EFFECTS OF MINERAL LEVELS ON
PHYSIOLOGY AND MORPHOLOGY
OF PLANTS

By

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TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS.....	ii
LIST OF TABLES.....	v
LIST OF FIGURES.....	vi
INTRODUCTION.....	1
REVIEW OF LITERATURE.....	2
Accumulation of Elements.....	2
Effect on Uptake and Distribution of Other Ions..	3
Toxic Effects.....	5
Cytological Effects.....	6
Effects on the Germination of Different Seeds....	7
Tolerance and Adaptation to Heavy Metals.....	8
Plasmatic Resistance.....	11
Long-Term Effects.....	13
Effects on Chromosome Aberrations and Plant Mutations.....	14
Interaction with Chelates.....	19
The Mode of Action on Chromosomal and Cellular Behavior.....	21
EXPERIMENTAL AND RESULTS.....	24
Part One.....	25
Experiment 1: Effect of Copper and Manganese Deficiencies on Soybeans.....	25
Part Two.....	30
Experiment 2: Effect of Concentrated Solutions of Copper, Manganese and Zinc Salts on Seeds of Soybean, Rye, Sorghum and Oats...	30
Experiment 3: Effect of Copper, Manganese and Zinc Solutions on Germination of Sorghum Seeds.....	36
Experiment 4: The Effect of Soaking Oat Seeds in Concentrated Solutions of Copper, Zinc and Manganese.....	38
Experiment 5: Effect of the Anions NO_3^- , Cl^- , SO_4^{--} on the Germination of <u>Portulaca oleracea</u> Seeds.....	39
Experiment 6: Effect of Various Concentrations of Copper and Zinc Solutions on Germina- tion and Further Development of <u>Portulaca</u> <u>oleracea</u> Seeds.....	41

TABLE OF CONTENTS (continued)

	Page
EXPERIMENTAL AND RESULTS (continued)	
Experiment 7: Effect of Lithium Chloride on <u>Portulaca oleracea</u>	44
Experiment 8: Effect of Various Levels of Mineral Elements on Plants and Progeny Seeds of <u>Portulaca oleracea</u>	70
Part Three.....	78
Experiment 9: Inducing Metal Tolerance in <u>Portulaca oleracea</u>	78
Experiment 10: Inducing Plasmatic Resistance in <u>Portulaca oleracea</u>	80
DISCUSSION.....	82
SUMMARY AND CONCLUSIONS.....	91
BIBLIOGRAPHY.....	93

LIST OF TABLES

TABLE	Page
1. YIELD DATA FOR SOYBEAN PLANTS DERIVED FROM ABNORMAL SEEDS DUE TO COPPER DEFICIENT TREATMENT AND NORMAL SEEDS.....	32
2. GERMINATION OF SOYBEANS, RYE, SORGHUM AND OAT SEEDS AFTER SOAKING IN VARIOUS CONCEN- TRATIONS OF COPPER, MANGANESE AND ZINC FOR 24 HOURS.....	34
3. GERMINATION OF SOYBEANS, RYE, SORGHUM AND OAT SEEDS AFTER SOAKING IN VARIOUS CONCENTRATIONS OF COPPER, MANGANESE AND ZINC FOR 24 HOURS, FOLLOWED BY DRYING.....	35
4. EFFECT OF STRONG SOLUTIONS OF COPPER, ZINC AND MANGANESE ON GERMINATION AND EARLY DEVELOP- MENT OF SORGHUM SEEDS.....	37
5. EFFECT OF DIFFERENT LEVELS OF POTASSIUM NITRATE, POTASSIUM CHLORIDE AND POTASSIUM SULFATE SOLUTIONS ON THE GERMINATION OF <u>PORTULACA</u> <u>OLERACEA</u> SEEDS.....	40
6. EFFECT OF DIFFERENT CONCENTRATIONS OF COPPER SULFATE ON GERMINATION, SHOOT LENGTH AND ROOT LENGTH OF <u>PORTULACA OLERACEA</u>	43
7. EFFECT OF DIFFERENT CONCENTRATIONS OF ZINC SULFATE ON GERMINATION, SHOOT LENGTH AND ROOT LENGTH OF <u>PORTULACA OLERACEA</u>	45
8. EFFECT OF TREATMENT WITH DIFFERENT IONS IN VARIOUS CONCENTRATIONS ON RATE OF SURVIVAL OF <u>PORTULACA OLERACEA</u> CUTTINGS.....	71
9. EFFECT OF TREATMENT WITH DIFFERENT IONS IN VARIOUS CONCENTRATIONS ON RATE OF SURVIVAL OF <u>PORTULACA OLERACEA</u> SEEDLINGS.....	73

LIST OF FIGURES

Figure	Page
1. Photomicrograph of longitudinal section through cotyledon of soybean seed (normal seeds).....	27
2. Photomicrograph of longitudinal section through cotyledon of soybean seed from manganese deficient plants.....	28
3. Forked stems in soybean as a result of manganese deficiency.....	29
4. Twin plants in soybean as a result of copper deficiency.....	31
5. <u>Portulaca oleracea</u> plant derived after seed treatment with 1 % lithium chloride.....	47
6. Flowers of wild and mutated types of <u>Portulaca oleracea</u>	48
7. Flower of mutated type of <u>Portulaca oleracea</u> produced after seed treatment with 1 % lithium chloride.....	49
8. Mutated branch of <u>Portulaca oleracea</u>	50
9. Cutting of mutated branch of <u>Portulaca oleracea</u> , showing reversion to wild type at base.....	52
10. Cutting of mutated branch of <u>Portulaca oleracea</u> , showing young wild shoot at base.....	53
11. Percent reversion from mutated to wild types in four generations of clones of <u>Portulaca oleracea</u>	54
12. Photomicrograph of stomata of lower leaf epidermis of mutated type of <u>Portulaca oleracea</u>	60
13. Photomicrograph of stomata of upper leaf epidermis from wild type of <u>Portulaca oleracea</u>	61

LIST OF FIGURES (continued)

Figure	Page
14. Photomicrograph of stomata of lower leaf epidermis from wild type of <u>Portulaca oleracea</u>	62
15. Photomicrograph of stomata of upper epidermis from mutated type of <u>Portulaca oleracea</u>	63
16. Photomicrograph of cross section through wild shoot of <u>Portulaca oleracea</u>	66
17. Photomicrograph of cross section through mutated shoot of <u>Portulaca oleracea</u>	68
18. Photomicrograph of cross section of half mutated (left) and half wild (right) stem tissues of <u>Portulaca oleracea</u>	69
19. Effect of pretreatment of boric acid on percent germination of <u>Portulaca oleracea</u> seeds.....	74
20. Effect of pretreatment of copper chelate on percent germination of <u>Portulaca oleracea</u> seeds.....	75
21. Effect of pretreatment of iron chelate on percent germination of <u>Portulaca oleracea</u> seeds.....	76
22. Effect of pretreatment of zinc chelate on percent germination of <u>Portulaca oleracea</u> seeds.....	77

INTRODUCTION

The influence of certain mineral deficiencies on the progeny seed from peanut plants has been described (44). The results of those experiments, together with reports in the literature on mutagenic effects of deficient and excessive mineral levels, prompted a study of these mineral effects on other plants in more detail.

The effect of mineral deficiencies on seed formation, seed characteristics and phenotype of subsequent generations in soybeans was studied here, because earlier reports (43) showed a very strong response of this plant to mineral deficient treatments.

Furthermore it seemed desirable to conduct experiments in which many elements were tested to induce mutations, especially in Portulaca oleracea, a plant especially suitable for such experiments because of its short life cycle.

The pronounced tolerance of certain plants to high levels of heavy metals (9, 11, 38), suggested the possibility of inducing metal tolerance in Portulaca oleracea. It seemed desirable therefore to treat cuttings of Portulaca oleracea with high levels of heavy metals and to study the adaptation to these high metal levels.

REVIEW OF LITERATURE

Many aspects of the effects of mineral elements on physiology and morphology of plants have been brought together. Most of the literature discussed here deals with the effect of high levels of elements. Accumulation, interaction with other ions and toxic action are reviewed. Effects on germination and tolerance to heavy metals are discussed. Most of the literature available on long-term effects and mutagenic action of elements has been cited. A short review on the mode of action of various elements on chromosome and cellular behavior concludes this literature survey.

Accumulation of Elements

Uptake and accumulation of various elements is strongly dependent upon the environment and is, furthermore, largely genetically controlled. Bertrand (4) showed that seeds of monocotyledons contained less lithium than those from the dicotyledons. The vegetative parts were in general four times richer in lithium than the seeds. Large differences existed also between the various plant families. Plants in the Papaveracea family, for example, were very high in lithium while those in the Rubiacea family were very low.

It has been shown by Zimmerman et al. (111) that several species in the family of Theaceae do accumulate fluoride in large amounts. In other species fluoride content could only be increased through additional amounts of fluorides as was shown in celery and

several other legumes (32). Robinson and Edington (86) reported an extreme variation in selenium content (from 0.1-15,000 ppm) in plants. Astragalus species were able to accumulate up to 4,000 ppm Se from a soil that contained only 2 ppm Se. Seeds of Neptunia from plants grown on soils rich in selenium contained 123 µg Se per seed (80). When these seeds were planted on normal soil the next generation seeds had only 0.45 µg Se per seed. However, analysis showed that the total amount of selenium in one plant was $\pm$ 0.100 mg. This could have come from entirely mobilized selenium in the seed.

Thomas and Baker (102) provided evidence that the level of chemical element accumulation in corn is under partial genetic control. Through plant breeding it is possible to obtain low accumulating genotypes for some elements. This may be of importance when varieties are required which accumulate less of a toxic or radioactive element as with strontium fallout.

Effect on Uptake and Distribution of Other Ions

A mineral excess or deficiency would be expected to affect the uptake, distribution and metabolism of other ions. Also the inter-relationship with other ions probably would differ with the metal tolerant species. Under copper deficiency young citrus plants accumulated very small amounts of phosphorus as was shown in autoradiographic studies with ^{32}P (79). Excess copper was accompanied by an almost normal distribution of phosphorus. However, a slight reduction in uptake of radioactive phosphorus was observed. Excess boron (22 ppm) also reduced considerably the uptake of phosphorus.

A lead-mine population of Agrostis was treated with different

levels of calcium and phosphorus (55). The lead-mine population did not respond to this higher level of fertility, whereas a pasture population (non-lead tolerant) responded favorably. Cu in concentration of 5 to 10 ppm had a lethal effect on young citrus roots but when used in combination with an Fe-EDTA treatment the toxic effect was reduced (95). Analysis showed that less copper had been taken up in the latter case.

The manganese and aluminum tolerance of certain alfalfa varieties was related to the calcium metabolism (77). Results indicated that toxicity symptoms in both tolerant and non-tolerant plants started at the same level of toxic concentration in the aerial organs. Ca ions were found to be particularly high in the roots of the tolerant species. It was believed that calcium lowered the uptake of manganese and aluminum or immobilized them within the roots. In strontium-adapted plants a correlation was found between the amount of strontium and calcium (21). Relatively low amounts of strontium in the plant corresponded to high amounts of calcium and vice versa. Ion substitution did take place within certain plants.

Calcium partially antagonized the toxic effect of lithium chloride (31). When 0.3 percent calcium nitrate was added to toxic lithium chloride solutions of 0.1 and 0.3 percent, plants survived for a longer period of time.

A high heavy metal concentration in a nutrient medium in general reduced the uptake of iron, resulting in chlorosis (30). This was shown for oats when Co or Cu was given in excess and for barley when high amounts of Cu and Ni were applied. With a high cobalt treatment the uptake of Ca and Mg in oats was higher than in the controls.

Toxic Effects

High levels of many metals and other elements are well known to be toxic to plants. Otto (76) in 1891 did probably one of the earliest experiments to prove the toxicity of a CuSO_4 solution on corn seedlings. The author worked with a concentration of 5 and 10 ppm Cu. Plant growth was greatly reduced. Analysis showed that the leaves had taken up only minute amounts of copper. As early as 1896 Haselhoff (46) reported the toxic effect of copper on plants. Solutions of 5 mg CuO per liter were toxic to Zea mays. Phaseolus vulgaris grew in a concentration as high as 10 mg CuO per liter before toxicity symptoms occurred.

The effect of 74 different acids and metal salts on root growth of Lupinus albus was tested (56). When different salts of the same element were used, the same concentration of the element in all cases caused injury. The metal ion action was independent of the salt used and was only determined by its actual molecular concentration. The same molecular concentration of cobalt, nickel, and iron gave the same toxic effects. The authors concluded that this was related to their atomic weights which were the same. Von Rosen's findings were similar (105). Heald (47) provided further evidence of the toxic action of nickel, cobalt, and copper on the seedlings of Pisum sativum and Zea mays. Partial recovery occurred when the plants were transferred from the toxic solution to distilled water. It was reported (45) that galvanized iron netting had a toxic effect on plants. From almost new wire 50 mg Zn per 100 g could be extracted by immersing the wire for 24 hours in water. Soils rich in nickel (100 to 400 ppm Ni) caused a considerable reduction

in growth of oats and the plants had a high amount of necrosis and chlorosis (50). A considerable concentration of nickel occurred in all parts of the plant, being higher in the young leaves and flowers. Moderately affected plants contained about 35 ppm Ni in dry matter. Severely affected plants contained 110 ppm Ni. When boron was supplied in supra-optimal quantities to plants, it was transported directly to the site of injury (74). Boron, in this case, probably moves mainly with the transpiration stream. Carnations were very tolerant and lemons very sensitive to high boron supplies. In general, necrosis may be expected when boron concentration in the leaves exceeds 1,500 ppm (dry wt.).

Zinc in concentrations of 1 to 10 ppm in polluted river water is a potential danger to vegetation on the side of rivers (71). Zinc from mine dumps was carried into the rivers as zinc sulphate. Terrestrial vegetation was severely damaged by smelter fumes which contained high amounts of sulfur (36). In some regions one million tons of sulfur per year were released into the air. Atmospheric fluoride in the form of cryolite particles produced no visible injury to plants (65). Physiologically, cryolite is a rather ineffective form of fluoride. Gaseous forms of fluoride are much more toxic (67).

