</td>
<td>25.3</td>
<td>5.1</td>
<td>3.7</td>
<td>55.7</td>
<td>2.238</td>
<td>0.404</td>
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<td>Chief</td>
<td>na</td>
<td>23.8</td>
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<td>4.099</td>
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<td></td>
<td></td>
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<td>a</td>
<td>24.5</td>
<td>6.2</td>
<td>4.5</td>
<td>63.3</td>
<td>3.896</td>
<td>0.791</td>
</tr>
</tbody>
</table>

*aMean of 24 plants

*bRating 1 to 5: 1 = Healthy leaf, 2 = Vein necrosis, light flecking or both; 3 = Approx. 1/2 leaf chlorotic or necrotic; 4 = Approx. 3/4 leaf chlorotic or necrotic; 5 = Entire leaf chlorotic or necrotic

*cna = Non-aerated; a = Aerated

dWeight of 5 plants

eLeaf: U = unifoliate; F = First trifoliate; S = Second trifoliate
trifoliate leaves of Ford plants treated with Agri-mycin were considerably less severe than in the control plants. Agri-Strep treatments were not effective. Most of the second trifoliate leaves of Ford plants developed no symptoms (Table 21).

I. Identification of Bacteria

Attempts were made to identify the bacteria isolates obtained from the nutrient solutions containing 40 and 320 ppm of Ca and from the roots of the plants grown in the same solutions in several of the previous experiments.

All isolates were Gram-negative and spore-forming. Several of the isolates were gas-forming, but none hydrolyzed starch. Results are summarized in Table 22. Data obtained were not enough to identify the organism, and further investigation is necessary for the identification of the bacteria.

Table 22. Reaction of the bacteria isolates obtained from nutrient solutions containing 40 and 320 ppm Ca and from the roots of the soybean plants grown in the same solutions

<table>
<thead>
<tr>
<th>Isolate No.</th>
<th>Gelatinase</th>
<th>Starch</th>
<th>Arabinose</th>
<th>Dextrose</th>
<th>Dulcitol</th>
<th>Galactose</th>
<th>Inulin</th>
<th>Nitrate</th>
<th>Gas Forming</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
A new and interesting approach to the study of disease development in plants has been established. Bacterial populations and leaf symptoms in soybeans are conclusively related to Ca nutrition.

Many beneficial effects to soils and plants have been attributed to Ca; however, high Ca nutrition in this particular study has upset the normal growth of soybean plants, and increased the bacterial populations in the nutrient solutions and in the roots of the plants.

High bacterial populations in the roots of Ford when grown in solutions containing high Ca, seem to indicate the cause of the leaf symptoms. Evidence that Chief did not develop symptoms and grew normally under high Ca nutrition reinforced the idea that bacteria, and not Ca, upset the normal growth of the Ford plants.

Results obtained with solutions containing high Ca may not be due to Ca only. Similar leaf symptoms have been obtained by Howell (1954) with solutions containing high P. Later, Dunleavy et al. (1964) have demonstrated a relationship between high P nutrition and the bacteria B. subtilis. Dunleavy's report on P nutrition and results obtained in these experiments with Ca indicate that the same phenomenon could occur in soybean plants grown in an unbalanced nutrient solution.

The absorption of other elements is often influenced by Ca. The accumulation of K and Br was demonstrated by Viets
(1944) to be accelerated by other metals. The uptake of K was either depressed or stimulated by Ca according to Overstreet et al. (1952). These reports suggest that a similar situation may exist in the present study. Consequently, the balance of the other nutrients as influenced by Ca could be related to the disturbance of plant growth which follows bacterial multiplication. This point could be illustrated by the fact that susceptible plants grown in solutions containing high Ca contained less N than the resistant plants. On the other hand, P content was considerably higher in the susceptible plants than in the resistant. The K content of the two varieties was not different.

Interesting enough, results obtained in this investigation may be projected to poor soybean growth in calcareous soils or in places where liming and fertilization has been carried to excess.

The fact that high bacterial populations existed in roots of the susceptible variety, and the pattern of disease development was from the cotyledon to the upper leaves indicate a vascular disease. Development of necrosis and eventual death of the leaves was decidedly associated with the bacteria, although attempts to isolate the bacteria from the leaves were futile. Presumably, the increase of bacteria populations in the roots of Ford, and especially leaf symptoms, were due to the presence of Ca. The plants in experiments in which Ca was lower (10 ppm) fail to produce the characteristic necrotic
symptoms in the susceptible plants.

Results obtained with mixtures of resistant and susceptible varieties grown together raise important questions in relation to the new approach that agronomists are taking in developing "composite varieties". Susceptible plants grown in the same pots with resistant plants, became more diseased than when grown alone. This type of experiments need further investigation to determine how varietal mixtures contribute to balance root microorganisms in a specific environment.

Questions of a biologically fundamental nature were raised with the results obtained with addition of R. japonicum to solutions containing high Ca and susceptible plants. The decreased symptoms in these plants cannot be explained with the information obtained, but several important points can be mentioned. Possible increase of the N content in the nutrient solutions by Rhizobium is remote because the Rhizobium suspension contained only 0.26 ppm of N.

There was no antagonistic effect between the Rhizobium and the bacteria isolated from roots. Attempts to prove that Rhizobium was systemic in the plants were negative. Nodule formation in the roots of treated plants was very insignificant and N fixation is questionable. This experiment suggests a future area of investigation.
The use of antibiotics reinforced the idea that bacteria are associated with the symptoms, and also presents a future area of investigation. Thus, the role of Ca on specific soybeans genotypes and bacterial populations seem to suggest that research designed to explore this relationship offers a fruitful and interesting field of plant disease studies.
VI. SUMMARY

Ford and Chief soybean plants were grown in the greenhouse in solutions containing high and low Ca concentrations. A relation was observed between Ca concentration, bacteria populations and the expression of leaf symptoms in Ford. Chief was resistant and did not develop leaf symptoms.

Ford plants grown in solutions containing 320 ppm Ca were shorter than Chief plants, but Ford plants grown in solutions containing 40 ppm Ca, were taller than Chief. Leaf length and width of Ford and Chief plants grown in solutions containing 320 ppm Ca were smaller than when grown in solutions containing 40 ppm Ca. Root length of Ford plants grown in solutions containing 320 ppm Ca was shorter than in Chief plants, but in solutions containing 40 ppm Ca, Ford had longer roots than Chief. The dry weight of tops and roots of Ford plants grown in solutions containing 320 ppm Ca was smaller than the dry weight of Chief plants, but in solutions containing 320 ppm Ca dry weight of Ford plants was greater.

Plants grew better in non-aerated solutions than in aerated solutions. Root length of Ford and Chief, and disease rating of Ford were higher in aerated solutions than in non-aerated.

When Ca(NO₃)₂ was substituted for CaCl₂, Ford plants developed symptoms. When Ca concentration was reduced from 320 ppm to 10 ppm and the Cl was maintained at high levels, plants developed no symptoms and variation in plant growth was small.
When one Ford plant was grown with several Chief plants, leaf symptoms on Ford increased; and growth was reduced. Chief plants developed no symptoms when grown in the same container with Ford plants.

Addition of *Rhizobium japonicum* to solutions containing 40 and 320 ppm Ca alleviated the symptoms in Ford plants. Antibiotics added to nutrient solutions retarded leaf symptoms.

Bacterial populations were generally high in solutions containing 320 ppm Ca in which Chief was grown, but low in the roots. Roots of Ford plants grown in solutions containing 320 ppm Ca had high populations of bacteria.
VII. LITERATURE CITED


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