CHLORIDE TOXICITY OF FLOWERING STOCK (MATTHIOLA INCANA, R. BR) AND SWEET PEA (LATHYRUS ODORATUS, L)

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By

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AN ABSTRACT

Submitted to the School for Advanced Graduate Studies of Michigan
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SAMUEL WIGDOR ABSTRACT

Greenhouse crops in the State of Michigan may be subjected to substantial amounts of the chloride ion which may come from the fertilizers and tap water used in their culture. The present work was designed to study the specific effects of the chloride ion on the growth of sweet pea (Lathyrus odoratus, L.) and flowering stock (Matthiola incana, R. Br.).

Six levels of chloride were used in different nutrient solutions, the basis constituents being those outlined by Hoagland and Arnon. The plants were grown on two different soil types and each treatment was replicated four times.

Growth was measured as fresh weight, dry weight, and number of leaves for each plant species. In addition, measurements were made on the number of branches and flower buds of sweet pea, and the number of open flowers, stem diameter, and mean internode length of flowering stock. Chloride determinations were made on both the soils and the above-ground plant tissue.

The results indicated that there was no reduction in growth attributed to the chloride ion within the concentration range studied. Foliar necrosis was noted on both sweet peas and flowering stock. In the latter instance, the incidence of necrotic leaves was related to the amount of chloride in the solution applied.

Granby sandy loam was found to inhibit the branching of sweet pea. This may have been due to adverse conditions of drainage and aeration, and pH.

Accumulation of chloride ion in the soil was found to be an exponential function of solution concentration, where the exponent is greater than one, while plant accumulation was found to be exponential as well, the exponent being less than one.

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INTRODUCTION

The chloride ion is a minor constituent of soils, and greenhouse soils may contain substantial additional amounts from fertilizer and tap water sources. In the first instance, greenhouse operators generally maintain adequate potassium levels in the soil by the application of potassium chloride. In the second case, sources of irrigation water may contain as much as 700 parts per million of chloride.[1]

Ground water high in chloride ion content is found in some areas in Michigan as a result of large salt deposits. In some locales, formerly productive salt mines have been abandoned, permitting the deterioration of equipment, especially piping, through which large quantities of salt may enter the water supply. These levels may result in suboptimal crop yields.

Although much research has been conducted on soluble salt toxicity, some of which has been concerned with the effects of specific salts, only a limited amount of research has been done on the toxic effects of the chloride ion itself. The present work was designed, insofar as possible, to isolate the specific effect of the chloride ion from those of increasing osmotic pressure, electrical conductivity, or the specific effects of another ion or mixture of ions. The plants used in this study were grown in soil and in environmental surroundings similar to commercial growing conditions.

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[1] See Appendix Table A.

LITERATURE REVIEW

The subject of chloride nutrition is one which has been investigated for many years. Observations have been made on the tolerances of different crops to the presence of various amounts of chloride in the solution associated with the growing medium, toxicity symptoms and levels, and the possibility of chlorine being an essential element for the growth of higher plants. To date, much is known about crop tolerances, especially in regard to the genus *Beta* (23, 25, 85, 97, 99). It is only within recent years that the physiological problems of uptake, translocation, and distribution of chloride within the plant have received serious attention.

Ion Absorption

The mechanisms of ion absorption and accumulation are not thoroughly understood at the present time. Several different systems have been proposed, and, although there is no complete agreement among the workers, differences in points of view are not as wide as may appear upon superficial examination.

The rate and extent of solute absorption are dependent on aeration, temperature, water and solute supply, etc. These are well known, and will not be elaborated here.

By using a technique which exposed narrow regions of an excised barley root to radioactive phosphorus, Kramer (61) has shown that most of the salts translocated upwards are absorbed in the root hair zone. The apical region of the root accumulated salts but did not release them to the shoot. In this region the xylem is not differentiated, hence there is no pathway for rapid upward translocation. The region of salt accumulation coincides with the region of greatest water entry.

Cell walls and cytoplasm occupy about fifteen percent of the volume of a wheat root cell (18). The water in the cell walls and cytoplasm may be regarded as continuous except for the Casparian strips (15). The vacuolar sap is discontinuous. Thus, ion transport to any individual cell may merely follow the transpiration stream.

Further evidence substantiates this view (52, 53, 79). By the use of respiratory inhibitors, it was demonstrated that some, but not all, ion uptake was stopped (53). Varying the rate of water transport by any of a number of means alters the amount of ion accumulation (15, 52, 79). Thus, one phase of ion uptake is transpiration-dependent, and another phase respiration-dependent.

To quote Hylmö (53): "The fact that the ion uptake component which is unaffected by inhibitors is directly proportional to the rate of water flow constitutes conclusive evidence that Cl^- ions are passively drawn with the transpiration stream from the medium through the root to the shoot, independently of the metabolism of the root. It does not support the theory that the ions are transported through an endodermal barrier and actively secreted to the xylem, unless we are willing to assume that the ion transport here is mediated by a metabolism which is not repressed by the inhibitors employed or by anaerobic medium."

Kinetic studies (25, 26, 27, 52, 65) have shown an initial rapid ion uptake followed by a slower, steady accumulation. The first phase involved an equilibrium between root and external medium which was established within sixty minutes (27). At equilibrium there is no competition among ions, and pH bears no influence. This may be regarded as diffusion into "outer" or "free space". The second process is irreversible and involves active transport.

Several theories exist concerning the mechanism of active ion transport. Most authors agree that transport is linear with time, it requires the expenditure of energy, it is selective with respect to ions, and the presence of some ions affects the uptake of other ions.

Lundegårdh's scheme (17) involves four assumptions. The absorption of anions is independent of the absorption of cations so that separate mechanisms exist for each. The absorption of cations takes place in two steps, the latter involving excretion into the vacuole and constituting real accumulation. Anion absorption involves a portion of total respiration referred to as anion respiration. It is distinct from ground respiration.

In essence, Lundegårdh proposed that anions are carried across the cytoplasm and into the vacuole by means of bridges of cytochrome--cytochrome oxidases which are not necessarily continuous. Ferric iron is formed by oxidation at the outside, accepts an anion, and reaches the tonoplast. Here the iron is reduced by accepting an electron and thus releasing the anion. Cations are bound to the cytoplasm by potential differences.

The data of Overstreet and his group (81, 82) and Epstein (24-27) cast some doubt on this theory. Dinitrophenol inhibits respiration via the phosphate system. This inhibits accumulation of ions and is not in accordance with Lundegårdh's theory (81). The relation between respiration and salt accumulation is confused (37). Lundegårdh's proposal does not explain why ions of similar charges would not compete with each other (e. g. 25).

Epstein's theory (25) involves a different mechanism. The cytoplasm constitutes part of the "outer space" to which ions are freely accessible. At the outer surface of the membrane (presumably the tonoplast) which is largely impermeable to free ions, the ions combine with the carriers; the carrier-ion complex traverses the membrane; and at the inner surface the ions are released in a rate-limiting, essentially irreversible step. The carrier is presumed to be enzymatic in nature.

