Inheritance and Physiology of Response to Low Boron in Red Beet (*Beta vulgaris* L.)[1]

G. Tehrani[2], H. M. Munger, R. W. Robinson[3] and S. Shannon[3]
Cornell University, Ithaca, New York

Abstract. Genetic variation in foliar symptoms of B deficiency in the seedling stage of red beet was observed among inbred lines and commercial cultivars grown in sand culture in growth chambers. F_1, F_2 and backcross populations between the most susceptible and tolerant lines, tested at .001 ppm B, indicated that susceptibility was conditioned primarily by a single dominant gene. No linkage was established between this gene and the gene controlling red leaf color.

The most susceptible line was inherently more vigorous than the most tolerant line but this relationship was not evident among the other inbreds and cultivars tested.

Boron deficiency sumptoms on leaves of both tolerant and susceptible lines were significantly accentuated by reducing the $Ca(NO_3)_2$ content of the nutrient solution from 8 to 7 mEq/litre without altering B content. At both Ca levels the B content was greater in the tolerant than in the susceptible line. When supplied with adequate Ca but low B the susceptible line contained higher Ca, Na and P and lower Mg than the tolerant line.

Black spotting in the growth rings of beet tap roots is a symptom of advanced B deficiency (14). Using this criterion cultivar differences in tolerance to B deficiency were observed in the field (5, 10, 16, 19). However, similar differences were not demonstrated in nutrient culture experiments conducted by Kelly and Gabelman (11)[4].

The commercial cultivars used by the above mentioned workers were extremely heterozygous because of the presence of self-incompatibility alleles. Self-compatible beet breeding lines which had been selfed for several generations recently became available. These lines seemed well suited for an extensive study of the inheritance of response to low B nutrition in this crop. Susceptibility to B deficiency in celery is determined by a single recessive gene (13). This study was undertaken to determine whether a genetic factor was responsible for controlling susceptibility to B deficiency in beets.

Methods and Materials

Source of plant material: Inbred lines, obtained from the University of Wisconsin, were selected because of their extreme differential response to .001 ppm B in a preliminary screening test. Three commercial cultivars and 13 inbreds were used in initial screening for response to low B. The last digit in the code of the inbred lines designates the number of generations of selfing in different years when the inbreds were used.

Pollination techniques: Since the lines were self-fruitful (*sf*) the inflorescences were covered with paper bags and selfing occurred naturally within the bags. For crossing the protandrous flowers were emasculated before the calyx opened.

Sand culture techniques: Seeds were sown directly in quartz sand in plastic transplanting flats. Six rows, generally of 10 seed balls each, were planted in each flat. Four or 5 seed balls per row were used for F_2 and backcross populations. In the initial screening, rows were thinned to 10 plants, 4 to 5 days after emergence. In later experiments 6 plants were usually left but F_2 and backcross populations were not thinned.

Hoagland's No. 2 solution was modified by substituting 2 mEq of $NaNO_3$ for 2 mEq of KNO_3 (6). A split plot technique was used with treatments as main plots and lines as subplots. Iron was supplied at a concentration of one ppm, in the form of Sequestrene NaFe and B at .001 ppm. Distilled water and reagent grade chemicals were used for making the solutions. The flats were supplied daily with 1–1/2 liters of solution which was sufficient to prevent salt accumulation in the flats.

Growth chamber conditions: Light intensity at plant level was 2400 and 100 ft-c fluorescent and incandescent light respectively; photoperiod was 16 hours light and 8 hours dark; temperature of 80°F (day) and 70°F (night) was used throughout the experiments. Preliminary test showed this gave the best definition of symptoms. Relative humidity was 80 to 90%.

The relation between B and chemical composition of plants was studied in a mobile growth chamber. For these experiments the light intensity at plant level was 1000 and 100 ft-c fluorescent and incandescent light respectively. The lower light intensity provided conditions which delayed the occurrence of B deficiency symptoms on the plants so that adequate amounts of healthy leaf could be obtained for analysis (7, 18).

Methods of classifying susceptibility to B deficiency: The beet seedling leaves were observed daily for appearance of macroscopic areas of tissue collapse. Classification of seedlings for B deficiency symptoms was repeated at 2 or 3 days intervals until sufficient data were obtained to classify each population.