Cytological Effects

A few studies have dealt with the effect of a mineral stress on fine structure of particular cell components. It was shown (103) that the absence of zinc blocked the full development of the grana fretwork in the chloroplast. Redistribution of zinc did not seem to

take place, as the older leaves appeared to be normally developed. Chloroplasts of iron deficient leaves of Xanthium (7) showed a lamellar structure considerably different from normal ones. The lamellae were reduced from a group of ten per granum to a group of two or three.

A cytological effect of calcium deficiency in the shoot apex of barley has been studied by Marinos (62). A frequent occurrence of an unevenness in the contour of the nuclear envelope, which became vesiculated with large gaps, was observed. Other cell membranes became disorganized under calcium stress. The evidence is fairly convincing that calcium is essential for the maintenance of structural integrity of different membrane systems.

Effects on the Germination of Different Seeds

In earlier studies the remarkable resistance of seeds to metallic poisons has been shown (19). Cress seeds were able to germinate in a 1/75 N zinc-sulfate solution. Copper proved far more toxic than zinc. The after-effect of strong copper solutions (1/75-1/600 N) was detrimental to the seedlings. On transfer to normal solutions the germinated seeds progressed no further. Ernst (26) found that seeds from zinc-tolerant plants of Silene and Minuartia species would germinate in very high concentrations of zinc. Sixty percent of Silene cucubalus seeds were still able to germinate in a 10,000 ppm Zn-solution. In contrast, the control plant Epilobium angustifolium was unable to germinate at 500 ppm Zn. Seeds of metal-tolerant plants also germinated faster and their total rate of survival was higher than in any other metal-sensitive plant.

An instant toxic effect has been reported for thallium where very low levels destroyed the vitality of the seeds (19). It has been suggested (38, 82, 84, 104) that the reaction of protoplasma with heavy metal causes the protein to precipitate in the outer layers of cells (shock-reaction). Further entry of the toxic metal is thus prevented. Seeds of Sabal minor were able to germinate after being immersed in seawater for four weeks (12).

Imbibition of seeds will be strongest in the lower salt concentrations where osmotic pressure of the solution is low. In very high concentrations imbibition is arrested which may result in a complete exclusion of the toxic ions.

Kisser and Lettmayr (59) tested the uptake of various metal ions on numerous agricultural seeds. They found that the differences in uptake are closely associated with the morphology of the seeds. The ion-absorption was strongly dependent on the rate of water uptake by the seeds. The faster this uptake, the more ions were absorbed.

Tolerance and Adaptation to Heavy Metals

Adaptation of plants to high metal concentrations has been studied by many investigators (11, 13, 27, 37). Most of these studies were concerned with so-called mine-plant populations. The diversity of the mining areas around the world has contributed very much to the knowledge and understanding of plant behavior under mineral excess. Increasing air and water pollution have become significant factors in providing excessive amounts of elements to plants (36, 65, 71).

Bradshaw et al. (10) discussed the evolutionary significance of heavy metals and the role they may have played in the evolution.

They pointed out the importance of the industrial environment where accumulation of certain waste products may result in certain selection pressures. The development of metal tolerance in Agrostis tenuis is one of adaption as a result of evolutionary changes. Tolerant and non-tolerant populations have sharp boundaries even if they grow close to each other. Wind pollination facilitates in such cases an ample gene exchange. The heavy metals of this environment play only a selective role and cannot in this case be considered as a mutagenic agent. There are several species of Agrostis, which are tolerant to high concentrations of Cu, Ni, Pb, and Zn (9, 10). The plants which were tolerant to these heavy metals originated from mine-plant populations. The Ni and Zn tolerance were correlated in one direction only. Zinc-tolerant plants were always tolerant to nickel but nickel-tolerance could be developed without tolerance to zinc.

Iron content was determined for several plants which grew in a soil very rich in iron (45 to 50 percent Fe) close to an old iron mine (8). Some plants here were particularly high in iron. Taraxacum officinale had four times as much iron as when grown in normal soil. Other plants such as Cirsium arvense had normal iron contents. The differences in iron uptake in both cases illustrate that the mechanism of tolerance must also be different.

The zinc-tolerant types of Silene, Campanula, Plantago and other plants have smaller cells than the normal types (92). Since these plants also grew on a very poor and dry soil, selection pressure in the direction of phenotypical smaller cells is very likely. The zinc-tolerant types were not in the least tolerant to lead, indicating

a very specific metal tolerance. Schwanitz and Hahn (91) found a difference in size of epidermis cells and of nucleus in various zinc-tolerant and normal forms of the same species of Silene. When both forms were grown under normal conditions the differences in size of cells and nuclei were maintained. A copper-resistant form of Silene inflata did not appear to be resistant to zinc. Broker (11) demonstrated that there was no correlation between the morphology of Silene inflata and the tolerance to high amounts of zinc. When grown under normal conditions, Silene inflata did not lose this tolerance. However, the plants were altogether bigger, with long stems and broad leaves in contrast with the more dwarf type grown on zinc soils. The tolerance to zinc is genetic in this particular variety, and is carried over dominantly.

Tillers of lead-tolerant Agrostis and tillers of pasture plants were transplanted to soil collected from an abandoned lead mine with 1.0 percent lead and 0.03 percent zinc (9). The pasture plants did not grow at all and 50 percent died. The lead-tolerant Agrostis grew normally. It was shown (27) that heavy-metal tolerance is particularly frequent in the Silene, Minuartia and Armeria species. Minuartia verna growing on a soil containing 4,626 ppm Cu accumulated 1,033 ppm Cu in the seeds. Silene cucubalis seeds accumulated only 374 ppm Cu from the same soil.

It has been found also (2) that the excess amount of zinc which is taken up by the zinc-tolerant species of Silene inflata Sm. is correlated to increased photosynthesis. CO₂ assimilation was not affected when non-tolerant plants were treated with higher levels of zinc. Prat (81) described a copper-resistant Agrostis and Melandrium variety, growing on a sandy soil very rich in copper. Melandrium

silvestre seeds were able to germinate in a mixture of 25 percent CuCO_3 and 75 percent soil. Seeds from the same variety grown in a low copper locality did not germinate at all. According to Prat, these copper resistant varieties can only be obtained through natural selection. In the F_1 generation the plants have kept this high copper tolerance. Extensive studies (25) have been made of the copper vegetation in Katanga. Total copper in this copper-rich region is present in amount from 500-100,000 ppm. Although the copper concentration is very high in the rhizosphere, the absorption is always limited. Differences however exist. Copper-resistant plants are able to exclude the copper. The so-called cuprophytes are able to accumulate 20 to 50 times the normal amount.

Very high amounts of strontium were reported in plants which had grown on strontium sulfate mines (21). The effect of different levels of zinc on the germination of seeds from various zinc-tolerant plants was determined (26). Germination was little affected when the nutrient medium contained less than 100 ppm Zn, but between 10,000 and 50,000 ppm Zn there was a rapid reduction in percent germination.

Plasmatic Resistance

It has been found by several authors (38, 84, 87) that plants which are tolerant to high metal concentrations also have a high plasmatic resistance or tolerance for the same metal. This means that cells of tolerant plants are able to perform normal plasmolysis after treatment with high levels of metal ions. Cells of metal-sensitive plants are instantly killed by such a treatment and consequently plasmolysis is no longer possible.

Gries (38) tested the resistance of protoplasm of different tolerant and non-tolerant species against high concentrations of zinc salt. Through plasmolysis experiments the author was able to show the great differences in zinc-tolerant and zinc-sensitive plants. The cytoplasm of zinc-tolerant forms withstood 100 to 1,000 times stronger concentrations of zinc than that of normal plants. The criterion for survival was the ability of the epidermal cells to perform plasmolysis in 1.0 M glucose solution.

The resistance of cell plasma against specific concentrations of vanadium varies from plant to plant (6). For some plants (Tradescantia zebrina) 0.0001 percent vanadium sulfate was harmful to the epidermis cells. Others (Rhoeo discolor) were able to support up to one percent vanadium sulfate. Various plants known to be Cu-tolerant showed high plasmatic resistance against toxic copper concentrations (84). The effect of heavy metals on plasmolysis has also been studied in several Bryophyta and higher plants (82). It was noticed that in these otherwise non-tolerant plants notable differences exist in plant resistance.

Otto (76), in experiments on copper toxicity, came to the conclusion that the living protoplasm formed a barrier for excessive amounts of copper. Only very small amounts of copper were found in the shoot and root of corn seedlings when treated with high levels of copper sulfate solution.

When the concentration of metal, e.g., copper, is very high, an impermeable layer is formed on the outside of protoplasm preventing further entrance of the toxic copper (104). There are suggestions (84) that plasmatic resistance can be built up, though no

experiments of this nature have ever been conducted. Vitis vinifera showed a high degree of Cu tolerance (84). Although the grape is propagated vegetatively it could well be that the many repeated copper sprays so commonly used in this culture have resulted in this high plasmatic resistance.

Growth retardant chemicals increased the tolerance of soybean plants against toxic levels of salt (64). The retarded plants, which could withstand an excessive amount of fertilizer, produced viable seed. Internal plasmatic changes may be responsible for this reaction. Similar results were obtained on kidney bean plants (75). Plants treated with the growth retardant B 995 tolerated twice as much salt as the untreated ones.

Long-Term Effects

Most of the experiments dealing with the prolonged effect of elements on plants have been limited to the effect of N P K treatments or radioactive element treatments. When applications of ^{32}P were made to young barley plants, fertility was reduced in the treated plants and in offspring (63). In subsequent generations the plants appeared to be normal and no long-term effect could be observed. Durrant and Tyson (24) reported an after-effect of N P K treatments on wheat in the first generation. The effect in the generation after treatment on yield depression was found for potassium deficiency, particularly. The effect was not correlated to differences in seed weight. In the second generations no effects of the original treatment could be detected. With different N P K treatments small and large forms of Linum plants, were obtained (23).

These variations remained stable for six generations. The heritable change is not directly associated with gene-mutations but is explained as possibly due to alterations in nuclear substances closely associated with chromosomes. Some comparison may be drawn here between this response and the induction of drought resistance in plants. According to some investigators drought hardening persisted through several generations (33). Differences in nitrogen base composition of nucleic acids and in composition of amino-acids were found (109).

Effects on Chromosome Aberrations and Plant Mutations

Chromosome aberrations and mutations have been obtained by many workers when plants were grown under mineral excess or stress conditions. Stockberger (101) in 1910 made a detailed study of the effect of CuSO_4 on mitosis in the root tips of Vicia faba. Dilute as well as strong CuSO_4 solutions, from 1/20,000 N - 1/12 N, were used. The weaker solution gave the same result as the stronger but it took a much longer time. Microscopic examination revealed an inhibited mitosis, a disorganization or interrupted formation of the spindle fibers, a non-continuous cell plate, and often an unusually large vacuole.

The mutagenic effect of a combination of copper and ethyl methane sulphonate (EMS) has been shown (1). When copper alone was used in concentrations of 10^{-3} M and 10^{-6} M no significant aberrations could be detected in the root tip cells of wheat, but when combined with EMS there was a very strong synergistic action resulting in many chromosome mutations. It seems here that the stimulating effect of Cu on plant growth in these low concentrations promotes the mutagenic

activity of EMS. Similar results were obtained with Vicia faba (69).

In contrast, when barley seeds were treated with EMS in the presence of Cu or Zn ions the total chromosome aberrations did not increase (68). However, the relative proportions of chromosome aberrations were changed. Effects of EMS alone, and in combination with Cu and Zn ions, were also studied on chlorophyll mutation rate in Arabidopsis (5). It was found that neither Zn or Cu in a concentration of 10^{-6} M induced genetic changes, but in combination with EMS they enhanced the mutagenic action. The maximum synergistic effect depended, furthermore, on the pH of the solution, and was found for the zinc solution at pH 7 and for the copper solution at pH 9. Cytological observations of onion root tip cells treated with sub-lethal concentrations of boric acid (1/100 M - 1/25 M) showed an arrested mitosis (22). Effects of some other ions (Ba, Ca, Mg) on the development of pollen mother cells of Tradescantia reflexa were reported (110). The reactions were described as a reversible coagulation of the chromosomes. The otherwise invisible chromosomes became visible in a light microscope without staining. Several chromosome aberrations could be induced in Allium cepa when roots were treated with a 10^{-2} solution of sodium fluoride (66). The presence of many bridges as a probable result of dicentric chromosomes indicates that fluoride may act on the chromosome during interphase. In another study the same authors (67) showed that permanent chromosomal aberrations could be induced when tomato plants were treated with gaseous fluoride. In the F_1 generation abnormal phenotypes were found. Hydrogen fluoride can, for this reason, be considered as a mutagenic agent.

Hyde and Paliwal (51) showed the effect of ethylene diamine tetra acetic acid (EDTA) and various levels of Ca and Mg on chromosome behavior. An excess of calcium, as well as a deficiency of calcium and magnesium, increased the chiasma frequency in Plantago ovata.

Deficiencies of N, P, K, Ca, Mg and S caused abnormalities in the chromosomes of Vicia faba (29). A shortage of K, P and Ca resulted in hyperchromatic chromosomes. The chromosomes, in this case, became shorter than usual with a general reduction in size of the nucleus and nucleoli. Hyperchromatic chromosomes were the result of sulfate stress. Three times as many mutations in the F₂ generation of Antirrhinum majus were obtained when the plants were grown under nitrogen, phosphorus and magnesium stress (20).

Abnormal cytological effects in Tradescantia were reported when plants were grown at deficient levels of sulfate (4 and 8 ppm) (97). The effect was primarily observed in the meiosis where tripolar spindles were formed. The presence of micronuclei in mitosis indicated a breakage of chromosomes.

Induction of chromosome breakage at meiosis was obtained by a magnesium (96) and calcium deficiency (98). Tests were performed on clonal material of Tradescantia (96). Chromosome fragments were nine times more frequent in the Mg deficient material. The high percentage of micronuclei was directly correlated to the high frequency of aborted pollen. Anthers from plants grown in very low calcium concentrations had only abortive cells (98). Microspore division was halted. In somewhat higher levels (10 ppm Ca) the pollen grain contained micronuclei 18 times more frequently than in normal cells. Since micronuclei are mostly formed from the acentric

chromosome fragments, their frequency is a good index for abnormal chromosome behavior. In another study (99) it was shown that calcium is able to affect the radiosensitivity in Tradescantia. In calcium deficient treatment both interchange and interstitial deletions were 40 percent higher than in the controls. Chromosomes are subject to breakage at low levels of calcium when irradiated.