The rate of ion absorption has been found to follow classical enzyme kinetics in its dependence on external ionic concentration and its response to interfering ions (26). The ion-binding sites exhibit high degrees of selectivity. Rb^+ competes with K^+ and Cs^+, but not with Na^+ or Li^+ (26). Br^- competes with Cl^- and I^-, but not with NO_3^- (24). Sr^{++} competes with Ca^{++} and Ba^{++}, but not with Mg^{++} (27). SO_4^{--} competes with SeO_4^{--}, but not with $H_2PO_4^-$ or NO_3^- (65). At high concentrations, normally noncompeting ions may compete for the same binding sites.

Epstein has left a few issues unexplained. Polyvalent cations, especially Ca^{++} stimulate K^+ uptake at certain K^+ levels (55, 82, 101) and depress it at others (82). The stimulatory effect is dependent upon respiration (101). Although Br^- uptake is independent of pH, within the range pH_4 to pH_6, K^+ uptake decreases with increasing pH (77). These bits of data should be consistent with any theory of ion uptake.

Salt Tolerance

One of the greatest obstacles hampering experimenters in their evaluation of the role of chlorine in plant growth is the inability to separate the effects of the chloride ion, per se, from those of increased osmotic pressure or electrical conductivity of the aqueous part of the growing medium. Electrical conductivity bears an empirical relationship to osmotic pressure (19, 60). Hence, these may be reduced to one variable.

Osmotic pressure of the soil solution is an important factor in the availability of water to a plant. At osmotic pressure values characteristic of non-saline soils, water is almost equally available between field capacity and permanent wilting percentage. As the osmotic pressure of the soil solution rises, the availability of water diminishes, and at high osmotic pressure values a linear relationship between soil moisture tension and soil moisture percentage exists (104). In some instances the stress may exceed fifteen atmospheres, a tension at which growth of most plants ceases (90).

The rate of water intake by a root is inversely proportional to the osmotic pressure of the medium regardless of what type of compound is used to raise the osmotic pressure (45, 70). Water uptake ceases when the osmotic pressure reaches 6.8 atmospheres (45). If an inorganic substance is used to raise the osmotic pressure, nutrient uptake may diminish, whereas an organic solute may not show this effect (70).

Salt tolerance of a plant may be defined as the capacity of that plant to produce a certain yield at a given level of salinity. An alternate definition of the tolerance of a plant is its performance at a certain level of salinity as compared to its performance on a non-saline substrate (47). The latter definition is considered more acceptable from the viewpoint of agriculture.

The United States Regional Salinity Laboratory has established standards to evaluate the tolerance of crops to saline conditions (44). Electrical conductivity of the soil solution is regarded as the most suitable criterion to judge soil salinity. Those crops which tolerate electrical conductivity values between 4 millimhos $x10^{-5}$/cm. and 8 millimhos $x10^{-5}$/cm. are moderately tolerant of saline conditions. Crops of poor tolerance do not grow well at electrical conductivity values in this range, and those crops which grow satisfactorily at values in excess of the upper limit are tolerant.

The tolerance of a plant to saline conditions is not necessarily uniform throughout the life history of the plant. While barley and corn may germinate better than alfalfa or beet at high osmotic pressures (5, 92), the latter two crops are more tolerant at advanced stages of growth (72). Beans may germinate well at high osmotic pressure, but their growth is severely restricted under these conditions (31, 72). Alfalfa, on the other hand, becomes more tolerant with age (56).

Crop tolerances vary widely from family to family (63, 73, 83, 109), genus to genus (e. g. 23, 3, 10, 68), species to species (3, 73, 83, 108), and variety to variety (6, 10, 11, 42, 47, 108). Thus, it may be stated that although growth of a plant may be reduced as the electrical conductivity of the soil solution is increased (10, 12, 23, 42, 43, 46, 59, 71, 73, 108, 109), the extent of the reduction in growth must be determined for any given plant.

Crop tolerances to saline conditions may be modified by some environmental factors. As the environmental temperature rises, plant tolerance decreases (1, 78), and growth may be severely restricted during the warmest part of the growing season (23). On stone fruits it has been observed that chloride toxicity is more severe on the exposed parts of the trees (14). A highly humid environment reduces salt uptake by plants (51). This evidence would seem to indicate that the greater the uptake of water, the greater the toxic effects of saline conditions.

Further evidence substantiates this hypothesis. The chloride ion is freely mobile in the soil (74). Accumulation studies (18, 29, 69) show that chloride absorption is a function of time and concentration in the substrate, and thus the ion, in the main, is passively absorbed by the roots. Hence, chloride toxicity, being a function of ion uptake, is related to water uptake, and thus to transpiration. It is interesting to note that in the selection of lettuce varieties for salt tolerance studies (6), heat resistant varieties were used. In the light of what has been previously stated, it must be recognized that if heat resistance is related to water economy, the conclusions reached may not be applicable to all varieties of lettuce.

Chloride Accumulation

Any factor which affects the mobility of the chloride ion or the availability of water is important in a study of chloride toxicity. This statement holds if the ion is in solution and is not associated with the colloidal fraction of the soil.

Reisenauer and Colwell (89) have shown that the colloidal fraction of a soil influences chloride toxicity. Applications of equal amounts of chloride to virgin and cultivated soils yielded different percentages of chloride in subsequent leaf analysis. On the cultivated soil considerably more chloride appeared in the leachate, hence less remained in the rhizosphere to be absorbed by the tobacco plant. On the virgin soil, which differed from the cultivated soil in that it contained more organic matter, less chloride appeared in the leachate. More was retained in the soil and more was taken up by the plants during the growing season.

Chloride accumulation may be related to the pH of the medium. The same workers found a highly significant correlation coefficient of -0.801 existed between accumulation and pH. A decrease of accumulation below a pH value of 4 and above 6 has been noted in another study (80). It has been postulated that a competing anion, HCO_3^- may inhibit Cl^- uptake in the pH range of 6 to 8.

Recent studies with radioactive Cl^-36 (62, 96) have shed much light on the mobility of chloride within the plant and the loci of maximal concentration. It was found that Cl^-36 was translocated both upward and downward when applied to the leaves of a plant. The ion was found to accumulate in conducting tissues and in those tissues having large cells. It was higher in the pedicels of tomato than in attached immature fruit, but these differences decreased as the fruit matured. The workers state that absorption of chloride is a linear function of its content in the substrate. However, the amount accumulated in the pulvini of lima beans appeared to be more in the nature of an exponential function of substrate concentration. The greatest amount of the ion accumulated there, followed by the mature flowers, and then the main veins.

Accumulation of Cl^-36 in alfalfa is mainly in the stems, petioles, and pulvini. In tomato the major concentrations are found in stems, leaf blades, and petioles below the fruit, especially below the lowest clusters. It may be noted that there is no particular concentration at the growing points. This finding has been supported elsewhere (14).

Chloride Toxicity

One of the most important toxic effects of saline soils is the reduction in growth and yield of the crops grown (6, 10, 11, 12, 59, 71). Any chloride salt, under certain conditions, may be found to bring this about. Specifically, the chloride ion is reported to cause a reduction in root growth (106). The condition is more severe if $CaCl_2$ is added rather than NaCl. Eaton, on the other hand, could find no reduction in root growth which could not be attributed solely to the increase of osmotic pressure of the substrate (22).