Analytical procedures: Samples of leaf blade were rinsed with distilled water and dried in a forced air oven at 70°F. The dried samples were ground to a fine powder in a Brabender grinder. Gram samples were dry ashed in covered porcelain crucibles at 550°C in a muffle furnace. The ash was dissolved in 20 ml of 1.0 N HCl and stored in air tight plastic bottles.

Boron was determined by a modification of the curcumin method proposed by Crawley (3). The changes included a 15 minute incubation of the complex forming mixture at 40°C and dilution of the cooled mixture with 50.0 ml of methanol. The absorbance of the resulting dye was measured at 550 mμ with a Bausch and Lombe "Spectronic 20" spectrophotometer. P was determined by the molybdate-vanadate method of Kitson and Mellon (12). K and Na were determined by flame emission spectrophotometry using a Beckman model DB. Ca and Mg were

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[2]The senior author acknowledges the generosity of W. H. Gabelman, University of Wisconsin in providing the inbred lines used in this study. Present address: Horticultural Research Institute of Ontario, Vineland Station, Ontario, Canada.

[3]New York State Agricultural Experiment Station, Geneva.

[4]Thompson, K. H. 1957. Studies on varietal susceptibility of *Beta vulgaris L.* to boron deficiency in water culture. M.S. Thesis, Cornell University, Ithaca, New York. 62 pp.

[5]Tehrani, G. 1967. The inheritance of susceptibility to boron deficiency in red beet, *Beta vulgaris L.*, Ph.D. Thesis, Cornell University, Ithaca, New York. 103 pp.

determined by atomic absorption spectrophotometry using the Perkin Elmer Model 303.

Results

Initial screening and selection of parental lines: In the original screening plants receiving nutrient solution with .001 ppm B[5] showed symptoms of deficiency as illustrated in Fig. 1.

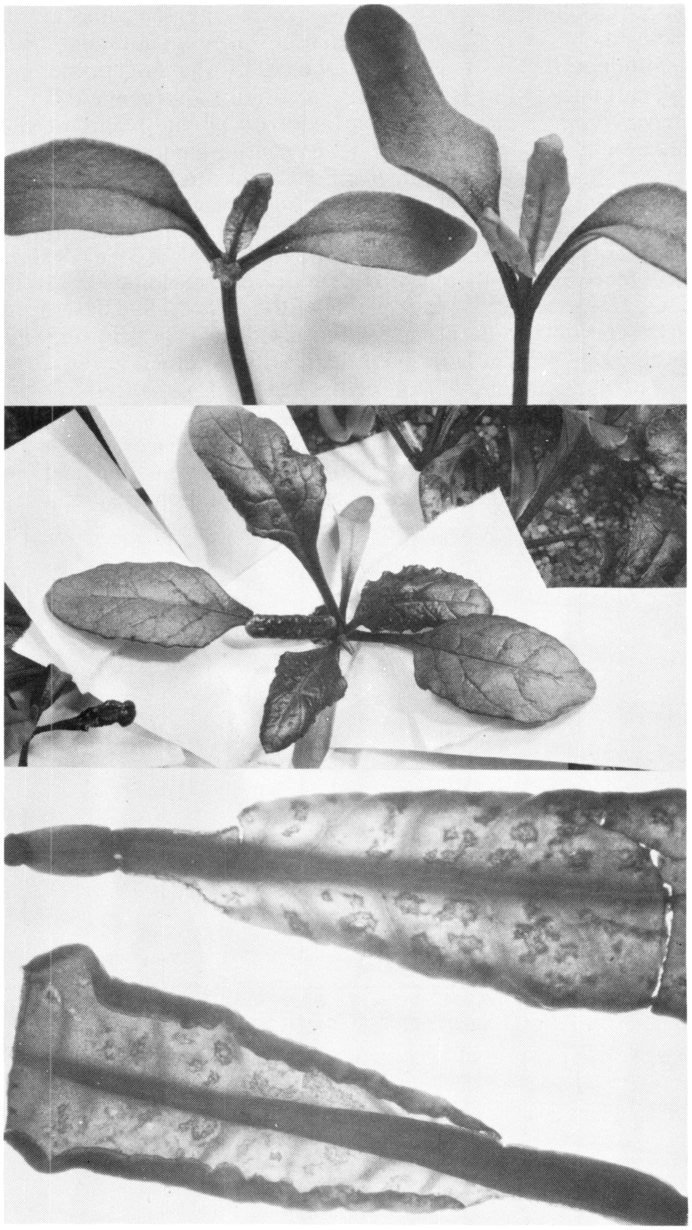

Fig. 1. Boron deficiency symptoms: Upper, first appearance of symptoms on first true leaves. Centre, severe necrosis on youngest leaves and the oldest leaves symptomless. Lower, enlargement of typical interveinal and marginal necrosis on young leaves.