In the experiments of Sigenaga (94) the young leaf epidermis and petal cells of Tradescantia reflexa were used. Both neutral salts and heavy metals were applied to plant material. The neutral salts in a concentration of 0.1 M - 0.3 M caused a decrease in the spindle volume. The chromosomes increased in refractivity and failed to migrate to the poles. Heavy metals, such as Pb, Zn, Fe, Sn, Ag and Cu, caused several abnormalities when used in relatively low concentration. $\text{Pb}(\text{NO}_3)_2$ and AgNO_3 yielded trinucleate cells and anaphase and telophase bridges. High concentration resulted in death of the cells.

Considerable research has been done on the action of several metals on the mitosis of root cells of Vicia faba (34, 35). The concentrations of the metals used ranged from 10^{-5} M to 10^{-2} M. In the lowest concentration, an increase in the mitotic activity was obtained, comparable to a minor element activation. The reactions described were very specific for each metal. Cadmium gave the highest percentage of pyknosis, whereby a complete destruction of the nucleic acids took place in the chromosomes. Cobalt gave the most achromatic chromosomes, copper increased considerably the stickyness, chromium treatment had the highest number of translocations, manganese seemed to interfere strongly with the coiling

of the chromosomes, and nickel affected the spindle formation resulting in tri- and multipolar cells. In general Mn and Zn gave the highest percentage of fragmentation. Here, fragmentation and translocation are the only chromosome aberrations considered as prerequisites for mutation. With the metal treatments the centromere of the SAT-chromosomes also became visible.

It has been reported (48) that a concentration of 0.1 - 0.2 percent $\text{Co}(\text{NO}_3)_2$ had a cytostatic effect on mitosis. A thickening and despiralization of the chromosomes in the root tip cells of Vicia faba was obtained. Scheibe (89) has shown that AlCl_3 and $\text{Al}(\text{NO}_3)_3$ could produce mutations in Melilotus albus. In an ingenious way the solutions were given directly to the plant by means of a split stem. One part of the stem sustained the plant as usual, the other split part was brought into the solution. Toxic solution could reach the cells in this way before meiosis took place. Most of the mutations were found in the second generation after treatment, especially the chlorophyll mutations where a single recessive factor is involved. Other types of mutations produced were low coumarin-containing plants especially found after treatment with 10^{-3} M AlCl_3 .

In the experiments conducted by Von Rosen (106, 107), the cytological effect of 55 elements of the periodic system on the mitosis in the rootlets of Pisum abyssinicum have been studied. Plants from treated seeds showed in the second generation chlorophyll aberrations and mutations such as a waxless type and a so-called Gigas-plant, with enlargement of all plant parts. Similar types of mutations could also be produced by radiation. Several phenotypically

recognizable mutations in summer wheat were obtained after treatment with AlCl_3 (90). Although aluminum chloride was very toxic, it had a high mutagenous effect. Most frequent mutations were square head types ears with short stem. AlCl_3 was introduced by the so-called "notching method" of Oehlkers (73), whereby part of the stem was split and immersed in AlCl_3 solution. Haarring (39) obtained several mutations in a hexaploid summer wheat variety using AlCl_3 in concentrations of 10^{-3} - 10^{-2} M. Zschege (112) used the same wheat variety for cytogenetical investigations and found several chromosome aberrations. Antirrhinum majus when treated with 10^{-2} M AlCl_3 also showed a considerable increase in mutation rate (73).

Interaction with Chelates

Introduction of chelates in plant studies has thrown new light on the role of metals in chromosomes. Cohn (15) studied the effect of chelation on the production of chromatid aberrations in Vicia faba. In these experiments, 2,2, bipyridine which complexes easily with iron was used. The result of the treatment was chromosome breakage in the heterochromatic regions of the chromosome. Found, also, were a significant number of achromatic lesions and a marked clumping of the chromosomes. If ionic linkages exist between the protein component of the chromosome and the DNA then iron may be one of them. These linkages could then be disturbed by a chelate such as bipyridine. It has been found that versene (a tetrasodium salt of ethylene diamine tetracetic acid) induced chromosome stickiness in neuroblast cells of the grasshopper (88). The rate of mitotic divisions was reduced. The chelating action of versene may

result here in binding elements such as calcium with a direct effect on cell division and chromosome behavior.

Rieger et al. (85) studied the interaction of ethylene diamine tetra-acetic acid (EDTA) and triethyl melamine (TEM) in root tips of Vicia faba. Simultaneous action of these two components increased the chromosome aberrations by 20 percent above the expected value. EDTA alone has only a weak mutagenic effect. Introduction of metal ions before or after treatment inhibited in some cases the actions of EDTA (complex forming). The mode of action of the chelate could be a direct one in the sense of a metal inactivation or metal removal leading to chromosome aberrations, or an indirect action by means of an inactivation of the enzyme system or by changing the ionic balance.

The specificity of certain chelates in the cells has enabled Phallusia mamillata to concentrate vanadium a million times more than the vanadium level in sea water (3). Bayer (3) synthesized a highly selective chelate for gold by polycondensation of di- and triaminothiophenols with glyoxal. In fact, gold could be removed from sea water without interference of other metals. A similar mechanism may be operative in plant cells.

The bivalent ions are also able to form chelates with protein (105). They belong to the group of metals with atomic weight of less than 65. Metals of higher atomic weight, such as mercury, are highly poisonous to the cells.

The introduction of heavy metal leads to alteration of the total balance of chelates with a direct effect on nucleic-acid and nucleotide metabolism (106). On the other hand, it was found (54)

that the resistance of plants to heavy metals was probably due to the reaction of certain chelates with these metals.

The Mode of Action on Chromosomal and
Cellular Behavior

The effect of Cu is probably related to a direct reaction with the SH-groups of the spindle fibers (101). In prophase of mitosis, spindle fibers contain only SH-groups which later in anaphase form solid S-S-bonds and these maintain fiber integrity. Also, the absence of sulphur probably affects the fiber chemistry of the spindle, resulting in its abnormal behavior (97). Histochemical tests (51) specific for sulfhydryl groups, gave a deeper staining of the nuclei in EDTA treated plants of Plantago ovata than in those not treated with EDTA. This leads to the inference that the structure of the sulphur containing proteins was modified, loosening the bonding between chromosomal RNA and associated proteins.

Selenium, since it is able to substitute for sulphur, may affect the structural chromosomal proteins (108). This could explain why a reduction in the rate of crossing over in barley was found when plants were treated with 0.5 ppm selenium.

The inhibitory effect of boron on starch phosphorylase was shown (93). Boron complexes either at the active site of this enzyme or with glucose-1-phosphate, the substrate.

It was suggested (22) that the formation of a boron ribonucleic acid complex in the interphase cell is responsible for blockage of mitosis. No chromosome aberrations were observed. The inhibiting effect of fluoride in concentrations of 2×10^{-4} and 10^{-3} M on growth of corn seedlings was demonstrated (14). This effect could

be related to inhibition of DNA-synthesis since less DNA was found in the fluoride treatment. Higher protein content in the roots of orange seedlings counteracted the toxic action of copper (95). Thus, more iron could be taken up. It was suggested that insoluble nitrogenous material could act as an absorbing complex for Cu.

Peterson and Butler (80) studied the incorporation of radioactive selenium into Neptunia, a selenium accumulator, and into non-accumulating species of wheat, rye grass, and clover. Although the amounts of selenium taken up from a culture solution was found to be the same for all plants the incorporation into various compounds was different. In plants of wheat, rye grass, and clover there was an extensive incorporation of selenium into the protein, as seleno-amino-acids. In Neptunia, however, the proportion of ^{75}Se bound to protein was much lower. Only a small proportion of the total radioactivity could be extracted by proteolytic enzymes (trypsin, chymotrypsin). A high percent was soluble in bromine water, indicating a high proportion of an insoluble inorganic selenium compound.

Radioactive calcium was incorporated into Lilium longiflorum (100), showing that on germination of the radioactive pollen the ^{45}Ca was firmly bound to the nuclei, indicating a possible binding with the chromosomes. It was suggested that the binding site for these and other metals is probably on the phosphate groups of the DNA.

Mutations, as they occur after certain mineral deficiencies, were explained as a result of stress in the so-called "Zuwachphase" (20). The duplication of chromosomal material in this phase is

short of the necessary elements and chromosome aberrations may occur. The direct effect of the mineral stress on the nucleic acid metabolism was correlated with the chromosome anomalies (29).

EXPERIMENTAL AND RESULTS

The experimental data presented here are divided as follows:

The first part deals especially with the effect of copper and manganese deficiencies on soybean plants. Abnormalities in progeny seed and plants are described.

The second part consists of a number of experiments dealing with the direct and prolonged effect of high mineral levels on plants and seeds from treated plants. Two approaches were made here.

First, seeds of sorghum, rye, oats and soybean and seeds of Portulaca oleracea were treated with various concentrations of mineral salts. Germination and further development of seedlings were investigated. Germination of Portulaca oleracea was also tested at various levels of potassium sulfate, potassium nitrate and potassium chloride to explore the levels of toxicity of the anions. Several generations in Portulaca were studied to obtain more information on the mutagenic effect of the elements. The rooting experiments with cuttings and leaves provided further information on the characteristics of the mutation. Grafting experiments were performed to investigate the possibility of a bacterium or viral infection. Morphological and histological differences between a mutated type of Portulaca (mutation induced by Li Cl) and the wild type were examined. Chromosome studies and leaf epidermis studies were conducted to obtain more information on the type of mutation produced.

The second approach was an attempt to induce mutations by direct treatment of seedlings and cuttings of Portulaca oleracea with higher levels of mineral elements. Seeds collected from treated plants were tested for germination and further development. Several generations were grown to find out if mutations had occurred.

The third part relates to the effect of various heavy metal ions on the induction of plasmatic resistance and metal-ion tolerance in Portulaca oleracea. The first experiment deals with the build up of this tolerance within the plant cell and the plasmolysis technique to test the viability of the treated cells. The second experiment relates to the tolerance of the whole plant as affected by a pre-treatment with heavy metals.

Since the methods varied for the different experiments, the details of the methods will be explained under the respective experiments. Throughout these experiments any mention of nutrient solution refers to the no. 2 solution of Hoagland and Arnon (49).

The results will be given following each experiment.

Part One

Experiment 1: Effect of Copper and Manganese Deficiencies on Soybeans

General methods

Soybeans, var. Hardee, were germinated on paper towels moistened with deionized water. The young seedlings were planted in big glass jars and grown in the open. Nutrient solution which was added to the jars was made up of all the usual major and minor elements, minus one element for a particular treatment. Minor element deficiencies were tested for Cu, Mn, Zn, Mo and Ca. A special study was made of the copper and manganese deficiencies.

Experimental procedure and results with manganese deficiency

Many seeds from plants which had grown under minus manganese conditions showed a very specific brown spot at the side of the cotyledons. Location of this particular spot was the same for each affected seed. To investigate the nature of this necrotic spot, microscopic examinations of the seeds were made. Seeds were soaked for 18 hours in water, fixed in Randolph's Modified Navashin fluid (52) and dehydrated with ethyl- and butyl-alcohol. Thereafter, seed was embedded in paraffin and cut into serial sections ten microns in thickness. A quadruple stain combination of Johansen (52) was used.

Microscopic studies indicated that the area which was affected in the manganese deficient seeds, consisted of a specific group of cells directly connected to the vascular bundles through thin parenchymatous cells (Figures 1 and 2). The size of these cell groups varied. On the average they were 20 to 30 cells long, 10 to 15 cells wide and 4 to 6 cells deep. Cell walls of these cells were thin compared to those of surrounding cells. The direct linkage of these cells with the vascular system suggested that they were involved in some way in water transport during development of the seed from the ovule. Deterioration of these cells with a manganese deficiency is not well understood. Plants grown under Cu, Zn, Mo, or Ca deficiency did not produce any seed of this kind. In a few cases the minus manganese treatment changed the morphology of the cotyledons. Tri-cotyledons were produced. In the check treatments hundreds of seeds were tested and no abnormalities were found. Upon further development most of the tri-cotyledon seeds grew into plants with twisted or forked stems (Figure 3). These deviations were never observed in the check plants.

Figure 1. -- Photomicrograph of longitudinal section through cotyledon of soybean seed (normal seeds). Thin parenchymatous cells are shown in A. This cell group is connected through cells of B with vascular system of the cotyledon (darker tissues). (95x)

Figure 2. -- Photomicrograph of longitudinal section through cotyledon of soybean seed from manganese deficient plants. White area indicates large destruction of cells. (95x)

Figure 3. -- Forked stems in soybean as a result of manganese deficiency. Picture at bottom shows a twisting of the forked stem.

Experimental procedures and results with copper deficiency

Seeds of soybean plants which had been grown in a nutrient solution without copper did not show any necrosis. However, many cotyledons were malformed, and plants grown from these seeds showed various abnormalities. There were many plants with a typical forked stem. In other cases two plants had developed from one seed. Sometimes the stems of these plants were united at the base or in the middle, forming twins. Where these twins were not united the roots were simply twisted around each other (Figure 4).

For further studies, six abnormal plants were retained and transplanted to containers filled with Leon fine sand. Nutrient solution at one half strength was added at the beginning. Tap water was used thereafter. From each plant, seeds were collected and pods and beans weighed. The results are shown in Table 1.

No definite conclusion can be drawn from these data since only single plants were used. Seeds from twin plants with twisted roots yielded the lowest average weight for beans plus pods. From each plant, forty seeds were planted in rows in the field. In all cases normal plants developed. It appears that the effect of the copper deficient treatment was only associated with the first generation plants.

Part Two

Experiment 2: Effect of Concentrated Solutions of Copper, Manganese and Zinc Salts on Seeds of Soybean, Rye, Sorghum and Oats

Material and methods

Mature seeds of soybean, rye, sorghum and oats were soaked for 24 hours in deionized water (control) and in strong solutions of copper, manganese, and zinc salts. The treatments were not aerated. Copper

Figure 4. -- Twin plants in soybean as a result of copper deficiency. All plants developed from one seed. Left: Roots twisted. Middle: Stems united in middle. Right: Base of stems grown together.