Foliar symptoms of chloride toxicity are clearly defined (4, 8, 14, 31, 39, 48, 94, 110). The tip of the leaf becomes necrotic and the "firing" may then extend to leaf margins. Older leaves are the first to be affected (31, 109). Occasionally, necrosis may proceed down the entire leaf blade (4). Eventually, the entire leaf blade may become necrotic and abscise (34, 39). On stone fruit trees twig dieback may accompany foliar signs (14).

Chloride toxicity may result in a thickened, flaccid leaf, accompanied by partial to complete cellular plasmolysis (8, 28, 41). On the other hand, a decrease in the succulence of barley has been reported (16). The turgidity

of a leaf may depend on the length of time the leaf has been subjected to toxic levels of chloride (109).

Healthy leaves generally contain somewhat less than 0.5% chloride (34, 87), while in injured leaves the concentration may vary between 0.4% and 3.5%, depending on the particular plant (4, 14, 34, 87). This may be the result of 150 to 200 parts per million or more of chloride in the irrigation water (57, 58, 88) or the application of as little as 150 pounds per acre of KCl (54).

The amount of chloride absorbed by any particular plant depends upon the source of the chloride. More is absorbed by the bean plant when it is supplied as NaCl rather than $CaCl_2$ (32). On the other hand, more chloride is absorbed by citrus and walnut seedlings from a substrate of $CaCl_2$ than from KCl, NaCl, or $MgCl_2$ (36). Stone fruit trees and orchard grass are more sensitive to $CaCl_2$ than to NaCl (14, 107, 108).

Chloride, especially in the form of $CaCl_2$, reduces the uptake of nitrate nitrogen (30, 36, 62, 106). NaCl causes a greater reduction in plant protein concentration than does $CaCl_2$ (106).

Potassium uptake by plants in the presence of chloride is variable. Increases have been noted when NaCl was added to the substrate and the reverse when $CaCl_2$ was added (20, 30). Conversely, the uptake of chloride is independent of potassium uptake (18, 39). Excess chloride accumulation may be accompanied by increased calcium absorption (75) or a depression in uptake (20). Magnesium uptake may be increased or unaffected (30). Hence, the balance between K, Ca, and Mg may be altered. This may be one cause of the injurious effects of high chloride levels (75).

The similarity of chloride toxicity symptoms to potassium deficiency symptoms may be due to insufficient amounts of potassium available to combine with all the chloride present (13). The addition of potassium alleviates chloride toxicity symptoms on buckwheat, but not on flax (76).

A high level of chloride accumulation in tobacco leaves is associated with a pronounced dissipation of the malic acid content of the leaves (28). Since the organic acids are the major components of the buffer system of plant cells, conditions affecting dissipation of organic acids significantly affect the pH control of the cell.

The chloride ion affects the normal amylolytic activity of the plant. The starch level rises markedly in the leaves (12, 28, 29). The sucrose level may decrease (12), although total sugars increase (29, 66, 102). The change in reducing sugars is not clear (102, 105). The enzyme systems responsible for carbohydrate metabolism are intact, as hourly fluctuations in carbohydrate levels still occur (29). There is no clue in carbohydrate metabolism as to the mechanism of chloride injury to plants (29).

Chlorides have been found to exert a stimulatory effect on respiration (91). With chloride salts of monovalent cations, high respiration persists although chloride accumulation may decline. With divalent cations an initially high respiratory rate falls to a level comparable to one in which no chloride has been added. Handley and Overstreet (37) postulated that respiration is affected by ions which are both rapidly absorbed and important in metabolism.

Chlorine - An Essential Element

A question which has appeared in the literature is the possibility of chlorine being an essential element. Tottingham (97) summarized early experimental work in which Nobbe and Siegert observed physiological disturbances of plants grown in chloride-free media. They reported that the need for chloride appeared in the fruiting stage, and concluded that this ion was essential in carbohydrate translocation. Subsequent experiments of Wagner and Aschoff, and Beyer led to conflicting results. Shulov found that chlorine was not essential for buckwheat nutrition. Tottingham criticized all the experiments on the basis that not enough was known about balanced nutrient solutions to permit the drawing of any valid conclusions. The purity of the chemicals used may be questioned as well.

The first more critical experiment was conducted on peas and buckwheat by Lipman (67). These plants were grown on chloride-free and chloride-added media. With buckwheat more seed production, better seed germination and subsequent development in the F_1 generation was noted. With peas no

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differences were observed until the seeds of the third generation were examined. Those on the chloride-free medium produced fewer, lighter and more shriveled seeds. These, and many other experiments, merely serve to indicate the possible beneficial effects of chlorine to some plants.

It was not until 1954 that experiments were conducted which proved the essentiality of chlorine according to the currently accepted criteria of essentiality. Broyer _et al._ (16) have shown that tomato exhibits nutritional deficiency symptoms when grown on a halide-free substrate. The deficiency symptoms are characterized by wilting of the leaflet blade tips, followed by progressive chlorosis, bronzing, and necrosis. Severely diseased plants bear no fruit. The workers found that growth was correlated with the amount of chloride in the culture. Adequate chloride additions to the culture prevented the trouble; severely deficient plants resumed good growth after chloride was added to the culture solution or through stem injection. Bromide may complement chloride when added at ten times the required chloride levels. They concluded that chlorine is an essential element, and certainly the naturally occurring essential halide.

It has since been shown that chlorine is essential to the nutrition of sugar beets (99). The authors reported that symptoms of chloride deficiency appeared first as a chlorosis on the leaf blades of the younger leaves. The main veins and even the minor veins remained green, while the interveinal areas became light green to yellow. They observed a netted mosaic pattern to transmitted light. The roots of the chloride-deficient plants became very stubby, the stubs having arisen from secondary roots to form a distinctive and abnormal root structure. Plant growth was retarded. The addition of chloride as $CaCl_2$ or NaCl to the culture solution resulted in significant increases in fresh and dry weights of both tops and roots. Under conditions of high potassium concentrations, chloride deficiency caused a decrease in sucrose content of the root. When potassium was low, a chloride deficiency caused an increased sucrose content in the root. The authors concluded that chlorine is associated with sugar formation rather than utilization. For plants deficient in both potassium and chlorine, added chlorine removes the stress on newly formed center leaves, and deficiency symptoms disappear. Once this stress is removed, the young leaves can utilize potassium from the older leaves, and growth is resumed normally.

Stout *et al.* (95) reported that lettuce and cabbage may display chloride deficiency symptoms as well. A lack of chloride caused a pattern of chlorosis followed by a necrosis of the leaf tissue. Added chlorine reduced the deficiency symptoms, and very small additions caused a delay in the appearance of these symptoms. Added bromine may delay some, but not all of the symptoms. However, a much greater amount of bromine must be added to the solution in order to substitute for the added chlorine.

The function of the chloride ion in plant tissue has not definitely been established. Aside from its postulated role in sugar metabolism, another possible function has been assigned to it. Arnon and Whatley (2) isolated chloroplasts from plant tissue and added cytoplasmic fluid to the suspension. In the presence of light the chloroplasts were inactivated, but the addition of chloride to the suspension prevented this inactivation. The authors reported that Warburg interpreted this as a possible function of chloride as a coenzyme of photosynthesis, but noted that the anions Br^-, I^-, and NO_3^- may serve this function as well. This effect bears similarity to salt respiration of washed root discs, and starch hydrolysis by dialyzed α-amylases (33). The stimulatory effects have a common in vitro basis, but it is a matter of conjecture as to whether this happens in vivo.