The most susceptible line was Czervma 1-OPMW167-6 (hereafter referred to as Czervma). It showed 71% necrotic plants 15 days after emergence. Thirty-two days after emergence 100% of these plants were necrotic. In contrast, inbred W163-8, the most tolerant line, showed no necrotic plants at 15 days and only 5% at 32 days. Czervma and W163-8 were selected for further genetic and physiological studies.

Inheritance studies: The susceptible and tolerant inbreds, together with their F_1 and F_2 populations were tested for response to low B (Fig. 2). The response of F_1 plants was similar to that of the susceptible parent. Twenty days after

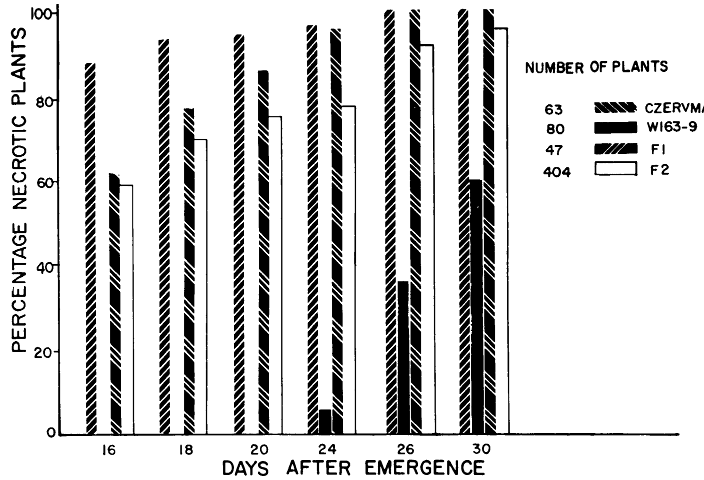

Fig. 2. Response to .001 ppm B of parental, F_1 and F_2 generations of Czervma and W163-9.

emergence 87% of the F_1 plants and 95% of the susceptible parent were necrotic and all plants of the tolerant parent were still normal.

The F_2 progenies segregated for response to low B in 3:1 ratio of susceptible to tolerant plants 20 days after emergence. At this time the parents showed maximum differences in percentage of necrotic plants. At 20 and 24 days after emergence the *P* value for a 3:1 ratio was acceptable. The non-cumulative percentages of necrotic plants at different days after emergence of parental, F_1 and F_2 generations are presented in Fig. 3. The response of the F_2 population to low B is bimodal. One peak coincides with the day at which 90% of the plants of the susceptible and 60% of the F_1 plants were necrotic. The other peak coincides with the days at which 36% of the plants of the tolerant parent were necrotic. The F_2 appears to consist of two different genotypes with responses corresponding to the two parental types.

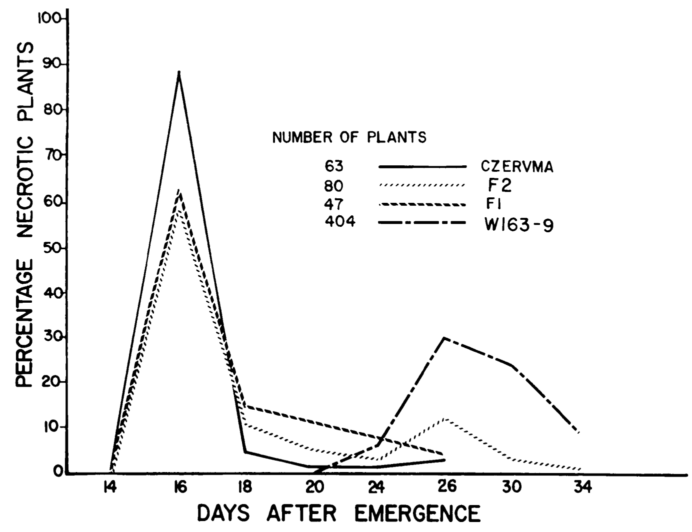

Fig. 3. The non-accumulative percentage of necrotic plants produced by Czervma and W163-9 and their F_1 and F_2 populations with .001 ppm B.

