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The interaction between calcium and boron in apple seedlings

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During the past several decades there has been much interest in certain apple fruit disorders that sometimes have been correlated with Ca and/or B nutrition. A recent review by *Faust and Shear* (1968) summarizes the extensive literature up to 1967 some of which dealt with the Ca:B ratio in the fruit and leaves and the amounts of these elements in the tissues. However, little attention has been given to the simultaneous absorption of these elements by apple trees, even though their effect on many apple varieties is known.

Brenchley and Warrington (1927) were the first to suggest that there exists a relationship between the amount of available B in a growth medium and Ca usage within plants. In the following decades *Marsch and Shive* (1941), *Reeve and Shive* (1943), *Jones and Scarseth* (1934), *Kepka* (1969) and *Gupta* (1972) and others studied the significance of the Ca: B ratio in the growth medium and the resulting ratios in experimental plants.

Many of their results have been contradictory. The different findings suggest that if boron influences Ca metabolism its effect may vary with species, the part of the plant examined, the condition of the plant, the level of nutrients already in the plant, the concentration of the nutrients in the substrate and possibly with the experimental techniques. *Minarik and Shive* (1939) reported that the amount of Ca in the leaves of soybeans was influenced by solution B concentration. Both toxic and deficient concentrations of B in the substrate were associated with subnormal Ca in plant tissue. There were no differences in the B levels associated with different Ca concentrations (0.0025 and 0.025M) of entire corn and tobacco plants when grown in nutrient solutions nor were there any differences in Ca levels when nutrient B concentrations were 0 and 1 ppm (*Drake et al.* 1941). These authors and also *Marsch and Shive* (1941) noted that higher B concentrations in the substrate were associated with more soluble Ca in plant tissue but that total Ca varied but little. With tomato, *Reeve and Shive* (1944) found that as Ca increased in the substrate there was a decrease in the B level of the plants. *Marsch* (1942) reported that the B content of a nutrient solution did not influence Ca absorption of either dicots or monocots.

In studies of the mineral balance as related to the occurrence of bitter pit in Baldwin apples *Garman and Mathis* (1956) stated that there was a mutually beneficial effect between Ca and B level in fruits. *Wightman et al.* (1970) showed that in the peel, flesh and core of

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fruit and leaves of York Imperial apple the B level was accentuated by Ca. The effect of B on Ca levels of leaves varied with sampling date.

This investigation was undertaken to study the interaction between Ca and B and their absorption and distribution in apple seedlings in a factorial designed experiment. Part of the study used ^{45}Ca to determine Ca uptake and distribution during a short period of time (24 h) after the plants had adapted to different Ca and B concentrations in the nutrient solution.

Materials and Methods

In January 1972 stratified apple seeds were germinated in sand and when 5 cm tall were transferred to a 1/2 x Long Ashton aerated solution in which the B concentration was 0,001 ppm. The seedlings were grown in a greenhouse having a night temp of 15 °C, day temp 24 °C, and a day length 16 h supplemented with fluorescent lamps (Osram-L-Fluora). Seedlings were selected for uniformity when 12 cm tall and differential treatments were started.

The variables were Ca and B concentrations in aerated full strength Long Ashton nutrient solutions. There were 3 concentrations of Ca: 160 ppm (Ca-160), 16 ppm (Ca-16) and no added Ca (Ca-0) and at each 3 levels of B: 1,0 ppm (B-1), 0,1 ppm (B-0,1) and 0,001 ppm (B-0,001). There were thus 9 treatments. A sufficient volume of nutrient solution for each Ca concentration was prepared and after 800 ml had been placed in a plastic container the appropriate amount of B was added. The lid of each container held 4 seedlings and there were 8 seedlings for each treatment, each of which was treated as a replicate. The solutions were changed every second day for 2 weeks. On the days the solutions were not changed the solutions were brought to volume by the addition of deionized water. The pHs of the solutions were measured when they were made and again when they were discarded.

Table 1: Changes in the nutrient solutions during the 24 h ^{45}Ca uptake period of 8 plants. The initial counts were taken 1 h after the addition of ^{45}Ca to the nutrient solutions containing the seedlings. The final counts were taken after the seedlings had been removed.

Nutrient Solution	^{45}Ca cpm ml ⁻¹		Volume decrease in 24 h - ml	pH	
	Initial	Final		Initial	Final
Ca-160	B-1	12 375	150 } 162 } 140 }	5,04	5,04 6,27
	B-0,1	12 538			
	B-0,001	12 019			
Ca-16	B-1	11 460	132 } 187 } 152 }	5,24	6,41
	B-0,1	11 773			
	B-0,001	11 366			
Ca-0	B-1	10 303	142 } 122 } 107 }	513	637 637
	B-1	10 338			
	B-0,001	10 688			

Groups followed by different symbols differ at the following levels of significance: a, b - 5 %; a, b - 1 %; and A, B - 0,1 %.

After the seedlings had been placed in the last change of nutrient solutions an equal amount of $^{45}\text{CaCl}_2$ (spez. activity 13,9 m Ci/mg) was added to each container. The initial specific activity of the Ca-160 solution was 80 cpm/ μg Ca and that of the Ca-16 solutions 757 cpm/ μg Ca. To follow the course of the total uptake for the different treatments a 2 ml sample was taken from each solution one h after addition and also at the end of the 24 h uptake period (Table 1).