TABLE 1
YIELD DATA FOR SOYBEAN PLANTS DERIVED FROM ABNORMAL
SEEDS DUE TO COPPER DEFICIENT TREATMENT
AND NORMAL SEEDS

	Total Number Pods	Total Number Beans	Weight Pods (gm)	Weight Beans (gm)	Total Weight Beans & Pods (gm)	Avg. wt. Pods Containing Beans (gm)
Twins united in middle	110	209	12.8	29.2	42.0	0.38
Twins united at base	47	72	6.4	12.1	18.5	0.39
Twins roots twisted	52	75	5.9	9.3	15.2	0.29
Plant from 3 cotyledon seed	96	160	10.9	25.8	36.7	0.38
Plant from 1 cotyledon seed	80	145	9.8	21.1	30.9	.039
Plant from misformed cotyledon seed	83	153	9.7	21.1	30.8	0.37
Plant from normal seed	71	127	8.9	22.2	31.1	0.44

and zinc were applied as sulfate, manganese was added as a chloride. Concentrations used were: for copper 36 and 288 ppm, for manganese 888 and 7104 ppm, and for zinc 188 and 1500 ppm. Half of the seeds from each treatment were transferred to Petri dishes on wet filter paper (series A) and germinated, while the other portion (series B) was dried without a preliminary washing and germinated at a later date. Germination was evaluated after 7 days. Criterion for germination in all cases was a distinct development of the radicle and appearance of the shoot.

Results

Percent germination after 7 days for each treatment is indicated in Tables 2 and 3. After 7 days no further increase in germination took place. The results indicate clearly that the sorghum seeds are very tolerant to high metal concentrations, especially in the series A. An 80 to 100 percent germination was found in these strong metal solutions. The oats in these series for all the treatments, gave a zero percent germination.

Comparison of the two series showed that drying the seeds after soaking with the toxic solutions had a favorable effect on the germination, especially in the case of oats and rye (compare Tables 2 and 3). Since drying took place on filter paper it is possible that a part of the toxic solution was drawn out. This could explain the differences between the two series. Highest percentage of germination for oats was found in the series B for the manganese treatment. It is possible that manganese is removed more easily from the oats than the copper and zinc. This confirms earlier reports (60) where absorbed manganese could be completely removed from peas and wheat seeds by washing. Absorbed zinc and copper ions could only be removed to a certain extent.

TABLE 2

GERMINATION OF SOYBEANS, RYE, SORGHUM AND OAT
SEEDS AFTER SOAKING IN VARIOUS CONCENTRATIONS OF COPPER, MANGANESE AND
ZINC FOR 24 HOURS

Concentration Elements	% Germination after 7 days			
	Soybeans	Rye	Sorghum	Oats
0 ppm	75	80	100	60
36 ppm Cu	75	50	100	0
288 ppm Cu	0	0	100	0
888 ppm Mn	75	40	80	0
7104 ppm Mn	0	60	80	0
188 ppm Zn	75	50	80	0
1500 ppm Zn	0	25	100	0

TABLE 3

GERMINATION OF SOYBEANS, RYE, SORGHUM AND OAT SEEDS AFTER
SOAKING IN VARIOUS CONCENTRATIONS OF COPPER,
MANGANESE AND ZINC FOR 24 HOURS,
FOLLOWED BY DRYING

Concentration Elements	% Germination after 7 days		
	Soybeans	Rye	Oats
0 ppm	75	80	60
36 ppm Cu	50	100	60
288 ppm Cu	0	100	50
888 ppm Mn	75	60	100
7104 ppm Mn	50	50	100
188 ppm Zn	50	100	80
1500 ppm Zn	50	80	80

Experiment 3: Effect of Copper, Manganese and Zinc Solutions on Germination of Sorghum Seeds

Material and methods

Since the sorghum seeds seemed more tolerant to the heavy metal solutions than the other seeds used (see Experiment 1), further experiments were done with sorghum seeds. Seeds of sorghum var. Lindsey 77 F, were soaked in concentrations of 288 ppm copper, 7,104 ppm manganese and 1,500 ppm zinc salt. Copper and zinc were used as sulfate, manganese as chloride. A total of 287 seeds were used for the copper treatment, 214 seeds for the manganese treatment and 454 seeds for the zinc treatment. After six days the seeds were taken out of the solutions and contrary with the first experiment thoroughly washed with distilled water and planted in glass trays which contained gravel saturated with nutrient solution. Germination was evaluated seven days after planting. Part of the sorghum plants derived from seeds that survived the treatments were later set out in crocks filled with vermiculite. Nutrient solution at one half strength was added.

Results

Total germination after seven days amounted to about 75 percent in the copper and zinc treatment and only 25 percent for the manganese treatment (see Table 4). The last results are not in accord with the first data (Tables 2 and 3). However, in this experiment the seeds were kept in the toxic solutions much longer. This could account for the differences found in the manganese treatments, especially since the manganese concentration was extremely high. Upon further development of the sorghum plants no abnormalities were found.

TABLE 4

EFFECT OF STRONG SOLUTIONS OF COPPER, ZINC AND MANGANESE
ON GERMINATION AND EARLY DEVELOPMENT OF
SORGHUM SEEDS

Degree of Germination	% Germination after 7 days		
	228 ppm Cu	1,500 ppm Zn	7,104 ppm Mn
Good germination (shoot 2 1/2-1 1/2 cm)	25	21	7
Less developed (shoot 1 1/2-1/2 cm)	42	39	13
Just emerging	9	15	5
No germination	24	25	75

Experiment 4: The Effect of Soaking Oat Seeds in Concentrated Solutions of Copper, Zinc and Manganese

Materials and methods

Oats were soaked for four weeks in concentrations of 375 ppm Zn, 1,776 ppm Mn and 72 ppm Cu. Zn and Cu were applied as sulfate, Mn was added as chloride. The strong concentrations of the solutions prevented germination of oats. After four weeks seeds were washed once with tap water and germinated on moist paper towels in glass trays filled with gravel. A part of the seeds would not germinate. The part that did germinate produced viable seedlings. These were transplanted in crocks filled with Leon fine sand. Crocks were placed outside and protected by wire netting to prevent bird damage. There were 14 plants per crock, and 2 crocks per treatment.

Results

These experiments showed a direct influence of the previous heavy metal treatment on the time of flowering in the plants. Blooming occurred 10 days earlier in the plants grown from seeds treated with 375 ppm Zn than those of the check treatment. The copper treatment did not appear to affect further plant development. The manganese treatment in contrast retarded the blooming by about ten days as compared with the check.

The reason for these differences must be directly or indirectly related to the accumulation of heavy metals in the seeds. No differences in plant height were observed between the treatments. Leaves of plants from copper treated oat seeds were somewhat darker than leaves from the other treatments.

Experiment 5: Effect of the Anions NO_3^- , Cl^- , SO_4^{--} on the Germination of *Portulaca oleracea* Seeds

Material and Methods

To explore the level of toxicity of the anions, seeds of *Portulaca oleracea* were treated with different levels of potassium nitrate, potassium sulfate and potassium chloride. The concentrations here used for each salt in ppm were 20,000, 10,000, 2,500, 625 and 300. Approximately 200 seeds were placed in Petri dishes. Five milliliters of one of the different levels of potassium salts were added to each dish. Germination was determined after 6 and 9 days. After 9 days the seeds were thoroughly washed with tap water and left in beaker glasses for further observation.

Results

In the nitrate and chloride treatment (Table 5) there was a complete inhibition of germination with the 20,000 ppm treatment. At this level 30 percent of the seeds germinated after 9 days in the sulfate series. However seedlings were small. In the nitrate and chloride series germination starts at a level of 10,000 ppm. It can be seen that the germination of *Portulaca oleracea* seeds was better in the nitrate series than in the others. It was found that after washing, the seeds did not germinate any further.

Anions can in general be tolerated in much higher concentrations by plants than the metal ions. It is obvious that when very high concentrations of metal salts are used, the anion part becomes also toxic. Any mutagenic or toxic effect at lower levels of metal salts solutions can be attributed solely to the action of metals.

TABLE 5

EFFECT OF DIFFERENT LEVELS OF POTASSIUM NITRATE, POTASSIUM
CHLORIDE AND POTASSIUM SULFATE SOLUTIONS ON THE
GERMINATION OF PORTULACA OLERACEA SEEDS

Treatment	Germination % after	
	6 days	9 days
Control (Water)	75	90
NO ₃ 20,000 ppm	0	0
10,000 ppm	43	62
2,500 ppm	76	88
625 ppm	77	91
300 ppm	68	96
Cl 20,000 ppm	0	0
10,000 ppm	19	43
2,500 ppm	55	76
625 ppm	52	72
300 ppm	69	98
SO ₄ 20,000 ppm	0	33
10,000 ppm	20	27
2,500 ppm	40	84
625 ppm	50	62
300 ppm	48	63

Experiment 6: Effect of Various Concentrations of Copper and Zinc Solutions on Germination and Further Development of Portulaca oleracea Seeds

In these experiments Portulaca oleracea was used. This plant has a short life cycle of six to seven weeks from seed to production of seed, with many advantages as well. Portulaca is a good seed producer. There are approximately fifty seeds in one seed head. Relatively small plants can easily produce a thousand seeds. Furthermore, the plant can be propagated very easily by cuttings. Clones of uniform material can be obtained in short time. Ten to twelve days after flowering the seeds are ripe. There is no dormancy and a close to 100 percent germination occurs in most cases. The plant requires little care and is able to survive through long periods of drought. Portulaca has a pronounced Circadian rhythm (57, 61) and is excellent for studying photoperiodicity (58, 61).

The effect of the copper solutions

Material and methods. -- One hundred seeds of Portulaca oleracea were placed in each Petri dish on Whatman no. 2 filter paper, using three papers per dish. Five milliliters of one of the copper sulfate concentrations were added. The following concentrations of copper were used in ppm: 4.5, 9, 18, 36, 72, 144, 288 and 576. To reduce the amount of evaporation, Petri dishes were placed on a moist sheet of filter paper and completely enclosed in a large plastic bag. It was found that the solution evaporated very fast when these precautions were not taken. Twelve hours of light and twelve hours darkness were provided for germination since Portulaca seeds germinated better under these conditions. Temperature was constant throughout the experiment at 25° C.

Germination was evaluated after 10 days and average shoot length and root length determined. Thereafter all the seeds, germinated and non-germinated, were transferred to water and washed. Further germination and development of seedlings was checked. Seeds were considered as germinated when either radicle or shoot or both had pierced through the seed coats. Portulaca seeds which had been killed in a 1 percent mercuric chloride solution, were not able to perform any similar breakthrough.

Results. -- The development of the seedlings in the first two treatments (4.5 and 9 ppm Cu) was not different from those of the check (see Table 6). However at 18 ppm Cu considerable reduction in root growth was observed. Increasing the amount of copper further reduced the root growth and at 36 ppm the shoot growth was affected. The germination percentage remained high until a concentration of 576 ppm Cu was reached. Higher concentrations inhibited strongly.

After washing, all seedlings developed in the 72 ppm Cu treatment died within a few days. This was also true for the seedlings grown in the 144 ppm Cu and 288 ppm Cu treatments. After washing a further germination took place of the seeds not germinated before. In the 576 ppm Cu treatment 70 percent of the seeds germinated and produced viable seedlings. These data showed that concentrated solutions of copper were able to inhibit strongly the germination of Portulaca oleracea seeds. No apparent damage was done to the seeds. Inhibition was removed as soon as the seeds were thoroughly washed in water. The seedlings treated with lower concentrations of copper (4.5, 9, 18, and 36 ppm Cu) all survived after being washed with water. Most damage was done in the middle concentrations

TABLE 6

EFFECT OF DIFFERENT CONCENTRATIONS OF COPPER SULFATE ON
GERMINATION, SHOOT LENGTH AND ROOT LENGTH
OF PORTULACA OLERACEA

Concentration Cu ppm	% Germination After 10 days	Avg. shoot Length (mm)	Avg. root Length (mm)
0	96	10	10
4.5	79	10	10
9	78	10	10
18	80	10	5
36	80	5	1.5
72	88	1	1.5
144	86	0.5	1.5
288	97	0	1
576	22	0	.5

(72, 144 and 228 ppm Cu) where copper concentrations were relatively high.

The effect of the zinc solutions

Material and methods. -- Similar experiments were carried out as in the preceding one, this time however with various concentrations of zinc sulfate solutions (see Table 7). Portulaca oleracea seeds were planted and treated in the same way as in the former experiment. Germination was determined after 10 days and root length and shoot length measured. All the seeds and seedlings were washed with tap water after 10 days and further development was checked.

Results. Data indicated (Table 7) that the percentage of germination was not greatly affected by the concentration of the treatment, but root and shoot growth were reduced. The first toxic effects were visible in the 94 ppm Zn solution. Tips of roots and cotyledons turned brown. From the 188 ppm Zn and stronger treatments all the roots and shoots became black, with a further reduction in size. When seedlings were later washed with water, all died which had been treated with higher than 47 ppm Zn. In the highest treatment of 1,500 ppm Zn, no further germination took place of the seeds that were inhibited before.

Experiment 7: Effect of Lithium Chloride on Portulaca oleracea

Material and methods

Portulaca oleracea seeds were treated with different concentrations of lithium chloride (1 - 2%). Two hundred seeds were soaked for 20 days in these solutions. Only part of the seeds germinated. After washing the seeds for two days with tap water,

TABLE 7

EFFECT OF DIFFERENT CONCENTRATIONS OF ZINC SULFATE ON
GERMINATION, SHOOT LENGTH AND ROOT LENGTH
OF PORTULACA OLERACEA

Concentration Zn ppm	% Germination After 10 days	Avg. shoot Length (mm)	Ave. root Length (mm)
0	91	10	8
24	85	8	5
47	87	5	1
94	89	4	0.5
188	83	4	0.5
375	82	4	0.5
750	85	0	0.5
1,500	76	0	0.5

germinated and non-germinated seeds were planted in small pots filled with sterilized soil.

Results

The non-germinated seeds started to germinate after four days. About 50.0 percent of the plants that developed from the seeds treated with 1 percent lithium chloride, bore branches with modified leaves and flowers (Figure 5). Leaves were more pointed than oval shaped. Differences were also found in the margins of the leaves. Margin cells of modified leaves were considerably reduced in size, giving a smooth appearance to the edge of the leaf. The petals of flowers on modified branches were incised, instead of having only one notch in the middle, as is the case with wild flowers (Figures 6 and 7).