Identification of a marker gene: A marker which proved to be extremely helpful for distinguishing true F_1 and F_2 populations from accidentally selfed seeds was observed in W163-9 soon after emergence of F_1 seedlings. This marker gave a uniform red pigment in the lower surface of the cotyledon. It was dominant over the uniform green color of Czervma and was conditioned by a single gene (Table 1). Twenty days after emergence a proportion of the F_2 plants segregating for color in

Table 1. Inheritance of color in the lower surface of cotyledon in beet inbreds and hybrids.

Line	Number of plants Red	Green	Total	X^2 3:1	P_{value}
Czervma 1-OPMW167-8	0	63	63		
W163-9	80	0	80		
F_2 obs.	298	106	404	0.106	.50-.75
exp.	303	101	404		
W16-8	0	34	34		
W163-9	33	0	33		
F_2 obs.	196	68	264	0.080	.98-.99
exp.	198	66	264		

the lower surface of the cotyledonary leaf (Table 1) and response to .001 ppm B (Fig. 2) was: 233 red necrotic: 65 red healthy: 72 green necrotic: 34 green healthy. A probability value of .02 - .01 for the χ^2 contingency test at 19:1 odds indicates a significant deviation from the expected ratio. However, since the deviation is the result of deficiencies in the parental genotypes, it must have been due to chance so no linkage was established. The appropriate crosses were not made to determine whether this gene is synonymous with or allelic to y^r (9).

Performance of backcross population: The backcross tolerant X (tolerant x susceptible), along with parental lines and F_2 were tested for their responses to low B (Fig. 4). The first symptoms of deficiency developed on plants of the susceptible line 23 days after emergence, compared to 16 days in the first test (Fig. 2). Slight contamination was suspected in the nutrient solution because the deficiency symptoms were delayed. At 40 days after emergence the F_2 progenies showed maximum difference in percentage of necrotic plants and segregated for low B response in the 3:1 ratio. The backcross population ratios did not deviate significantly from the 1:1 ratio at 28, 35 and 40 days after emergence. The difference between the parents for percentage of necrotic plants was essentially the same on all these days. It is concluded that a single major gene pair determines the difference in response of the susceptible and tolerant lines to low B, and that susceptibility is dominant.

Nutritional studies: At 7 mEq of Ca all the plants of the susceptible line were necrotic 12 days after emergence (Fig. 5). If the same line was given 8 mEq Ca the first necrotic plants appeared 17 days after emergence and not until 24 days were 94% of the plants necrotic. The plants of the tolerant line likewise showed more necrosis with 7 mEq Ca. At 19 days after emergence 34% of these plants were necrotic reaching 70% at 25 days. However, none of the plants of the tolerant line developed any symptoms of deficiency within 24 days at 8 mEq Ca. This finding was in accordance with the results presented in Fig. 2.

The mineral composition of the leaf blades, the fresh weight of the tops of susceptible and tolerant lines, and 3 Ca treatments are shown in Table 2. One month after emergence only 3 out of 36 seedlings of the susceptible line and no plants of the tolerant line receiving 7 mEq Ca showed symptoms

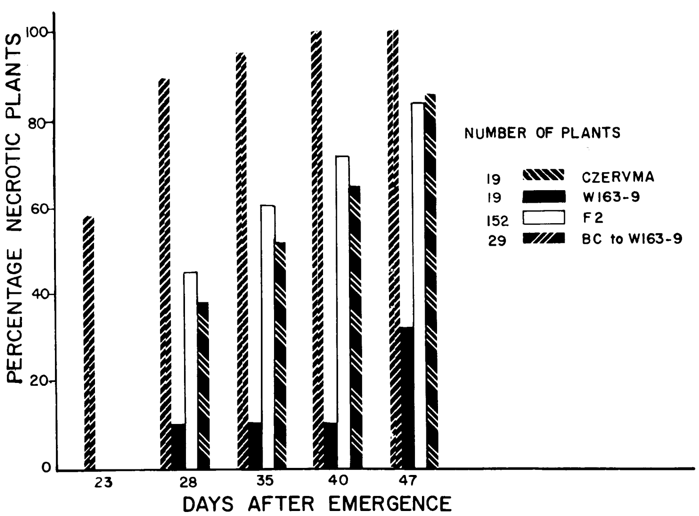

Fig. 4. Response of parental, F_2 and backcross generations to .001 ppm B.