MATERIALS AND METHODS

In order to determine whether the soil type alters the toxic effects of the chloride ion, two different soils were used. Brookston clay loam and Granby sandy loam were selected, as these soils are comparable to those used by commercial greenhouse establishments. The soil was placed into eight-inch clay pots, which had previously been coated on the interior surfaces with asphalt paint. The soil and pots were steam pasteurized on January 29, 1957. After pasteurization was completed, the soil was thoroughly leached with distilled water each day for the next four days.

Seeds of Ball non-branching Number 1 Lilac-Lavender Improved flowering stock (Matthiola incana, R. Br) were sown directly into half of the pots on February 4, 1957. Seeds of spring-flowering Cuthbertson strain Lois variety[1] of sweet pea (Lathyrus odoratus, L) were sown into the remainder of the pots on February 16, after twenty-four hours' soaking in distilled water. The pots were watered as required with distilled water. The greenhouse was maintained at a minimum night temperature of 50 degrees Fahrenheit.

On March 10 the stock were thinned to six plants per pot, and the sweet peas to two plants per pot, on the basis of uniformity of length. The sweet pea pots were staked by placing three rose stakes in each pot and joining them at the top. From this date the plants were treated with half-strength nutrient

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[1] Courtesy of George J. Ball and Company, Chicago, Illinois.

solutions, every second or third day, depending on the rate of water loss.

Six levels of the chloride ion were used, namely, 0, 1, 2, 3, 4 and 5 milli-equivalents per liter of solution.[1] Each replicate consisted, then, of the six levels of chloride and the two soil types, or twelve pots for each of the two species. Each treatment was replicated four times, making a total of forty-eight pots per species. The pots were randomized as to treatment using a set of random number tables. Thus, a "random block" experimental design was employed.

On April 1, the plants were thinned to one plant per pot, selection again based on uniformity of length. Full-strength nutrient solutions were added as of this date. Solutions were added almost every day due to the high amount of insolation during the month of April and the beginning of May. Each addition consisted of one-half liter of solution.

The experiment was terminated on May 11, at which time the first of the stocks would have been ready for harvest had the plants been grown on a commercial basis. At this time the sweet peas were just beginning to bloom. The stocks were cut at the cotyledonary node, and the sweet peas at soil level. All visual abnormalities were recorded at this time. Measurements were made as to fresh weight, total length, number of leaves, number of flowers, both mature and immature, for both species. In addition, stem

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[1] Composition and characteristics of the nutrient solutions may be seen in Appendix B.

diameter at the cotyledonary node was recorded for the stocks. The plants were then placed in a 70 degree Centigrade forced-draft oven for forty-eight hours. Dry weights were recorded, and the plant tops were ground to pass a 20-mesh screen in a Wiley mill. The percentage chloride was measured for both the dried plant tissue and the air-dry soil[1] by a modification of the technique outlined by Samson (93).

Electrical conductivities and pH values were made at the conclusion of the experiment using the Industrial Instruments Inc. "Solu-Bridge Soil Tester" model RD15, and a Beckman H2 model pH meter, respectively.

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[1] See Appendix C.

RESULTS

General Observations

Brookston clay loam had the appearance of a well-aggregated, well-drained soil. Granby sandy loam appeared to be poorly aggregated. Subsequent steam pasteurization seemed to promote the deflocculation of soil aggregates. Consequently, drainage and aeration were impeded.

No differences were noted as to speed or percentage of germination of the seeds germinated in the two soil types. Uniformity of stand prevailed until the final selection of plant material on April 1.

About two weeks after this date differences in growth of the sweet peas became apparent. Those plants grown in Granby sandy loam exhibited less branching and linear growth. Flowering stocks exhibited no such differences.

On April 21 spots were observed in interveinal areas on the lower leaves of some of the sweet peas and flowering stocks. The original appearance was that of water-soaked areas, but these rapidly became necrotic. The size of each spot did not change with time, but the number of such areas did increase. The leaves on the left in Figure 1 show the symptoms observed. The occurrence of injured leaves was sporadic on the sweet peas. There was a significantly greater percentage of injured leaves of flowering stock appearing on the three higher chloride levels than on the three lower levels.

FIGURE 1

Appearance of leaves of stock (above) and sweet pea, those on the left showing interveinal necrosis, and the stock leaf on the right showing tip chlorosis.

The other injured leaf which appears to the right of the three stock leaves in the figure is representative of a tip necrosis observed on the lower leaves of some of the plants. This injury is significantly correlated with the chloride level of the applied solution, the correlation coefficient being +0.863. Its occurrence was more prevalent on Brookston clay loam than on Granby sandy loam.

Judging on the basis of the characteristics measured and the size and placement of the flowers of stock, these plants were of good quality. The sweet peas grown on Brookston clay loam were of satisfactory quality, while those grown on Granby sandy loam would be judged inferior.

Sweet Peas

Figure 2 illustrates the growth of sweet peas on 1, 3 and 5 milliequivalents per liter of added chloride on Brookston clay loam and Granby sandy loam. The growth of the plants appeared to be similar regardless of the chloride level applied, however, those plants grown on Brookston clay loam appeared more dense than those grown on Grnaby sandy loam. The difference in appearance was due to the greater number of branches that developed on the plants grown in the Brookston soil.

Table 1 indicates that there were no differences in any growth characteristic of sweet pea which may be attributed to the chloride level of the solution applied. Differences in fresh weight, dry weight, number of branches, number of flower buds, number of leaves, total length, and internode length were significant at the 1% level. Growth on the Brookston soil was significantly greater than that on the Granby soil.

The most important single difference appeared to lie in the number of branches per plant. A recalculation of the data on a "per branch" basis showed that the differences due to soil type vanished. Thus, the extent of branching is dependent on soil type, and other characteristics are dependent upon the number of branches produced. Granby sandy loam inhibited branching in some manner, while the individual branches manifested the same growth habits regardless of soil type.

FIGURE 2

Appearance of sweet pea at the termination of the experiment. Left to right--1, 3 and 5 milliequivalents per liter of added chloride. Top--plants grown on Brookston clay loam. Bottom--plants grown on Granby sandy loam.

TABLE 1

Growth of Sweet Peas as Related to Chloride Concentration of the Growing Medium.