Further experimental procedures and results

Rooting of cuttings. -- In order to check the constancy of the mutated branches, cuttings were made. A record was kept for each plant from where cuttings were taken and drawings made (Figure 8). On each drawing was indicated from where the cuttings originated. Main top shoots were indicated by A, B, C, etc. Going towards base of plant, leaf axil indicated as A', A'', etc. Main top shoot of left branch from A' is A'L. The different leaf axils on this branch indicated were as A'L, A'L'', etc. Left and right branches from A'L' are then A'L'r and A'L'l branches. Following this system it is easy to label the whole plant. In this way all the clones have a known origin, and they carry the same letters as the branches from where they were derived.

It was found that most cuttings kept their morphological features.

Figure 5. -- Portulaca oleracea plant derived after seed treatment with 1 % lithium chloride. Note the two mutated branches at the left and right. In the middle the original wild type is shown.

Figure 6. -- Flowers of wild and mutated types of Portulaca oleracea.
Left: Flowers of wild type. Leaves are here obovate and
petals lobed. Right: Two smaller flowers of the mutated
type. (Approximately natural size.)

Figure 7. -- Flower of mutated type of Portulaca oleracea produced after seed treatment with 1% lithium chloride. Leaves are elliptical and margin of the petals is incised. (5x)

Figure 8. -- Mutated branch of *Portulaca oleracea*. Cuttings were taken at the dotted lines. Numbering system is explained in the text.

In approximately 30 percent of the cuttings, however, branches of the original wild type reappeared (see Figures 9 and 10). In four generations of cuttings tested it was never possible to obtain a pure clone of the modified Portulaca type. The percentage of reversion for four generations is shown in Figure 11 for four different clones. The high percentage of reversion in the beginning is probably due to the low number of observations since only a few cuttings were available. With the next generation the number of cuttings increased. Consequently, more observations could be made, resulting in more reliable percentages.

Plants grown from seeds collected from the mutated branches did not show abnormalities but resembled the original wild type. Also, no difference was found in the second and third generation plants. It seems likely that we have here a chimera phenomenon. Part of the somatic tissue is probably mutated. If all cells were mutated in a deviated branch then we should at least find some plants of the mutated type in the next generation. Most gene mutations go from dominant to recessive (89), so that in most cases we cannot, in the first generation, recognize the modified types. In the next generation, however, double recessive types are readily recognizable as the mutated ones. In the case of a chimera, especially the periclinal type, it will never be possible to obtain seeds reproducing the same modified tissue.

Microspores and megaspores are initially formed from the sub-epidermal cell layers in the corpus. The tunica cells only divide in one direction. The cells of the corpus divide in two directions. The only case in which it is possible to obtain seeds from the mutated

Figure 9. -- Cutting of mutated branch of Portulaca oleracea, showing reversion to wild type at base. Left branch with obovate leaves. (Approximately natural size.)

Figure 10. -- Cutting of mutated branch of Portulaca oleracea, showing young wild shoot at base. The young shoot developed in the axil of the third pair of leaves is from the mutated type. (Approximately natural size.)

Figure 11. -- Percent reversion from mutated to wild types in four generations of clones of Portulaca oleracea. Code number for each clone is indicated at left of curve.

tissue will be when tunica cells are given off to the corpus cells. Usually this does not occur.

Rooting of leaves. -- Leaves can also be rooted easily by sticking them in coarse gravel moistened with nutrient solution in a tray covered with plastic. If the petiole is left on the leaf it will root much better. Cutting off the petiole and the lower part of the leaf makes the chimera type leaves more difficult to root than the wild ones. However, an addition of 15 ppm indole acetic acid (IAA) to the nutrient solution will considerably promote the rooting of both types. It is not necessary to dissolve the IAA in alcohol as is usually done. When added directly to the concentrated nutrient stock solution (ten times Hoagland's solution) it is easily dissolved by stirring with a magnetic rod.

Results indicated that roots were formed at the base of the leaf from the vascular bundles. In three weeks these leaves grew much larger and increased their fresh and dry weight five times or more. Sometimes new sprouts developed at the base of the leaf. These sprouts usually had the same leaf type as the mother leaf. Seeds were collected from such branches and the progeny studied.

It was found that seeds obtained from the mutated leaf sprouts reproduced only the wild types. This indicates that two different cell tissues, wild and mutated, must be present in these leaves. The periclinal chimera type continued in the structure of the leaves.

Virus studies and grafting. -- When making clones from the chimera and wild form an occasional infection showed up in the new growth. This was probably caused by a virus or bacterium. The new leaves were considerably reduced in size and typically elongated.

Infection could have taken place by a contaminated razor blade or directly from outside into the cut surface.

In order to check the possibility of a viral or bacterial infection being responsible for the formation of the pointed leaf type in Portulaca, several plants of the wild type were mechanically infected with a leaf sap extract. The diseased leaves were ground in a porcelain mortar together with a few milliliters of 0.05 M KH_2PO_4 . The juice was applied to the cut surface of a stem directly above a branch or to the cut surface of a leaf base where a young bud was just beginning to develop.

Tobacco plant leaves were also treated with the extract. The leaves were first dusted with a light cover of carborundum (600 mesh) and the infected sap applied by a slight pressure with a piece of cheesecloth. The same procedure was repeated using chimera type leaves.

After several weeks of growing no signs of virus infections were observed. The treated plants developed in a normal way and no leaf deformity could be detected.

In addition to these experiments several graftings were made.

If a virus had been responsible for this modified leaf type the chances of transmission through the graft would have increased. The easiest way to perform the grafting was to insert the stem portion into a pre-made hole in the stock. The opening of the hole should be slightly smaller than the diameter of the scion. Graftings can be made onto any part of the receiving plant. It is not necessary to bind the grafted sections together with string or to use waxed tape.

The results indicated a normal development of the grafts. The wild form was not affected and continued to develop normal wild branches. The grafted pointed leaf type kept the typical morphological features in the same way.

Chromosome studies. -- It was to be expected that chromosome differences exist between the two types of Portulaca oleracea. The extreme smallness and high number ($2n = 54$) of chromosomes in Portulaca oleracea made it difficult to detect variations, unless the mutation occurred in the form of a polyploid. The adventitious root tips of Portulaca formed an excellent material to study the chromosomes. These roots in general were thicker and easier to handle than the finer roots of the seedlings. Rooting of the cuttings started in about 5 days. Roots developed fast at the beginning. The best stage for taking material was when the roots were about a half inch long.

When dealing with a chimera, in which a periclinal mutation existed, it was difficult to obtain root tips from both mutated and wild tissues. Adventitious roots in Portulaca oleracea originated in the pericycle and interfascicular cambium. Unless some mutated tissue was present in these regions no difference would be found in chromosomal composition in the root tips of both types. Nevertheless an attempt was made to analyze root tip material of Portulaca on chromosome abnormalities. Root tips were fixed in a mixture of five parts ethyl alcohol (95%), one part chloroform, one part glacial acetic acid, and one part formalin (40% formaldehyde) for five minutes. After fixation root tips were washed and hydrolyzed in 1 N HCl solution for ten minutes at a temperature of 60° C. To avoid fluctuation in

temperature the small vials containing the root tips were placed in a pyrex tray of sand and kept in an oven at a constant temperature of 60° C. After hydrolysis, root tips were transferred to Schiff's reagent (52). A deep red color developed after 10 to 15 minutes after which the root tips were ready for examination of chromosomes. Root tips were then transferred to a slide, a drop of aceto-carmin added, and the tips squashed between slide and cover slip.

Several methods for preparation of Schiff's reagent were tested. The best combination, with longest preserving time and fastest reaction was prepared as follows: Dissolve 0.5 gram basic fuchsin in 50 ml boiling water, filter at 60 to 80° C and add one gram sodium metabisulfite together with 10 ml 1 N hydrochloric acid. Let stand overnight to decolorize and filter after shaking for one minute with 300 mg powered charcoal.

The results indicated that the wild type and the mutative type had the same number of chromosomes, namely 27. Since chromosomes are very small it was not possible to determine any abnormalities.

Studies on leaf epidermal cells. -- It is known that stomata of polyploid forms are larger than the ones from the same diploid types (18). If the mutant in Portulaca was the result of polyploidy then it should be possible to recognize this by the larger stomata in the leaf blades. For this reason comparisons were made on the microscopic structure of the leaf epidermal cells of both types of Portulaca.

Epidermal cells were removed very easily by a small scalpel.

Staining was performed at the same time by dipping the scalpel in a 1.0 percent Erythrosine solution. After the stain had dried, the scalpel was worked under the epidermis which was cut off with a sharp razor blade on both sides. A perfect, live stained section was thus obtained. Transfers and damages were avoided this way. Sections were transferred to a drop of water on a slide. Water to which a detergent had been added was used. This released surface tension and avoided air bubbles. For better contrast and observation, sections were dried several hours under cover glass. This intensified the colors and sharpened the contrast between the cell walls and the rest of the cells.

Examination of the epidermis showed that the stomata were more abundant in the upper epidermis than in the lower epidermis (1 1/2-2 as many). All epidermal cells, including guard cells and accessory cells, were slightly smaller in the upper epidermis. Per unit of surface there was approximately the same number of stomata in both Portulaca types. No difference in size or shape of comparable cells could be detected (see Figures 12, 13, 14 and 15). Since the corpus cells contribute to the inner structure of the leaves and tunica cells determine the structure of epidermis, it is evident that two kinds of tissues in a leaf of chimera must be present.

Furthermore, it can be seen that when rooting such a leaf, it must have been possible to obtain both types of shoots, depending on the organization of the two different tissues. Rooting experiments with Portulaca oleracea leaves have indeed shown that both types of shoots could be produced.

Figure 12. -- Photomicrograph of stomata of lower leaf epidermis of mutated type of Portulaca oleracea. (540x)

Figure 13. -- Photomicrograph of stomata of upper leaf epidermis from wild type of Portulaca oleracea. (540x)

Figure 14. -- Photomicrograph of stomata of lower leaf epidermis from wild type of Portulaca oleracea. (540x)

Figure 15. -- Photomicrograph of stomata of upper epidermis from mutated type of Portulaca oleracea. (540x)

Histological differences in the chimera and wild type. -- In order to have a better understanding of the chimera phenomenon, it was necessary to study the initial development of the shoot. The apical meristem is located at the top of the shoot. The peripheral portion of the apical meristem is formed by repeated cell division of the apical meristem. Foliar primordia and cortex originate from this peripheral portion of the apical meristem.

Leaf primordia are formed from the surface layers. If the tunica is several cell layers deep the entire leaf may be derived from this zone. Leaf primordia may also originate in the corpus. In the case of a chimera this offers various possibilities. Leaves of different origin may be found on the same branch opposite each other. In case of the chimera type Portulaca oleracea this typical situation has repeatedly been found by the author in young shoots. After giving rise to a pair of pointed leaves the shoots of Portulaca subsequently produced only the wild type.

The anatomy of the vascular connections between leaf and stem can be best studied in the region between the internodes. The vascular bundles (leaf traces) may travel as independent bundles through several nodes. Somewhere they are linked to another part of the vascular system. However, the branch traces are located in the nodal region with vascular bundles connecting the main stem with the branch.

In Portulaca branch traces are formed slightly below the insertion of a leaf. Cross sections through the nodes reveal the various aspects of branching.

Axillary buds are formed in the axils of the leaves. In general,

they originate in the superficial layers of the young stem. Periclinal and anticlinal divisions precede the protrusion of the buds. Axillary buds may develop later into lateral shoots having the same general structure on the main stem. Since different cell layers can participate in the formation of lateral shoots it is evident that, in case of periclinal chimeras, wild types as well as mutated types may originate from these sites.

Many comparisons at microscopic level have been made between the two types of Portulaca. However, when young tissues were examined no differences could be established. Axillary buds in early stages of development could not be recognized externally as wild or mutated. Consequently, microscopic comparisons of those structures were useless.

The older stems of both types did not show any anatomical differences. Also, the flower buds have essentially the same cell structure with no particular deviation being detected. However, when young mutated and wild branches originating from the same mutated stem were compared definite anatomical differences could be established. Most of these differences were found in the zone where the shoot joins with the main stem. Thin wall parenchymatous cells fill the gap between the two vascular bundles of main shoot and lateral shoot.

Orientation and structure of the first four to five cell layers of the cortex were not the same for both types of shoots. In wild shoots these cortical cells are flattened and form a distinctive ring around the vascular bundles (see Figure 16). In many places these cells are broken. This leaves the impression that the cell

Figure 16. -- Photomicrograph of cross section through wild shoot of Portulaca oleracea. Note typical ring of flattened cells around the pericycle. In a few places (A) cells are disrupted. (150x)

walls are thinner and are more easily damaged by the cutting process. Outside this ring the cortical cells appear to be normal. Measured across, toward the epidermis, this zone has about five to six cells.

In the mutated shoot we do not find this ring structure of flattened cells (see Figure 17). The cell arrangement is more open without further differentiation of the cortical cells.

In further development this structural difference is lost. Sections from wild lateral shoots above the axil cannot be recognized from those of mutated lateral shoots.

Cross sections were also taken from stems which carried the two different kinds of leaves at the same level opposite each other. It was found that one-half of the section had disrupted cells primarily derived of epidermis and cortical cells (see Figure 18). These differences must be linked to the two different cell tissues present in this section. One part carried the mutated leaf, the other part carried the wild leaf type. Disruption of the cells could be the result of thin wall cells in one part of the section. In the process of cutting or dehydration these cells are probably damaged. Thus, there must be a correlation between the disrupted cells and the appearance of wild leaves or shoots in altered branches.

Microscopically, cell differences can be detected only in the young developing wild tissues within a chimera. In the older tissues, however, structural differences between the two Portulaca types cannot be established.

Figure 17. -- Photomicrograph of cross section through mutated shoot of Portulaca oleracea. Cortical cells show a normal development. Note the many starch grains that fill cells of the first cortical layers. (150x)

Figure 18. -- Photomicrograph of cross section of half mutated (left) and half wild (right) stem tissues of Portulaca oleracea. Note the disruption of epidermis and cortical cells on the right. (150 x)

Experiment 8: Effect of Various Levels of Mineral Elements on Plants and Progeny Seeds of Portulaca oleracea

Effect on cuttings and seeds thereof

Material and methods. -- Cuttings of Portulaca oleracea were grown for 4 weeks in various concentrations of salts of different elements. Cu was supplied as sulfate, Li and Zn as chlorides, B as boric acid and Se as selenumoxychloride. All dilutions were made in one-half strength nutrient solution. Five cuttings were placed in each jar with 135 ml of solution through a 2 mm opening in the lid. Concentrations and elements used are listed in Table 8. A plus sign indicates that all plants did survive and were able to form seeds. A minus sign indicates that all plants died. Where flowers were formed and seed setting took place, the seeds were harvested. Further germination of seeds from the above plants and development was tested. Young plants were set out in a greenhouse in small plastic pots filled with sterilized soil.