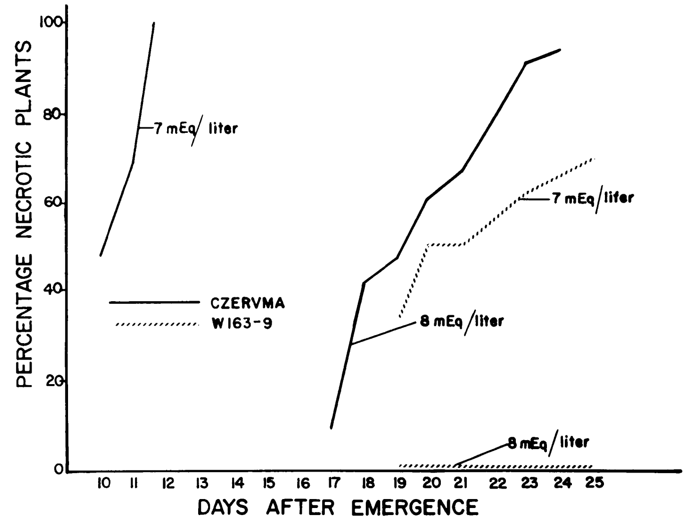

Fig. 5. The response of Czervma and W163-9 lines at .001 ppm B to 7 and 8 mEq/liter calcium nitrate in nutrient solution.

Table 2. Mineral element composition, total cation content, and fresh weight of tops of susceptible and tolerant inbred lines of beet at .001 ppm B.

Component	Ppm B	Percent of dry matter^x Ca	Mg	Na	K	P	Ca/B	Total cations /100 g dry matter (mEq)	Fresh tops wt/plant (g)
Ca levels *(mEq/liter)*									
7	7.7	1.00	1.42a	1.62	4.42	1.80	1,332	352.7	8.38a
8	7.8	0.99	1.39a	1.72	4.64	1.77	1,307	362.5	10.91b
9	8.2	1.02	1.58b	1.68	4.58	1.83	1,318	374.3	9.23a
Beet lines									
Czervma	6.6A	1.03b	1.38A	1.71b	4.44	1.85b	1,574B	357.1A	12.25B
W163-9	9.2B	0.97a	1.55B	1.64a	4.65	1.74a	1,064A	369.2B	6.76A
Interaction									
Treat. X line	*	NS	**	NS	NS	*	NS	**	NS
CV(b) (%)	4.7	4	2.7	3.0	0.6	4.4	5.57	1.39	16.0

*Significant at the 5% level.
**Significant at the 1% level.
^{x}Unlettered means, or means having letters in common, are not significantly different. Capital letters indicate significance at the 1% level, small letters indicate significance at the 5% level by Duncan's Multiple Range Test.

of B deficiency at low light intensity (1100 ft-c). At 7 and 8 mEq Ca the plants of the susceptible line were more vigorous than those of the tolerant line. The top fresh weight of Czervma was twice that of W163-9 at 7 mEq Ca. The Ca concentration of leaf blades of the susceptible line was higher than that of the tolerant line. However, increasing the Ca in the nutrient solution did not change the Ca content of the leaves.

The significant interactions are shown graphically in Fig. 6. When external Ca concentration was increased, B content of the blades of the tolerant line was proportionally increased, whereas B content of the susceptible line did not increase. The susceptible line contained 30% more total B in its leaf blades than the tolerant line, because the greater dry weight more than compensated for lower B percentage. The Ca level X line interaction for Mg was due to the higher Mg content of the tolerant line at 9 mEq Ca. As Ca level increased, P increased in the susceptible line and decreased in the tolerant line. As Ca level increased, total cations increased in the tolerant line.