Measurement (means of 4 replicates)	Brookston Clay Loam (ME Cl^-/Liter)						Granby Sandy Loam (ME Cl^-/Liter)						Significant Differences Between		
	0	1	2	3	4	5	0	1	2	3	4	5	Soils	Solutions	Interaction
Fresh weight (grams)	80.5	86.5	78.8	63.9	82.1	75.3	31.3	46.5	34.6	42.0	40.6	46.5	1%	NS*	NS
Dry weight (grams)	11.5	13.4	11.5	8.8	12.0	10.4	5.3	7.3	5.4	6.6	6.3	7.3	1%	NS	NS
No. of branches	5.8	6.0	5.5	5.3	5.0	5.0	2.5	3.8	3.8	2.8	3.0	2.8	1%	NS	NS
No. of flower buds	37.5	36.5	28.3	26.0	35.5	25.5	18.0	18.0	11.0	26.8	17.3	22.0	1%	NS	NS
No. of leaves	86.8	89.3	83.8	73.5	75.0	71.5	38.8	47.5	42.3	44.0	39.0	47.5	1%	NS	NS
Total length (cm)	418.3	464.8	449.3	388.5	407.0	373.3	176.0	224.8	203.0	208.3	176.3	224.5	1%	NS	NS
Internode length (cm)	19.2	21.0	20.9	21.2	21.6	20.9	18.1	19.0	18.7	18.7	17.9	18.6	1%	NS	NS
Fresh weight per branch	14.3	14.2	14.5	12.1	16.2	15.5	13.4	12.9	9.1	14.8	14.5	16.8	NS	NS	NS
Dry weight per branch	2.1	2.4	2.2	1.7	2.4	2.2	2.2	2.0	1.5	2.3	2.3	2.6	NS	NS	NS
Flowers per branch	6.8	6.3	6.1	4.9	6.9	5.2	8.0	5.2	3.2	9.1	6.3	7.6	NS	NS	5%
Leaves per branch	15.2	14.8	16.1	13.9	14.7	14.8	16.0	13.1	11.8	15.9	13.5	17.3	NS	NS	NS
Length per branch	72.7	71.4	83.4	73.8	79.6	77.0	72.9	63.3	54.6	77.1	62.3	80.7	NS	NS	NS

*Not significant

Flowering Stock

Figure 3 depicts the growth of flowering stock at 1, 3 and 5 milliequivalents of added chloride on Brookston clay loam and Granby sandy loam. From the photograph no differences are apparent in the growth of these plants under the conditions of the experiment.

Table 2 indicates that the only differences found were between fresh weight, dry weight, and stem diameter of the plants grown on the two soil types. The difference in dry weight is accounted for by the difference in stem diameter, and not in terms of leaf number or stem length.

Flowering stock is harvested when there are between eight and ten open flowers per stem. No treatment resulted in a significantly earlier date of harvest, as there were no differences in the number of open flowers at the termination of the experiment.

Chloride addition had no significant effect on fresh weight, dry weight, leaf number, stem diameter, length to the top leaf, or the mean internode length within the range of concentration studied.

FIGURE 3

Appearance of flowering stock at the termination of the experiment. Left to right - 1, 3 and 5 milliequivalents per liter of added chloride. Top--plants grown on Brookston clay loam. Bottom--plants grown on Granby sandy loam.


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TABLE 2

Growth of Flowering Stock as Related to Chloride Concentration of Growing Medium.

	Measurement						
	Fresh Weight (gms)	Dry Weight (gms)	Length of Top Leaf (cm)	No. of Open Flowers	No. of Leaves	Stem Diam. (cm)	Mean Internode Length
Brookston Clay Loam (ME Cl^-/Liter)							
0	102.3	12.8	42.7	9.5	24.3	9.5	1.9
1	96.9	12.4	39.3	11.3	24.8	10.0	1.6
2	105.3	12.9	44.5	9.3	24.5	9.3	1.8
3	94.9	12.3	43.7	11.3	24.0	10.1	1.8
4	98.4	12.1	42.6	10.3	25.3	9.6	1.7
5	111.6	13.4	43.4	10.8	24.5	10.4	1.8
Granby Sandy Loam (ME Cl^-/Liter)							
0	87.8	11.6	42.7	10.0	25.0	9.4	1.7
1	93.0	11.9	42.5	9.5	25.5	9.4	1.7
2	76.8	10.9	45.7	10.5	25.8	9.0	1.8
3	80.6	10.3	39.9	10.0	23.8	8.7	1.7
4	74.3	10.1	38.4	10.3	26.0	8.8	1.5
5	82.5	11.1	42.1	9.0	25.8	9.1	1.6
Significant Differences							
Soil	1%	5%	N.S.	N.S.	N.S.	1%	N.S.
Solutions	N.S.*	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Interaction	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

*Not significant

Chloride Accumulation

The chloride content of the plants and the two soil types at the end of the experiment may be seen in Table 3. As the solution chloride increased from 5 parts per million to 182.5 parts per million, the amount of chloride in Brookston clay loam increased from 3 parts per million to 115 parts per million, and from a trace to 180 parts per million in Granby sandy loam. The chloride content of flowering stock increased from 0.193 percent to 1.108 percent on the Brookston soil, and from 0.237 percent to 0.966 percent on the Granby soil, while the chloride content of sweet pea increased from 0.213 percent to 1.180 percent on Brookston clay loam, and from 0.193 percent to 0.859 percent on Granby sandy loam.

The amount of chloride in the plant tissue is closely related to the amount of chloride added in solution. The correlation coefficients are given:

Comparison	Correlation Coefficient
Solution chloride and stock chloride	+0.895
Solution chloride and sweet pea chloride	.800
Solution chloride and plant chloride on Brookston soil	.903
Solution chloride and plant chloride on Granby soil	.865

All correlation coefficients are significant at the 1% level.

TABLE 3

The Relation Between Solution Chloride and Chloride Accumulation in Soil and Plant.

Solution Number	Ppm Chloride in		% Dry Weight Chloride in	
	Solution*	Soil	Stock	Sweet Pea
		Brookston Clay Loam		
1	5	3	0.193	0.213
2	40.5	11	.698	.809
3	76	30	.749	.770
4	111.5	59	.948	1.060
5	147	89	.956	1.090
6	182.5	115	1.108	1.180
		Granby Sandy Loam		
1	5	tr.	0.237	0.193
2	40.5	15	.730	.412
3	76	61	.677	.521
4	111.5	75	.833	.543
5	147	150	.908	.745
6	182.5	180	.966	.859
L.S.D. soils		21.1	N.S.[1]	0.081
1% levels		67.4	0.172	0.219
interaction (soils x levels)		N.S.	N.S.	0.219

*Includes 5 ppm in distilled water

[1] Not significant

The correlation coefficients between solution chloride and soil chloride at the end of the experiment were +0. 988 for Brookston clay loam and . 979 for Granby sandy loam. Both correlation coefficients are significant at the 1% level.

On the basis of the experimental evidence, chloride retention in both soil and plant is directly related to the amount of chloride applied in the irrigation water, at least within the concentration range studied.

Mean chloride retention by the two plant species may be seen in the following table:

Flowering Stock		Sweet Pea	
Brookston clay loam	Granby sandy loam	Brookston clay loam	Granby sandy loam
0. 775%	0. 725%	0. 853%	0. 545%

L. S. D. = 0. 175%

Thus it is seen that retention by sweet pea is significantly less on Granby sandy loam than on Brookston clay loam. These plants also contained less chloride than flowering stock grown on either soil type. Combining the results, there is no difference between species uptake, but retention was greater on Brookston clay loam than on Granby sandy loam.

Figures 4, 5, and 6 illustrate the relationship between the amount of chloride applied and the amount of chloride retained in soil and plant. Soil retention appears to be an exponential function of solution concentration where the exponent is greater than one, while plant accumulation is exponential as well, the exponent being less than one.