Results. -- In the course of the experiment it was found that extremely high concentrations could be tolerated by the cuttings. Copper was relatively toxic and zinc to a lesser degree. Seeds that were harvested from the treated plants showed in most cases a normal development.

Selenium in selenumoxychloride proved to be very toxic. In the higher concentrations plants died within a few hours. Many seedlings died after the two cotyledons appeared. Cotyledons were very dark red in color in contrast to a normal dark green. High amounts of selenium were probably incorporated in the seeds. This could explain its devastating action on germination.

TABLE 8

EFFECT OF TREATMENT WITH DIFFERENT IONS IN VARIOUS
CONCENTRATIONS ON RATE OF SURVIVAL OF
PORTULACA OLERACEA CUTTINGS

Treatment	Rate of Survival	Treatment	Rate of Survival
Cu ppm		Li ppm	
4.5	+	26	+
9	+	52	+
18	+	104	+
36	+	208	+
72	-	416	+
144	-	833	+
288	-	1,666	+
B ppm		Se ppm	
7	+	4.5	+
15	+	9	+
31	+	18	+
62	+	36	+
125	+	72	-
250	+	144	-
500	+	288	-
Zn ppm			
24	+		
47	+		
94	+		
188	+		
375	-		
750	-		
1,500	-		

Effect on seedlings and seeds thereof

Material and methods. -- In a similar experiment young seedlings of Portulaca oleracea were used. Roots were cut to a length of one-half inch. Concentrations and elements used are indicated in Table 9. All the dilutions were made up with one-quarter strength nutrient solution. Copper, zinc, and iron were given as chelates, lithium as chloride, aluminum as sulfate, boron as boric acid, and selenium as selenioxychloride. Observations were made at regular intervals of three days.

Results. -- After 14 days a definite separation could be made into surviving and dying plants (see Table 9). A plus sign indicates that no noticeable effect from the solution was observed. A minus sign indicates that all plants died. A plus and minus sign means that plants were slightly affected. After 20 days most of the plants which survived started to bloom and seeds were harvested 20 days later.

Further experimental procedure. -- From each of the above treatments that produced seed 25 seeds were taken and planted in small pots filled with sterilized soil. A square piece of carton, perforated with 25 small holes, served as a guide for planting. Seeds are extremely small and they need to be guided in order to obtain a regular planting. Percentage of germination was determined after 7, 14, and 21 days.

Results. -- Results are given in Figures 19, 20, 21 and 22. Pretreatment of iron and boron had a direct effect on the germination at the beginning. It took the seeds from these treatments much longer to germinate than those from the plants which were treated

TABLE 9

EFFECT OF TREATMENT WITH DIFFERENT IONS IN VARIOUS
CONCENTRATIONS ON RATE OF SURVIVAL OF
PORTULACA OLERACEA SEEDLINGS

Treatment	Rate of Survival	Treatment	Rate of Survival
Cu ppm		Li ppm	
4	+	42	+
8	+	208	-
24	+	416	-
80	-	833	-
400	-	1,666	-
Al ppm		Fe ppm	
8	+	4	+
27	+	8	+
68	-	21	+
135	-	70	-
270	-	350	-
B ppm		Se ppm	
25	+	12	+
62	+	46	-
125	+	115	-
250	+	231	-
500	-	1,156	-
Zn ppm			
4	+		
8	+		
24	+		
82	+		
410	-

Figure 19. -- Effect of pretreatment of boric acid on percent germination of *Portulaca oleracea* seeds.

Figure 20. -- Effect of pretreatment of copper chelate on percent germination of *Portulaca oleracea* seeds.

Figure 21. -- Effect of pretreatment of iron chelate on percent germination of *Portulaca oleracea* seeds.

Figure 22. -- Effect of pretreatment of zinc chelate on percent germination of Portulaca oleracea seeds.

with an excess of copper or zinc. The data showed, furthermore, that the total percentage of germination, which remained constant after 21 days, was very much affected by the concentrations of the solutions and of the elements used. Final germination percentage for the boron pretreatment was rather low (64%). Pretreatment with zinc did not seem to affect the total germination. Upon further development of the plants none showed abnormalities.

Part Three

Experiment 9: Inducing Metal Tolerance in *Portulaca oleracea*

The possibility was investigated whether the resistance of cells to high concentrations of metal salts solutions could be artificially induced. For this reason several concentrations of copper, zinc, and iron chelate solutions were prepared. The idea was to allow *Portulaca oleracea* cuttings to develop in high concentrations of metal ions for several weeks. Thereafter, cuttings of these plants were to be transplanted to the same or even higher salt concentrations. If some cellular resistance to high salt concentrations had been developed, the cuttings of the treated plants would then probably do better in concentrated solutions of metal ions than non-treated plants.

Material and methods

Chelated copper was used for this experiment in concentrations of 8, 24, and 80 ppm, chelated zinc in concentrations of 24, 82 and 204 ppm, and chelated iron in concentrations of 21, 35 and 70 ppm. Preliminary experiments, in which a wide concentration range of metal chelates were used, indicated that these concentrations were most suitable for this experiment. The various

concentrations were made up in one-quarter strength nutrient solution. Cuttings of clonal material of Portulaca oleracea were taken and planted four per jar in 135 ml of the solution. Plants were kept in this solution for two months. These experiments were carried out in duplicate.

Results

In the iron series root development took place on all the cuttings. However, there was a gradual retardation of plant growth in the higher concentrations. In the copper series only the 80 ppm solution showed a severe inhibition on growth. In spite of this inhibited growth, roots were formed even in this high concentration of Cu. Plants showed severe retardation in growth in the concentration of 204 ppm zinc solution. Roots were formed but six of the eight plants died.

Further experimental procedures and results

Cuttings of plants which had grown in the 8 ppm Cu solution were placed in a Cu concentration of 80 ppm and 160 ppm. A similar scheme was worked out for the rest of the treatments. In summary they were:

Cuttings from 8 ppm Cu solution placed in 80
and 160 ppm Cu

Cuttings from 24 ppm Cu solutions placed in 80
and 160 ppm Cu

Cuttings from 35 ppm Fe solution placed in 70
and 140 ppm Fe

Cuttings from 70 ppm Fe solution placed in 70
and 140 ppm Fe

Cuttings from 24 ppm Zn solution placed in 82
and 164 ppm Zn

Cuttings from 82 ppm Zn solution placed in 82
and 164 ppm Zn

For comparison, cuttings of control plants were included and received the same treatment as the cuttings derived from the metal chelate solutions.

After two weeks, observations were made on root development and general appearance. The results indicated that the control plants developed slightly better in the 82 ppm Zn and 140 ppm Fe solutions. In the 70 ppm Fe and the 160 ppm Cu solutions, cuttings from plants grown in high Cu and Fe solutions grew better.

Definite conclusions cannot be drawn from this experiment. It seems that the control plants grew better in some cases. This might be because they were stronger at the start of the test. The plants which had grown in the high concentrations of metal ions had suffered definite growth retardation. On the other hand, it was shown that cuttings from plants having grown in 24 ppm Cu were able to form complete roots in a solution of 160 ppm Cu. Control plants did not develop roots in this high concentration, but produced a stunted growth. The leaves also came off these plants. This indicates that a certain tolerance against high concentrations of copper, at least at the beginning, may have been developed.

Experiment 10: Inducing Plasmatic Resistance in *Portulaca oleracea* Material and methods

Cuttings of *Portulaca oleracea* were grown for two months in a concentrated solution of 82 ppm Zn made up in a 1/4 strength nutrient solution. After this the cuttings were transferred from the greenhouse and set outside, in the sun to develop enough anthocyanin. The epidermis cells which turn red through pigmentation of the vacuolar contents were then easier to examine for further

plasmolysis. Cell membrane may be seen pulling away from the cell wall.

Small pieces of stem from control plants, and from plants having received 82 ppm Zn, were excised. They were put into zinc sulfate solutions of 1, 10^{-1} , 10^{-2} and 10^{-3} M strength. After 24 hours pieces were taken out. Epidermis cells were removed and treated with 5.0 percent KNO_3 solution to test the viability of the cells. Deplasmolysis was performed with a 3.0 percent sugar solution. In order to obtain a good section of the epidermis for examination the piece of stem was held steady with a needle in the center and small incisions were made with a razor blade about 1 mm apart. The epidermis layer was then removed with a small scalpel between the points of incision.

Results

It was found that epidermis cells from plants treated with 82 ppm ZnSO_4 still plasmolized in a 5.0 percent KNO_3 solution after a 24 hour treatment with 10^{-3} M zinc sulfate. Epidermis cells from control plants could not always be plasmolyzed and if they could the plasmolysis reaction time was slowed down. After plasmolysis all cells deplasmolyzed with a 3.0 percent sugar solution.

In the 1 M, 10^{-1} M, and 10^{-2} M zinc sulfate solution most of the cells died. Anthocyanin had leaked from the cells. A few single cells derived from the 82 ppm zinc treatment underwent plasmolysis after treatment with 10^{-2} molar zinc sulfate solution.

Results of this experiment indicated that a certain cell tolerance for zinc could be built up by growing the plants in concentrated zinc solutions.

DISCUSSION

Whenever elements are found in excess or deficient quantities, it is to be expected that they affect plant growth; the nutrient balance is disturbed and result in abnormalities in growth. This direct effect has been studied by many authors (44, 50, 76).

The prolonged effect on seed and following generations is more difficult to interpret. We have to distinguish between cell disturbances in seeds as a result of a particular excess or deficiency in the parent plant and between the genetic effects of such a treatment. Seeds are probably affected directly by a mineral excess or deficient treatment. Several reports have dealt with the effect of a mineral deficient treatment on seed quality and germination (42, 44). The "hollow heart" defect of boron deficient peanuts may in severe stages destroy the tips of the young plumule (44). Peanut plants grown under calcium deficient conditions produced seeds which showed severe vascular deterioration.

It is evident that in most cases the germination of such seeds is also affected. Harrington (42) found that seeds from carrots and lettuce grown on a low calcium level did not germinate as well as the ones from a complete nutrient solution. Deficiency or excess of certain ions may result in bad or retarded germination. It may affect the formation of the young embryo, resulting in deformed cotyledons (41), or in some cases it may affect the apical

system. Shoot formation becomes abnormal, unusual branching and twisting of stems occurs.

In the experiments conducted in part one on soybeans, many of these disturbances in seeds have been found with a manganese or copper deficient treatment. The occurrence of twin embryos in soybean, as a result of a copper deficient treatment, has never been reported before. The natural occurrence of twin plants in a particular line of soybean was described by Owen (78). In many other soybean varieties tested no twin plants were found. The twins that were grown to maturity showed an independent segregation of genetic characteristics. This means that the twin plants were not identical and probably two eggs must have been fertilized independently.

It was known that treatments such as 2-4 D could produce twinning in plants (40). However, where such plants have a common root, we may assume that the apical meristem is damaged, resulting in a take-over by the lateral shoots. The young embryo is here affected in the later stages of development. This is also the case where very young developed seeds of Eranthis hiemalis were treated with excess lithium carbonate (41).

Polyembryony as a result of a treatment with growth-promoting substances has been reported by Johri (53). A certain combination of casein hydrolysate, yeast extract and indolacetic acid induced polyembryony in young excised pollinated embryos of Anethum graveolens.

Where soybeans were grown under copper stress, the young embryo is probably affected in a very early stage, before any differentiation of cotyledons took place. A longitudinal cleavage of the multi-cellular

embryo gave rise to these twins. They may be completely separated or may remain attached with a few cells in the middle (incomplete cleavage). A deficiency of copper at the very early stages of embryo development was probably responsible for this cleavage of cells. Since copper is a component of ascorbic acid oxydase, it might well be that the function of this enzyme was inhibited by a copper deficiency. Ascorbic acid oxydase along with numerous other enzymes was found in the primary cell wall of the plant (70). Although the proper function of ascorbic acid oxydase in cell walls is not known, it might be involved in synthesis of wall components. This could possibly explain why under a copper stress a cleavage of cells may occur. On the other hand, since polyembryony in soybean may result from double fertilization (78) the possibility cannot be excluded that a deficiency of copper promotes this anomaly.

The symptoms of manganese deficient soybeans can be related to the destruction of certain cell tissues in the early phases of seed development. The impression is that tissues of the apical meristem and certain areas of the cotyledons are particularly affected. The typical forked stem, which sometimes remains twisted, is a direct proof that the apical meristem has been damaged. We must here clearly distinguish between the later branching where a main shoot is present and between the forked stem where no main shoot is found.

In contrast to the copper deficiency in soybean the deficiency of manganese is here manifest at a much later stage of embryo development. Particular cell groups, probably fully developed, are disturbed in the cotyledons.

In the experiments of part two some of the direct effects of metal excess on seed germination have been investigated. It was found that germination of different seeds in various metal salt solutions was not the same for all the seeds. Some seeds like sorghum were able to germinate in much higher concentrations of metal salts than rye, oats or soybean. This does not necessarily indicate that the sorghum plant is more tolerant to high concentrations of metals than the other plants tested. However, a certain relationship may exist between the ability to germinate in a concentrated metal salt solution and the tolerance to this specific metal.

In the experiments conducted here it was found that very high concentrations of certain mineral elements prevented the germination of Portulaca oleracea seeds. The vitality of these seeds however remained normal. It is a common fact that unfavorable conditions, as they often occur in nature, inhibit the germination of seeds. Seeds of Sabal minor, the stemless palmetto, survived a four weeks' soaking in seawater (12).

Genetic effects of a mineral excess or deficient treatment have been studied by many authors (89, 90, 106, 107). Several mutations have been obtained. The mutations could also be produced by means of irradiation or other agents (107). This means that the mutation caused by a treatment of heavy metals is not necessarily tolerant to this metal.

Results of the experiment conducted here indicated that the Portulaca mutants obtained after treatment with 1.0 percent lithium chloride was less tolerant to high concentrations of lithium chloride than the wild type.