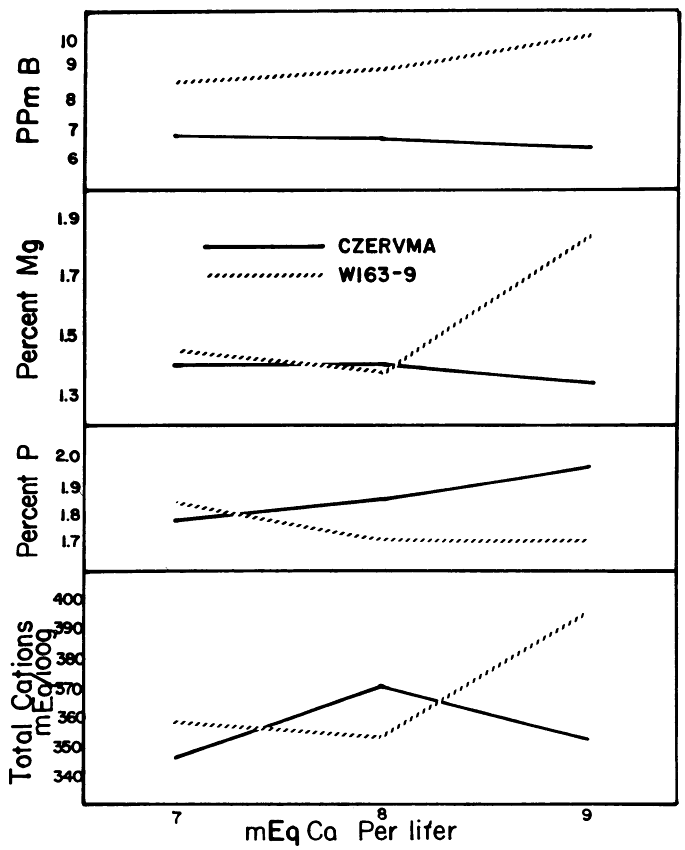

Fig. 6. Mineral element composition interactions between Czervma and W163-9 lines and Ca level in nutrient solution at .001 ppm B.

Discussion

This study indicates that susceptibility to low B was dominant and conditioned by a single major gene. However, the presence of modifying genes is indicated by the existence of lines showing an intermediate response and the slight delay in response of the F_1 when compared with the susceptible parent. Kelly and Gabelman (11), concluded that the genetic system controlling tolerance is probably complex. Their conclusions were based on the wide and continuous range of tolerance shown by different cultivars and strains of beet grown under B deficient conditions in the field. They observed the symptoms on the roots, while in this study, the plants were indexed on the basis of foliar symptoms of B deficiency in seedlings. Consequently, the simple type inheritance reported here between lines exhibiting extremes in response to low B does not contradict the conclusion of Kelly and Gabelman (11).

Although the plants of the susceptible line were inherently more vigorous than the plants of the tolerant line, it cannot be concluded that the difference in growth rate was responsible for the different response to low B levels (11). Among the 13 inbred lines that were tested, some were more vigorous but less susceptible to low B than the susceptible line.

Several workers, using different species of plants, have reported varied results on the inter-relation between mineral composition of plant tissue and B deficiency. No correlation was found in beets between B content of the leaves of cultivars and strains and the incidence of black spotting of the roots (11). Wall and Andrus (20) reported that the leaves of the brittle stem mutant of tomato contained much less B than the normal cultivar when grown at high but not at low concentrations of B. In the present study, at .001 ppm B, the tolerant line had significantly higher total B than the susceptible line irrespective of Ca treatment. The B concentration of the susceptible line remained unchanged when the external B supply was deficient and Ca was adequate. Apparently under low B conditions the tolerant line is able to maintain a higher B concentration and thus withstand B deficiency longer than the plants of the susceptible line.

A highly significant correlation between B deficiency symptoms of the roots and Ca content in the leaf tissue of beets was reported by Kelly and Gabelman (11). Apparently B deficiency symptoms in roots, are accentuated by high Ca content in the leaf tissue. In contrast, when $Ca(NO_3)_2$ was reduced from 8 to 7 mEq/liter in the nutrient solution containing .001 ppm B, B deficiency symptoms were greatly accentuated on the leaves of both lines without altering B content significantly. In the tolerant line, B concentration was greater at both Ca levels. However, the Ca content of the tolerant line was lower than the susceptible line. The combination of higher B and lower Ca content of the tolerant line may have enabled the tolerant line to withstand low B for a longer period after emergence. The functions of B and Ca in the plant are not fully known. Reed (15) does not believe that ions are linked to internal B activity. On the basis of similarity of symptoms of B and Ca deficiency and the confinement of both to meristematic regions of the plant, it is generally accepted that B plays an important role in the functional processes of Ca in plants.