FIGURE 4

Graph of Chloride in Solution Versus Accumulated Chloride in Brookston Clay Loam and Granby Sandy Loam.

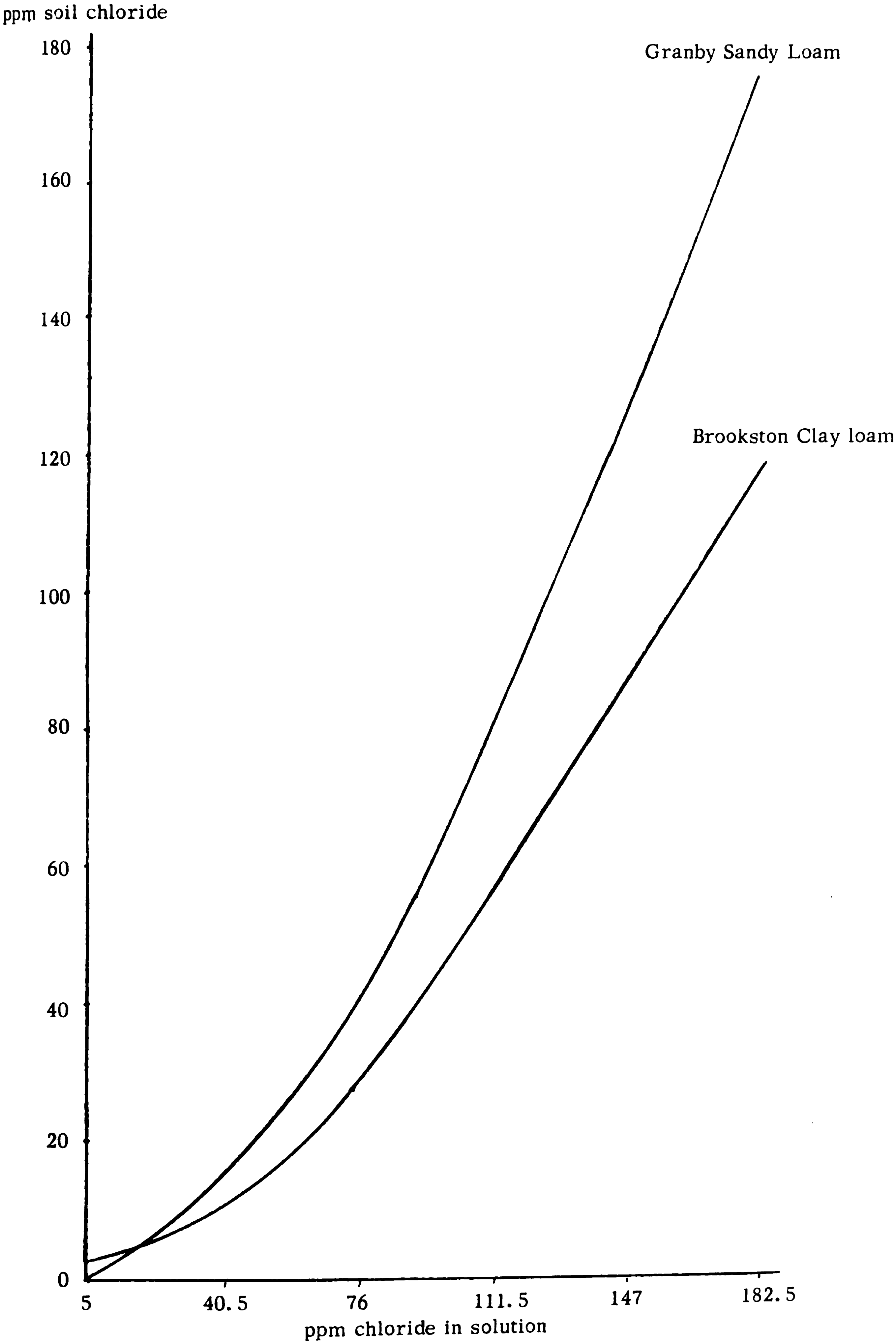

ppm soil chloride
180
160
140
120
100
80
60
40
20
0
5
40.5
76
111.5
147
182.5
ppm chloride in solution
Granby Sandy Loam
Brookston Clay loam

FIGURE 5

Graph of Chloride in Solution Versus Chloride in Tops of Sweet Peas Grown in Brookston Clay Loam and Granby Sandy Loam.