We must sharply distinguish between the mutagenic action of an element and the selection pressure executed by the same element.

It was already known that lithium produced abnormalities in the cotyledons of plants (41). Malformations resulting from excess of lithium were also found in young animal embryos (83). It was reasonable to believe therefore that similar results might be expected when young seeds of Portulaca oleracea were treated with high amounts of lithium chloride. It could also be seen that since the seed contains a completely developed embryo it would be difficult genetically to affect the mainshoot and cotyledons. All abnormalities observed in these regions are probably due to the direct toxic action of lithium chloride.

In the initial stage of branching, buds are formed from cells in the superficial layers of the tunica and deeper layers of the corpus. It is probably at this stage, where only a few cells are involved, that lithium chloride can act. The first four branches in Portulaca oleracea are in general much more developed than the later ones.

It was among these four branches only that the mutations were found, after Portulaca oleracea seeds had been treated with a 1 percent lithium chloride solution. The number of mutated branches in each plant varied from one to three.

Reversion to wild type in the mutated branches indicated that a typical chimera type was produced. Therefore mutation took place only in the superficial cell layers of the tunica. Chlorophyll mutation in Portulaca oleracea observed after treatment with 1 percent lithium chloride, indicated a similar mutagenic action on cells in the tunica.

Dermen (17) in studies on periclinal cytochimeras in cranberry could clearly distinguish between the different polyploid types which were obtained from treatment with colchicine. Histological studies on the apical dome and tissues of the stem showed the various differences in structural make-up of the cell layers of the chimeras.

In the experiments with Portulaca a similar attempt was made to identify the various tissues in young growing buds. Comparisons were made between the wild and mutated types. If the Portulaca chimera was the result of polyploidy then distinct histogenic layers must be found. The polyploid layers have in general larger cells than the diploid cell layers (18). The same is true for stomata of the leaf epidermis (17). If this cell layer was derived from polyploid cell tissue then the stomata should have been larger than normal. The results of microscopic examinations of different tissues and epidermis cells in Portulaca showed no difference in cell size, when similar cells were compared in wild and mutated types.

This leads to the conclusion that the Portulaca chimera type is not the result of polyploidy.

The action of lithium chloride was rather specific. That is, besides some chlorophyll mutations, only one type of somatic mutation namely an elliptical leaf type, was produced. Since polyploidy is probably not involved here, the mutagenic action must have been on a specific gene.

There are a few instances in which spontaneous mutations have been found in the Portulaceae. Somatic mutations in Portulaca

grandiflora have been described by Faberge and Beale (28). That mutation was caused by an unstable gene which was responsible for production of colored spots on stems and petals. The mutation rate was considerably reduced with rising temperatures.

Spontaneous mutations in epidermis cells of adult leaves of Portulaca grandiflora have also been described by Czeika (16). Mutations produced were of polyploid nature and occurred during mitosis of adult epidermis cells.

The effect of an excess mineral element treatment can sometimes be related to a deficiency of certain elements. It was found that chlorosis often occurs as a result of toxic action of a high metal concentration (30). Heavy metals interfered with the uptake of iron.

It was found in the experiments of part two (the second approach) that percentage germination of Portulaca seeds grown from plants treated with high concentrations of copper, zinc, iron and boron was considerably reduced. The effect was specific for each ion. The time required for germination seemed much more prolonged than in control treatment. It took about seven days for normal treated Portulaca seeds to reach a germination percentage of ninety. After 14 days a 30 - 40 percent germination was found for seeds from plants treated with different iron chelate solutions. There was a retardation which could also be observed in the other treatments. It is not known how much of the different elements had been taken up by the seeds. But it seems likely that some accumulation took place.

Seeds of Neptunia grown on soils rich in selenium contained

123 μg selenium per seed (80). The normal amount is only 0.45 μg per seed.

It was found that Portulaca seeds derived from the plants treated with high levels of selenium produced a high percentage of empty seeds. Many seeds which did germinate died in an early stage of growth. It seems that a heavy accumulation of selenium by the young seed must be responsible for the early death of the seedling. Selenium is extremely mobile in plants. The effect of its uptake is very pronounced. In only a few hours after introduction of selenium into plants, the leaves are discolored and produce the typical odor of a selenium solution. It must be emphasized that under very toxic conditions fertilization could be arrested. Incompletely developed embryos may also be produced.

In the experiments conducted in part three a particular metal tolerance could be built up by the plant cell if plants were grown in high levels of mineral solutions. It should be emphasized that this induction is not an alteration of the genetic constitution of the cell. The greater resistance of the cell to certain metals is probably due to a protoplasmic reorganization. This build up of tolerance to a certain metal under excess metal conditions can be compared with the effect of other environmental factors resulting in a structural reorganization of the cell. Since high tolerance to metals corresponds to high metal content in the plant (87), we may assume that the mechanism for tolerance probably involves a precipitation or adsorption of the toxic ions. Where adsorption takes place, special organic chelates are probably involved. It was also found that the presence of anthocyanin

increased the metal tolerance in plants (87). Anthocyanin may act as a metal binding agent. High osmotic pressure in cellsap could also be partly responsible for the increased metal tolerance.

SUMMARY AND CONCLUSIONS

The effect of various mineral levels on physiology and morphology of different plants was studied.

In a copper and manganese deficient nutrient medium, soybean plants produced seeds which were typically affected. Syncotyledons and tricotyledons were produced. A high percentage of twin plants developed from these abnormalities in the copper deficient treatment. In the manganese deficient treatment many so called "forked stems" developed from these seeds. Effect of the manganese deficient treatment could be related to the destruction of certain cell tissues in the young embryo. Effect of the copper deficient treatment may inhibit cell wall formation resulting in an early cleavage of the zygote and formation of twin plants.

The effect of various salt solutions on germination of seeds, particularly on seeds of Portulaca oleracea, was studied. It was found that high concentrations of salt solutions inhibited the germination without destroying the vitality of the seed. Sorghum seeds were able to germinate in much higher concentrations of metal salts than rye, oats or soybean seeds.

Soaking oat seeds in concentrated solutions of zinc and manganese retarded the blooming in the zinc treatment and advanced the blooming in the manganese treatment.

When Portulaca oleracea seeds were treated with a 1 percent lithium chloride solution, mutations were produced with elliptical

leaves and incised petals. Reversion to the wild type in the cuttings indicated a chimera characteristic. The mutation was shown not to be a result of polyploidy. At certain stages of development marked histological differences could be shown in the cortical cells of both the mutated and wild types. Grafting experiments performed between the two types of Portulaca, also showed that the modification found in the leaves and flowers of Portulaca, was not a result of virus or bacterium infection.

Plants which had grown in high concentrations of metal ions produced seeds that showed a characteristic reduction and retardation in germination. No mutation effects were observed.

It was found that a tolerance for zinc can be built up in the cells of Portulaca oleracea, when growing these plants in concentrated zinc solutions. Plasmolysis technique was used to measure the viability of the cells. Pretreatment of Portulaca oleracea cuttings with concentrated solutions of heavy metals increased the resistance of the cuttings to high metal concentrations in some cases. Cuttings from plants having grown in 24 ppm Cu were able to form complete roots in a solution of 160 ppm Cu. Control plants did not develop any roots in this concentration.

In conclusion, when plants are grown under excessive or deficient levels of mineral elements, abnormalities in the progeny seed may result. As another alternative, mutations may occur, especially after treatment of seeds with high levels of mineral elements. The induction of tolerance to high levels of heavy metals is probably due to protoplasmic reorganization.

BIBLIOGRAPHY

1. Bari, Ghulam. 1963. The mutagenic effect of ethyl methane sulphonate alone and in combination with copper on wheat. *Caryologia* 16:619-624.
2. Baumeister, W. 1955. Über den Einfluss des Zinks bei Silene inflata Smith. *Ber. Deut. Bot. Ges.* 67:205-213.
3. Bayer, E. 1964. Structure and specificity of organic chelating agents. *Angew. Chem. Int. (Eng. ed.)* 3:325-332.
4. Bertrand, Didier. 1959. Nouvelles recherches sur le lithium des graines. *Compt. Rend. Acad. Paris* 249:331-332.
5. Bhatia, C. R., and K. R. Narayanan. 1965. Genetic effects of ethyl methane sulfonate in combination with copper and zinc ions on Arabidopsis thaliana. *Genetics* 52:577-581.
6. Biebl, R. 1949. Über die Resistenz pflanzlicher Plasmen gegen Vanadium. *Protoplasma* 39:251-259.
7. Bogorad, L., G. Pires, H. Swift, and W. J. McIlrath. 1958. The structure of chloroplast in leaf tissue of iron deficient Xanthium. *Brookhaven Symp. Biol.* 11:132-137.
8. Bollmann, A., and F. Schwanitz. 1957. Über den Eisengehalt in Pflanzen einer eisenreichen Standortes. *Z.f. Bot.* 45:39-42.
9. Bradshaw, A. D. 1952. Population of Agrostis tenuis resistant to lead and zinc poisoning. *Nature* 169:1098.
10. Bradshaw, A. D., T. S. McNeilly, and R. P. G. Gregory. 1965. Industrialization, evolution and the development of heavy metal tolerance in plants. In *Ecology and the Industrial Society*. 5th Brit. Ecol. Soc. Symp.: 327-343. Blackwell Scientific Publications.
11. Bröcker, Werner. 1963. Genetisch-psysiologische Untersuchungen über die Zinkverträglichkeit von Silene inflata Sm. *Flora* 153:122-156.
12. Burk, Carl John. 1966. Dynamics of plant distribution. 1. Sabal minor. In *Advancing Frontiers of Plant Sciences* 14:1-4.

13. Cannon, Helen L. 1960. Botanical prospecting for ore deposits. *Science* 132:591-598.
14. Chang, Chong W., and C. Ray Thompson. 1966. Effect of fluoride on nucleic acids and growth in germinating corn seedling roots. *Physiol. Plant.* 19:911-918.
15. Cohn, N. S. 1961. The effect of chelation on the production of chromatid aberrations in Vicia faba. *Exp. Cell Res.* 24: 596-599.
16. Czeika, G. 1958. Über das spontane Auftreten von Mitosen und inäqualen cytokinesen in der endopolyploiden Epidermis von Portulaca grandiflora. *Planta* 51:566-574.
17. Dermen, H. 1947. Periclinal cytochimeras and histogenesis in cranberry. *Amer. J. Bot.* 34:32-43.
18. Dermen, H., and H. F. Bain. 1944. A general cytological study of colchicine polyploidy in cranberry. *Amer. J. Bot.* 31:451-463.
19. Dilling, W. J. 1926. Influence of lead and the metallic ions of copper, zinc, thorium, beryllium and thallium on the germination of seeds. *Ann. Apl. Biol.* 13:160-167.
20. Döring, H. 1937. Über den Einfluss der Ernährung auf die Mutationshäufigkeit bei Antirrhinum majus. *Ber. Deut. Bot. Ges.* 55:(167)-(182).
21. Drescher-Kaden, F. K., and F. Schwanitz. 1956. Über den Strontium und Calcium-gehalt in Pflanzen eines Strontium-haltigen Standortes. *Z.f. Bot.* 44:501-504.
22. Du Bois, Anne M., Gilbert Turian, and Alain Gonet. 1957. Recherches sur l'action mitostatique de l'acide borique dans le meristème racinaire d'Allium cepa L. *Caryologia* 10:102-110.
23. Durrant, A. 1962. The environmental induction of heritable change in Linum. *Heredity* 17:27-62.
24. Durrant, A., and H. Tyson. 1964. The effects of fertilizers on two subsequent generations of winterwheat. *Der Züchter* 34:41-45.
25. Duvigneaud, P., and S. Denayer-de Smet. 1963. Cuivre et végétation au Katanga. *Bull. Soc. Roy. Bot. Belg.* 96: 93-231.
26. Ernst, Wilfried. 1965. Über den Einfluss des Zinks auf die Keimung von Schwermetallpflanzen und auf die Entwicklung der Schwermetall-pflanzengellschaft. *Ber. Deut. Bot. Ges.* 78:205-212.

27. Ernst, Wilfried. 1966. Ökologische-soziologische Untersuchungen an Schwermetallpflanzengesellschaften südfrankreichs und des östlichen Harzvorlandes. *Flora* (B.) 156:301-318.
28. Faberge, A. C., and G. H. Beale. 1942. An unstable gene in Portulaca: mutation rate at different temperatures. *J. of Genet.* 43:173-187.
29. Fink, Hanneliese. 1950. Experimentelle Untersuchungen über die Wirkung des Nahralszmangels auf die Mitose der Wurzelspitzen von Vicia faba. *Chromosoma* 3:510-565.
30. Forster, W. A. 1954. Toxic effects of heavy metals on crop plants grown in soil culture. *Ann. Appl. Biol.* 41:637-651.
31. Frerking, H. 1915. Über die Giftwirkung der Lithiumsalze auf Pflanzen. *Flora* 108:449-453.
32. Garber, K. 1962. Pflanze und Fluor. *Qual. Plant. et Mater. Veg.* 9:33-44.
33. Genkel, D. A., R. S. Morozova, and N. D. Pronina. 1962. Synthetic ability of drought hardened tomato plants. *Fiziol. Rastenii* 9:57-61.
34. Gläss, E. 1955. Untersuchungen über die Einwirkung von Schwermetallsalzen auf die Wurzelspitzenmitose von Vicia faba. *Z.f. Bot.* 43:359-403.
35. Gläss, E. 1956. Die verteilung von Fragmentationen und achromatischen Stellen auf den Chromosomen von Vicia faba nach Behandlung mit Schwermetallsalzen. *Chromosoma* 8:260-284.
36. Gorham, E., and A. G. Gordon. 1960. The influence of smelter fumes upon the chemical composition of lake waters near Sudbury, Ontario, and upon the surrounding vegetation. *Can. J. of Bot.* 38:477-487.
37. Gregory, R.P.G., and A. D. Bradshaw. 1965. Heavy metal tolerance in populations of Agrostis tenuis Sibth. *New Phytologist* 64:131-143.
38. Gries, Brunhild. 1966. Zellphysiologische Untersuchungen über die Zinkresistenz bei Galmeiökotypen und Normalformen von Silene cucubalus Wib. *Flora* 156(B):271-290.
39. Haarring, R. 1962. Mutationauslösung durch Chemikalien bei Weizen. 3. Unterschiedliche Folgen einer mutagenen Behandlung von verschiedenartigen Zellen. *Z.f. Pflanzenz.* 48: 117-142.
40. Haccius, B. 1955. Experimentally induced twinning in plants. *Nature* 176:355-356.