The tolerant line W163-9 had lower Na and P, but higher Mg content than the susceptible Czervma. These results are somewhat contrary to those of Kelly and Gabelman (11) who reported that the strains of beet most susceptible to root B deficiency had the highest Mg in the leaf tissue. They also found that the tolerant lines had higher Na and K in their leaves. In the present study no significant difference in K concentration of the two lines was found. Possibly differences in mineral composition of the two lines, singly or in combination are involved with the differential response of the lines to low B. However, none of these elements has been reported to enhance the severity of B deficiency symptoms. Smith and Reuther (17) noted that in citrus a low supply of B associated with an increased level of P in plant tissues reduces the occurrence of B deficiency symptoms. Similar results have been reported for annual crops (1, 21).

Total cation content was not associated with the differential response of the two lines to low B because tolerant and susceptible lines had similar ion accumulation. The ratio between the different elements, however, or the ratio of B to other elements might be responsible for their differential response. It was noted that the tolerant line had a lower Ca:B ration than the susceptible line irrespective of Ca treatment. This is in accordance with the findings of others (2, 4, 8).

Nutritional mutants reported in this study may prove useful in studies of the function of boron and its interrelations with anions and cations. Certainly, the use of mutants, particularly in micro-organisms, have been useful in elucidating pathways for synthesis and metabolism of organic compounds. The possibility of using a comparable approach in studies of inorganic nutrition seems worthy of consideration.

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Hormone-Directed Transport of ^{32}P in *Malus sylvestris* Seedlings[1]

A. H. Hatch[2] and L. E. Powell
Cornell University, Ithaca, New York

Abstract. Studies were conducted to determine the effect that plant hormones such as auxins, gibberellins, and cytokinins might have on the mobilization of ^{32}P in apple seedlings grown in the greenhouse. Prior to the hormone treatments the seedlings were defoliated and the apex removed to avoid competition. Experiments with ^{32}P indicated that auxins, gibberellins, and cytokinins could direct the transport of ^{32}P, especially when used in various mixtures. It was found that ^{32}P could be mobilized acropetally, basipetally, and into and out of leaves depending on the area treated with the hormones. Their effect in mobilizing ^{32}P was less evident in seedlings that had ceased growth, with subsequent terminal bud formation, than in growing seedlings. It was also found that ^{32}P was mobilized through the phloem and required the synthesis of protein.

The translocation of solutes into certain plant parts was once thought to arise from the movement of a solute from a region of higher to a region of lower concentration. There are examples, however, which indicate that concentration gradients do not apply in many instances of translocation (1).

In recent years attention has been directed towards the ability of auxins, gibberellins and cytokinins to influence the movement of nutrients, with special reference to ^{32}P, in an attempt to explain these observations (2,3,5,6,7,8). The data suggest that regions of high hormone content could cause movement of nutrients into such regions from parts of the plant where the hormone content was lower. Such a process may be responsible for the preferential movement of nutrients into meristems and developing organs where the hormone content is typically high.

The present investigation was designed to study the role that plant hormones may have in mobilizing ^{32}P. in apple seedlings. Previous workers have generally confined their research to effects of a single hormone on distribution of ^{32}P, with one notable exception (8). However, their work was performed on a herbaceous plant. These studies were conducted to determine if indoleacetic acid (IAA), gibberellins A4 and A7 ($GA_{4\&7}$), and benzyladenine (BA) could produce similar responses in apple seedlings.

Materials and Methods

All experiments were performed on seedlings of *Malus sylvestris*, cv. Northern Spy, grown from seed in the greenhouse. The seeds were dusted with Captan 50% and stratified for 6-8 weeks at 5°C in damp peat moss. After chilling, the seeds were germinated in a mixture of soil, peat moss, and sand (3:2:1, v:v:v). When the seedlings were 5-10 cm tall they were

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[2]Present address: Department of Horticulture, Pennsylvania State University, University Park.

[3]The term "hormone", in the context of this paper, refers to hormone-like compounds as well.

[4]The $GA_{4\&7}$ mixture had a GA_4:GA_7 ratio of 69:31 as determined by S. D. Seeley of the Pomology Department using gas chromatography.