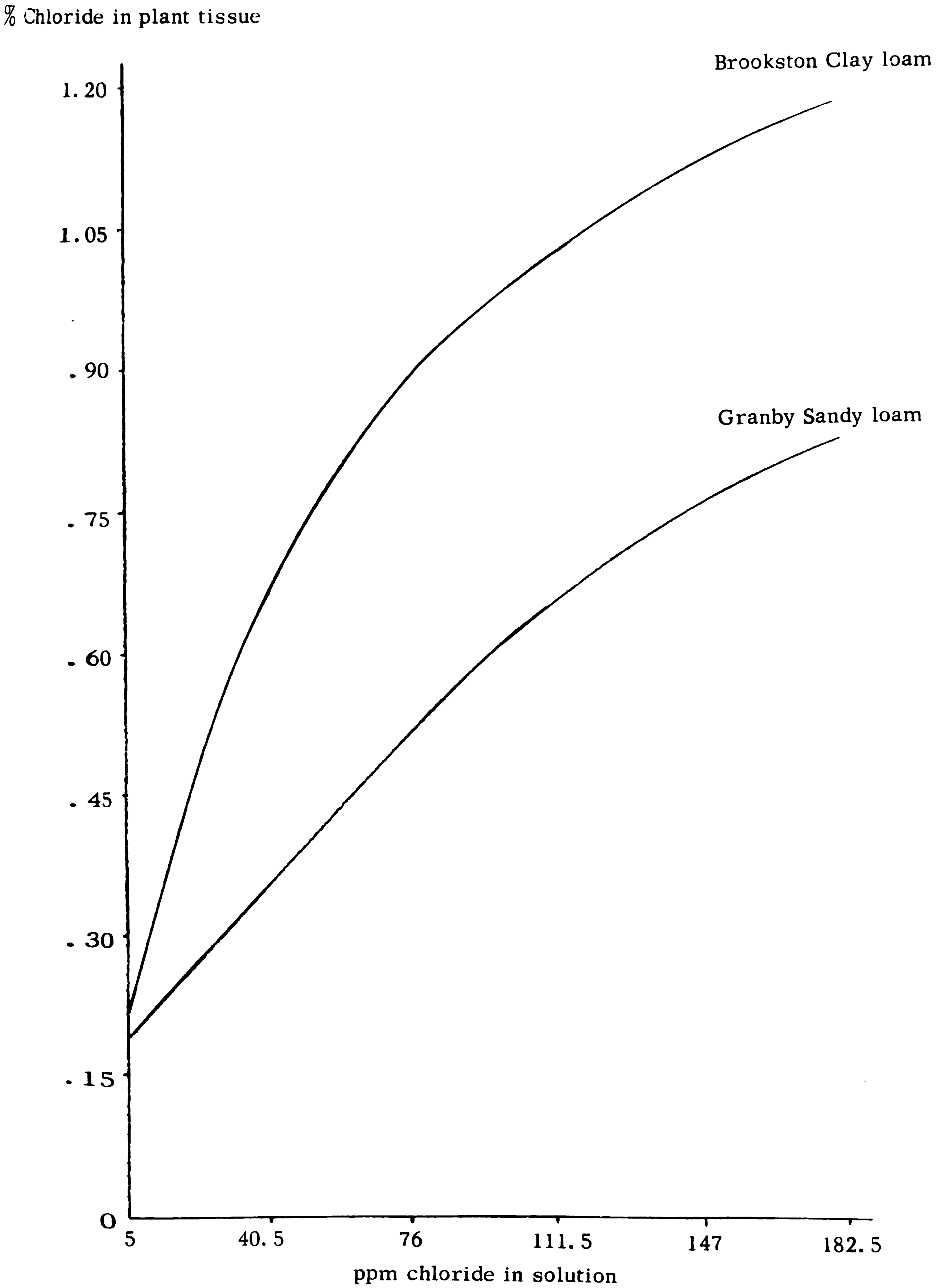
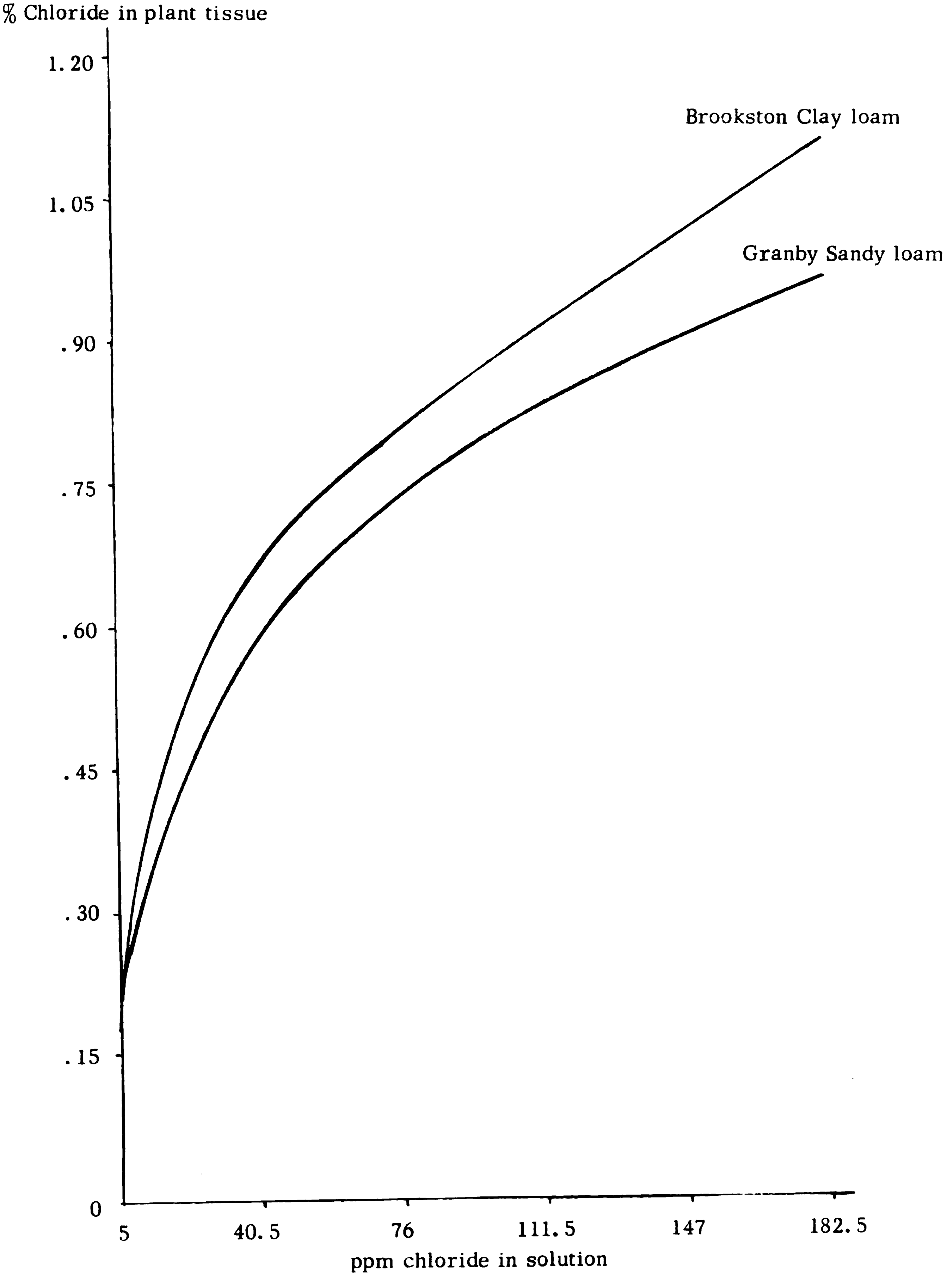

% Chloride in plant tissue
Brookston Clay loam
Granby Sandy loam
1.20
1.05
.90
.75
.60
.45
.30
.15
0
5
40.5
76
111.5
147
182.5
ppm chloride in solution

FIGURE 6

Graph of Chloride in Solution Versus Chloride in Tops of Flowering Stock Grown in Brookston Clay Loam and Granby Sandy Loam.


% Chloride in plant tissue
1.20
1.05
.90
.75
.60
.45
.30
.15
0
Brookston Clay loam
Granby Sandy loam
5
40.5
76
111.5
147
182.5
ppm chloride in solution

DISCUSSION

This study has indicated that the spring flowering Cuthbertson strain of sweet pea and the non-branching lilac-lavender variety of flowering stock did not manifest any reduction in growth due to additions of the chloride ion up to a concentration of 182 parts per million when grown in Brookston clay loam and Granby sandy loam. Visual signs which appeared on the leaves of both plant species appeared more frequently on the leaves of flowering stock grown at higher chloride levels, and appeared sporadically on the leaves of sweet pea. Chloride ion accumulation in soil and plant were directly related to the concentration in the solution.

Within the concentration range of chloride studied, neither plant manifested any reduction in growth. The species, evidently, are tolerant of the chloride ion at least up to a concentration of 182 parts per million. Flowering stock is reported to be moderately tolerant of high salt concentrations (71). Although the present study is of a different nature and can neither confirm nor deny this evidence, perhaps tolerance of saline conditions and to the chloride ion are related. Tolerance to the chloride ion in the substrate is not a blocking mechanism as plant and solution concentrations are directly related.

The more important difference found in plant growth was a reduction of branching of sweet peas grown in Granby sandy loam. Two major differences exist between the soil types, namely pH and aeration. Post (85) states that the best pH range for sweet pea growth is between pH 6.0 and pH 7.5. The pH of Granby sandy loam exceeds the upper limit of the favorable range. All sources consulted (9, 64, 85, 100) agree that sweet pea soil should be well drained to a considerable depth. This condition did not exist in the Granby soil. The inhibition of branching could not have been due to a low level of potassium, as this ion is in greater abundance in the Granby soil. Aeration and/or pH or other conditions may have caused the observed reduction in branching.