41. Haccius, B. 1956. Über die Beeinflussung der Morphogenese pflanzlicher Embryonen durch Lithium-ionen. Ber. Deut. Bot. Ges. 69:87-93.
42. Harrington, James F. 1960 Germination of seeds from carrot, lettuce and pepper plants grown under severe nutrient deficiencies. Hilgardia 30:219-235.
43. Harris, Henry C., 1965. The interrelated effects of mineral nutrient deficiencies, environment, and heredity on the nitrogen metabolism of plants. Ann. Rep. 1965, Fla. Ag. Exp. Sta. 59.
44. Harris, Henry C., and John B. Brölmann. 1966. Comparison of calcium and boron deficiencies of the peanut. 2. Seed quality in relation to histology and viability. Agron. J. 58:578-582.
45. Harris, T. M. 1946. Zinc poisoning of wild plants from wire netting. New Phytologist 45:50-55.
46. Haselhoff, E. 1892. Über die schädigende Wirkung von Kupfer-sulfat- und kupfer-nitrathaltigen Wasser auf Boden und Pflanzen. Landwirt. Jahrb. 21:261-271.
47. Heald, F. D. 1896. On the toxic effect of dilute solutions of acids and salts upon plants. Bot. Gaz. 22:125-153.
48. Herich, Rudolf. 1965. The effect of cobalt on the structure of chromosomes and on the mitosis. Chromosoma 17:194-198.
49. Hoagland, D. R., and D. I. Arnon. 1950. The water-culture method for growing plants without soil. California Agr. Exp. Sta. Circ. 347 (Rev.).
50. Hunter, J. G., and O. Vergnano. 1952. Nickel toxicity in plants. Ann. Appl. Biol. 39:279-284.
51. Hyde, B. B., and R. L. Paliwal. 1958. Studies on the role of cations in the structure and behavior of plant chromosomes. Amer. J. Bot. 45:433-438.
52. Johansen, Donald A. 1940. Plant microtechnique. Mc-Graw-Hill Book Company, Inc., New York.
53. Johri, B. M., and C. B. Sehgal. 1963. Chemical induction of polyembryony in Anethum graveolens L. Naturwissenschaften . 50:47-48.
54. Jowett, D. 1958. Populations of Agrostis spp. tolerant of heavy metals. Nature 182:816-817.
55. Jowett, D. 1959. Adaptation of a lead tolerant population of Agrostis tenuis to low soil fertility. Nature 184:43.

56. Kahlenberg, Louis, and Rodney H. Ture. 1896. On the toxic action of dissolved salts and their electrolytic dissociation. Bot. Gaz. 22:81-124.
57. Karve, A. D., and S. G. Jigajinni. 1965. Circadian rhythm in Portulaca. Z. Pflanzenphysiol. 53: 169-172.
58. Karve, A. D., and S. G. Jigajinni. 1966. Leaf movements of Portulaca under different photoperiods. Z. Pflanzenphysiol. 54:270-274.
59. Kisser, J., and K. Lettmayr. 1933. Untersuchungen über die Absorption von Salzen durch Samen. Z. f. Pflanzenernährung, Dungung und Bodenkunde. 29A:195-210.
60. Kisser, J., and K. Lettmayr. 1934. Untersuchungen über die Auswaschbarkeit der von Samen absorbierten Salze und ihre Bedeutung für die Samenstimulation. Z.f. Pflanzenernährung, Dungung und Bodenkunde. 34A:172-181.
61. Lebrun, J. 1965. Le mouvement d'ouverture et de fermeture des fleurs chez diverses Portulacacées. Bull. Soc. Roy. Bot. Belg. 90:19-35.
62. Marinos, N. G. 1962. Studies on submicroscopic aspects of mineral deficiencies 1. Calcium deficiency in the shoot-apex of barley. Amer. J. Bot. 49:834-841.
63. Marschner, H. 1960. Über die Nachwirkung von P^{32} in der 2 Generation von Sommergerste. Die Kulturpflanze 8:90-92.
64. Marth, P. C., and J. R. Frank. 1961. Increasing tolerance of soybean plants to some soluble salts through application of plant growth retardant chemicals. J. Agr. Food Chem. 9:359-361.
65. McCune, D. C., A. E. Hitchcock, Jay S. Jacobson, and Leonard H. Weinstein. 1965. Fluoride accumulation and growth of plants exposed to particulate cryolite in the atmosphere. Contrib. Boyce Thompson Inst. 23:1-11.
66. Mohamed, Aly H., Howard G. Applegate, and James D. Smith. 1966. Cytological reactions induced by sodium fluoride in Allium cepa root tip chromosomes. Can. J. Genet. Cyt. 8:241-244.
67. Mohamed, Aly H., James D. Smith, and Howard G. Applegate. 1966. Cytological effects of hydrogen fluoride on tomato chromosomes. Can. J. Genet. Cyt. 8:575-583.
68. Moutschen, J., A. Moes, and J. Gilot. 1964. Some meiotic consequences of ethyl-methanesulfonate and the interaction of copper and zinc. Experientia (Basel) 20:494-495.

69. Moutschen-Dahmen, J., and M. Moutschen-Dahmen. 1963. Influence of Cu^{++} and Zn^{++} ions on the effect of ethylmethanesulfonate (EMS) on chromosomes. *Experientia* (Basel) 19:144-145.
70. Newcomb, E. H. 1963. Cytoplasm-cell wall relationships. *Ann. Rev. Plant Physiol.* 14:43-64.
71. Newton, Lily. 1944. Pollution of the rivers of West Wales by lead and zinc mine effluent. *Ann. Appl. Biol.* 31:1-11.
72. Niethammer, A. 1930. Studien über die Beeinflussung der Pflanzen durch Schwermetallverbindungen. *Protoplasma* 8:50-57.
73. Oehlkers, Friedrich, 1956. Die Auslösung von Mutationen durch Chemikalien bei Antirrhinum majus. *Z. Vererbungslehre* 87:584-589.
74. Oertli, J. J., and H. C. Kohl. 1961. Some considerations about the tolerance of various plant species to excessive supplies of boron. *Soil Sci.* 92:243-247.
75. Ota, T. 1964. Increasing tolerance of kidney bean plants to toxic levels of sodium chloride and ammonium sulfate through application of B 995. *Plant and Cell Physiol.* 5:255-258.
76. Otto, R. 1893. Untersuchungen über das Verhalten der Pflanz-enwurzeln gegen Kupfersalzlösungen. *Z. f. Pflanzenkrankh.* 3:322-334.
77. Ouellette, G. J., and L. Dessureaux. 1958. Chemical composition of Alfalfa as related to degree of tolerance of manganese and aluminium. *Canad. J. Plant Sci.* 38:206-214.
78. Owen, F. V. 1928. Soybean seeds with two embryos. *J. Heredity* 19:373-374.
79. Parkash, Ved, and B. V. Subbish. 1965. Radioisotopes in micro-nutrient studies. 1. Distribution pattern of ^{32}P in citrus seedlings under deficient or toxic conditions of different micronutrients. *Soil Sci. and Plant Nutrition* 11:1-8.
80. Peterson, P. J., and G. W. Butler. 1962. The uptake and assimilation of selenite by higher plants. *Aust. J. Biol. Sci.* 15:126-146.
81. Prát, S. 1934. Die Erbllichkeit der Resistenz gegen Kupfer. *Ber. Deut. Bot. Ges.* 52:65-67.
82. Pringsheim, E. G. 1924. Über Plasmolyse durch Schwermetall-salze. *Beih. Bot. Zentralbl.* 41:1-14.

83. Raven, Chr. P. 1952. Lithium as a tool in the analysis of morphogenesis in Limnaea stagnalis. *Experientia* (Basel) 8:252-257.
84. Repp, G. 1963. Die Kupferresistenz des Protoplasmas höherer Pflanzen auf Kupferböden. *Protoplasma* 57:641-659.
85. Rieger, Rigomor, Hristo Nicoloff and Arnd Michaelis. 1963. Chelatbildung als Sensibilisierungsfactor bei der chemischen Auslösung von Chromosomenaberrationen. *Biol. Zentralbl.* 82:393-412.
86. Robinson, W. O., and G. Edington. 1945. Minor elements in plants and some accumulator plants. *Soil Sci.* 60:15-28.
87. Ruther, F. 1967. Vergleichende physiologische Untersuchungen über die Resistenz von Schwermetallpflanzen. *Protoplasma* 64:400-425.
88. Sarkar, Ira. 1957. Effects of versene-induced calcium deficiency in living neuroblasts of the grasshopper embryo. *Cytologia* 22:370-379.
89. Scheibe, A., and G. Hülsmann. 1958. Mutations-auslösung durch Chemikalien beim Steinklee (Melilotus albus). *Z.f. Pflanzenz.* 39:299-324.
90. Scheibe, A., and H. Meyer. 1962. Mutationsauslösung durch Chemikalien bei Weizen. 1. Die Anschnittmethode und erste Ergebnisse. *Z. f. Pflanzenz.* 47:181-192.
91. Schwanitz, Fritz, and H. Hahn. 1954. Genetisch-entwicklungsphysiologische Untersuchungen an Galmeipflanzen. 1. Pflanzengrösse und Resistenz gegen Zinksulfat bei Viola lutea Hudson, Alsine verne L. und Silene inflata Sm. *Z. f. Bot.* 42:179-190.
92. Schwanitz, Fritz, and H. Hahn. 1954. Genetisch-entwicklungsphysiologische Untersuchungen an Galmeipflanzen. 2. Über Galmeibiotypen bei Linum catharticum L. Campanula rotundifolia L., Plantago lanceolata L., und Rumex acetosa L. *Z.f. Bot.* 42:459-471.
93. Scott, E. G. 1960. Effect of supra-optimal boron levels on respiration and carbohydrate metabolism of Helianthus annuus. *Plant Physiol.* 35:653-661.
94. Sigenaga, M. 1944. Experimental studies of abnormal nuclear and cell division. 1. Observation with living cells of the effects of neutral salts and heavy metal salts. *Cytologia* 13:380-404.
95. Smith, P. F. 1953. Heavy metal accumulation by citrus roots. *Bot. Gaz.* 114:426-436.

96. Steffenson, D. 1953. Induction of chromosome breakage at meiosis by a magnesium deficiency in Tradescantia. Proc. Nat. Ac. Sci. 39:613-620.
97. Steffenson, D. 1954. Irregularities of chromosome division in Tradescantia grown on low sulfate. Exp. Cell Res. 6: 554-556.
98. Steffenson, D. 1955. Breakage of chromosomes in Tradescantia with a calcium deficiency. Proc. Nat. Ac. Sci. 41:155-160.
99. Steffenson, D. 1958. Chromosome aberrations in calcium deficient Tradescantia produced by irradiation. Nature 182:1750-1751.
100. Steffenson, D. 1959. Divalent metals in the structure of chromosomes. In The cell nucleus: 216-221. Proc. Faraday Society. Butterworths & Co., London, publisher, Ed. J. S. Mitchell.
101. Stockberger, W. W. 1910. The effect of some toxic solutions on mitosis. Bot. Gaz. 49:401-429.
102. Thomas, W. I., and D. E. Baker. 1966. Application of biochemical genetics in quality improvement and plant nutrition. 2. Studies in the inheritance of chemical element accumulation in corn (Zea mays L.). Qual. Plant. Mater. Veg. 13:98-104.
103. Thompson, William W., and T. E. Weier. 1962. The fine structure of chloroplasts from mineral deficient leaves of Phaseolus vulgaris. Amer. J. Bot. 49:1047-1055.
104. Url, W. 1956. Über Schwermetall- zumal Kupferresistenz einiger Moose. Protoplasma 46:768-793.
105. Von Rosen, G. 1954. Breaking chromosomes by the action of elements of the periodical system and by some other principles. Hereditas 40:258-263.
106. Von Rosen, G. 1957. Mutations induced by the action of metal ions in Pisum. Hereditas 43:644-664.
107. Von Rosen, G. 1964. Mutations induced by the action of metal ions in Pisum. 2. Further investigations on the mutagenic action of metal ions and comparison with the activity of ionizing radiation. Hereditas 51:89-134.
108. Walker, G. W. R., and Keh-Ping Ting. 1967. Effect of selenium on recombination in barley. Can. J. Gen. Cyt. 9:314-320.
109. West, S. H. 1962. Protein, nucleotide and ribonucleic acid metabolism in corn during germination under water stress. Plant Physiol. 37:565-571.

110. Yamaha, G., and T. Ishii. 1932. Über die Ionenwirkung auf die Chromosomen der Pollenmutterzellen von Tradescantia reflexa. Cytologia 3:333-336.
111. Zimmerman, P. W., A. E. Hitchcock, and J. Gwirtsman. 1957. Fluorine in food with special reference to tea. Contrib. Boyce Thompson Inst. 19:49-53.
112. Zschege, Christa. 1963. Mutationsauslösung durch Chemikalien bei Weizen 4. Cytogenetische Untersuchungen an Mutanten. Z. f. Pflanzenz. 49:122-160.

BIOGRAPHICAL SKETCH

Johannes Bernardus Balthasar Brölmann was born November 20, 1920, in Hilversum, The Netherlands. He was graduated from High School in Hilversum in 1939, and received States Diploma for Physics and Mathematics in 1941 at the Hague, the Netherlands, entering the University of Wageningen, the Netherlands, in 1941. His studies were interrupted during World War II and he reentered the University of Wageningen in 1945, receiving the Master of Science degree in Tropical Agriculture with a major in plant breeding and soil chemistry in 1952. From 1952 until 1960 he worked as plant breeder and chemist at the Agricultural Experimental Station near Arles, Southern France. He entered the University of Florida in 1962, while being employed as Senior Laboratory Assistant at the Agricultural Experiment Station and has been employed in this capacity to the present time.

Mr. Brölmann was married to the former Antoinette Van Rhyn in 1952, and is the father of four sons.

This dissertation was prepared under the direction of the chairman of the candidate's supervisory committee and has been approved by all members of that committee. It was submitted to the Dean of the College of Agriculture and to the Graduate Council, and was approved as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

August 1968

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