Both fresh weight and dry weight of flowering stock were significantly less in Granby sandy loam. This was found to be due, in part, to a reduction in stem diameter. Fresh weight differences were greater and were not wholly dependent on stem diameter. From this evidence it is concluded that water uptake by flowering stock grown in Granby sandy loam was inhibited. This may have resulted in a reduction in cell enlargement. The restriction of aeration in Granby sandy loam may have been the causal factor.

Visual signs which appeared on some lower leaves of both plant species were not similar to those reported by most authors (4, 14, 31, 39, 48, 94, 110) but resembled those found by Dilley (21) on grape and peach when irrigated with 315 parts per million of chloride, and 624 parts per million of sulfate. Leaf injury found in the leaf of flowering stock at the right of

Figure 1 is similar to that described by Baker et al. (14) who noted tiny yellow-brown necrotic spots at the leaf tip caused by a local high concentration of soluble salts at the hydathodes. This may be an early condition of the signs reported by the other workers. Although growth of plants was not reduced, it is possible that leaf signs were due to toxic levels of chloride as chloride may concentrate in the leaf blades (14,62, 96).

In this study, correlation coefficients between substrate concentration and plant accumulation were highly significant. This finding substantiates those of other workers (18, 29, 84). Toth and Kretschmer (96) and Kretschmer et al. (62) stated that chloride absorption is a linear function of its content in the substrate. However, accumulation in the pulvini of lima beans (96) appeared to be more in the nature of an exponential function. This study supports the latter finding.

Chloride accumulation is mainly a function of transpiration (79), the transpiration stream accounting for up to 60 percent of the total chloride accumulated. There is no logical basis to assume that there are transpirational differences between plants grown on different chloride levels, hence the differences must be attributed to the different substrate concentrations.

Significantly greater amounts of chloride were found in both Brookston clay loam and Granby sandy loam with additions of larger amounts of chloride. The chloride ion does not accumulate in the soil (103) possibly due to its great mobility (74). However, the type of soil colloid bears a slight influence on the amount of chloride retained (111). Present evidence would indicate that substrate concentration is as important a factor in chloride retention as is the soil type.

On an overall basis, chloride retention by plant tissue was greater on Brookston clay loam than on Granby sandy loam. Conversely, soil chloride retention was greater on the Granby soil. Total chloride applied was the same for any treatment level, irrespective of species or soil type, and might be represented thus: Total Cl = plant Cl + soil Cl. Plant chloride is a function of the dynamic factors of growth. Hence, soil chloride differences may be regarded as a residual effect.

Differences in chloride accumulation in plants due to soil type are significant at the 1% level for sweet pea. Accumulation is higher in plants grown in Brookston clay loam, the soil with the larger percentage of colloids. Reisenauer and Colwell (89) have shown that the colloidal fraction is influential in chloride accumulation by tobacco, the plants having accumulated more on the soil with more colloidal matter. It is interesting to note that this effect was not observed with flowering stock in this experiment. The reason is unknown.

SUMMARY

The specific influence of the chloride ion on the growth of sweet pea (*Lathyrus odoratus*, L.) and flowering stock (*Matthiola incana*, R. Br.) was studied. The plants were grown on Brookston clay loam and Granby sandy loam, soils similar to those used in commercial greenhouses. A random block design was employed, using six levels of chloride each replicated four times. The solution composition, in other respects, was similar to that outlined by Hoagland and Arnon (50).

Growth was measured as fresh weight, dry weight and number of leaves for both plants. Additional measurements were made as to the number of branches and flower buds on sweet pea, and number of open flowers, stem diameter, and mean internode length of flowering stock. Chloride was determined as percent dry weight of the above-ground portion of the plants and as parts per million of air-dry soil.

No significant reductions in growth were noted on either plant which were due to the application of chloride.

Interveinal necrotic areas were found to be more prevalent on the leaves of flowering stock grown at the three higher levels of chloride than at the three lower levels. This injury appeared sporadically on some sweet pea leaves. A foliar tip necrosis was displayed by flowering stock. Its incidence correlated very significantly with the amount of chloride applied in the nutrient solution.

Granby sandy loam severely inhibited the branching of sweet pea. The cause is unknown, although it may have been due to restricted drainage and aeration, too high a pH, or a combination of both factors.

The chloride ion accumulated readily in both soil and plant. Significant positive correlation coefficients were found between solution chloride and chloride accumulation. Accumulation in the soil appeared to be an exponential function of solution concentration where the exponent is greater than one, while plant accumulation appeared to be exponential as well, the exponent being less than one.

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APPENDIX - TABLE A

Amount of Chloride in the Tap Water Used by Greenhouses in Some Michigan Communities

Community	Number of Growers[1]	PPM Chloride in Water[2]
Adrian	6	8
Almont	1	700
Ann Arbor	6	10
Battle Creek	7	4
Bay City	8	30
Detroit	21	8
Flint	6	25
Grand Rapids	16	5
Jackson	9	55-295
Kalamazoo	10	8
Lansing	6	11
Monroe	5	18
Mount Clemens	8	7.5
Muskegon	6	4
Plymouth	6	280
Saginaw	11	210
Ypsilanti	4	27

[1] Michigan State Florist Ass'n Membership Directory, P. R. Krone, Managing Editor, May, 1957.

[2] Chemical Analyses of Public Water Supplies in Michigan. Michigan Department of Health Engineering Bulletin no. 4, 1948.

APPENDIX - TABLE B

Constituents and Characteristics of Nutrient Solutions

Chemical	Milliequivalents per Liter					
	Solution Number					
	1	2	3	4	5	6
KH_2PO_4	1	1				1
$NH_4H_2PO_4$			1	1	1	
KNO_3	5	4	4	3	2	
KCl		1	2	3	4	5
NH_4NO_3		0.5		0.5	1	2.5
$Ca(NO_3)_2$	5	5	5	5	5	5
$MgSO_4$	2	2	2	2	2	2
Electrical Conductivity (millimhos x 10^{-5})						
	265	270	260	290	270	300
pH						
	5.0	5.1	5.4	5.4	5.6	5.1

Iron was supplied as "Na Fe Sequestrene" at the rate of 1 milliliter of stock solution per liter. The stock solution contained 42 grams chelated iron per liter. All minor elements were supplied as prescribed by Hoagland and Arnon (50).

Soil Typ
Brookston
Granby
Brooks
Granb

APPENDIX - TABLE C

Soil Properties at the Termination of the Experiment

Soil Type	Solution Number	Cl(ppm)	Electrical Conductivity (Millimhos x 10^{-5})	pH
Brookston Clay Loam	1	3	24	6.5
	2	11	18	6.5
	3	30	25	6.6
	4	59	22	6.2
	5	89	16	6.6
	6	115	18	6.3
Granby Sandy Loam	1	tr.	10	8.0
	2	15	10	8.1
	3	61	15	8.2
	4	75	16	7.8
	5	150	24	8.1
	6	180	24	7.9

	% Sand	% Silt	% Clay	lb/A available		C.E.C. (M.E./100g)
				P_2O_5	K_2O	
Brookston Clay Loam	35.6	30.6	33.8	132	148	22.0
Granby Sandy Loam	62.0	25.2	12.8	126	165	18.5

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