At the end of the 24 h uptake period the shoot (leaves and stem) was separated from the root and one shoot from each treatment was freeze dried and an autoradiograph made. The remaining 7 shoots of each treatment were kept separate and divided into leaves and stem and dried for chemical analyses. There were 7 samples per treatment. The roots from each seedling were rinsed for 15 min in an inactive solution with nutrient concentrations equal to the growing medium. They were then dipped in 0,5 N HCl, washed with de-ionized water and dried for analysis. There were 8 root samples per treatment.

After the removal of the roots the solutions used in the 24 h uptake period were made to volume and an aliquot (24 h, in Table 1) was tested for ^{45}Ca . A 100 ml portion of each solution was transferred to a flask and 2 newly cut shoots from seedlings that had been growing in $1/2 \times$ Long Ashton nutrient solution (80 ppm Ca; 0,001 ppm B) were placed in this portion. After 24 h these shoots were removed and one of each pair was freeze dried and autoradiographed. The other was separated into leaves and stem and dried for analyses. At the completion of the autoradiograph the tissues were also separated for analysis. There were 2 replicates of each of the shoots from each treatment (Table 3).

Analyses for ^{45}Ca , Ca, Mg, K and B were made on the ash solutions of the dried tissues: ^{45}Ca by liquid scintillation; Ca, Mg and K by atomic absorption and B by the curcumin method (Dible et al. 1954). The results were evaluated by the 't' test.

Results

The leaves appeared normal throughout the experiment but the roots in the Ca-0:B-0,001 solutions when the experiment was terminated were short. There were few long roots or root hairs suggesting Ca and/or B deficiencies. Differences in weight were few (Table 2).

The ^{45}Ca in the nutrient solutions (Table 1) was determined one h after its addition and the differences were possibly due to the more rapid adsorption or absorption by those plants in the low Ca solutions. This trend continued during the 24 h experimental period. Less nutrient solution was absorbed by the plants growing in the Ca-0 solutions. There were no differences in pH due to the different levels of Ca. Each solution, when discarded, was 24 h old and during this period its pH had changed from approximately 5.1 to 6.3 (Table 1). It is doubtful if this change had a very great effect upon Ca and B uptake even though some workers (Gupta 1972, and Wear and Patterson 1962) found a decrease in B as pH increased. They were working with limed soils rather than with nutrient solutions.

The levels of Ca and B in the tissues varied with their respective concentrations in the nutrient solutions (Tables 2). The statistical evaluation in this respect (not shown in detail) resulted in a significant difference of the Ca content of all plant parts at least for a level $P < 0,5\%$ at each constant B but

Table 2: The mineral levels and dry weight of parts of apple seedlings grown in nutrient solutions at 3 concentrations of Ca each at 3 concentrations of B. The values listed are the means of 7 samples of leaves and stems and 8 samples of roots.

Nutrient	Solution	Ca % dry wt			μg Ca absorbed and/or Translocated during 24 h			B ppm dry wt		
		Leaves	Stem	Root	Leaves	Stem	Root	Leaves	Stem	Root
Ca-160;	B-1	,872 a A ^z	,855 a	,205	2,07 a A	8,60 a A	8,08 a a	72 a	40	51 a A
	B-0,1	,817 a A	,793 a b	,189	2,38 a A	8,96 a A	9,60 c b	31	23	30 a A
	B-0,001	,648 B B	,647 b	,163	1,10 B	5,90 B B	9,43 b c	16	11	16
Ca-16;	B-1	,518	,402 a	,039	,64 a	3,99 a	3,93	49 b	41	27 b B
	B-0,1	,456	,290 b	,056	,71 a	3,09 b	4,59	29	29	17 b B
	B-0,001	,468	,326 a b	,045	,36 b	2,76 b	4,59	9	11	10
Ca-0;	B-1	,274 a	,125	,007 a				49 b	44	37 a b
	B-0,1	,220 b	,090	,003 b				27	21	22 a b
	B-0,001	,230 b	,125	,004 a b				8	10	16
K % dry wt										
		Mg % dry wt			K % dry wt			Dry wt g		
		Leaves	Stem	Root	Leaves	Stem	Root	Leaves	Stem	Root
Ca-160;	B-1	,214	,170	,166	1,67 a	1,38	1,96	,99	,29	,17
	B-0,1	,194	,168	,160	1,52 b	1,22	1,83	1,08	,36	,17
	B-0,001	,187	,168	,163	1,52 b	1,27	1,90	1,20	,39	,13
Ca-16;	B-1	,231 a	,271 a	,151 a	1,72	1,25 a b	1,73	,97 a	,25 a A	,16 a
	B-0,1	,199 b	,225 b	,153 a	1,58	1,44 b	1,48	1,19 b	,39 b B	,22 b
	B-0,001	,222 a b	,268 a	,188 b	1,63	1,15 a	1,84	1,06	,28 a	,18 a b
Ca-0;	B-1	,211 a b	,414 a	,254	1,73 a	1,58	1,33 a	1,10	,31	,18
	B-0,1	,221 a	,428 a	,261	1,78 a	1,41	1,44 A	1,05	,29	,18
	B-0,001	,188 b	,362 b	,302	1,54 b	1,37	1,10 b B	1,22	,33	,15

^zAll data are compared within each group, however B values between each group. Means in each column followed by different symbols differ at the following levels of significance: a, b, c - 5 %; a, b, c - 1 %; and A, B, C - 0,5 % and A, B, C - 0,1 %.

Table 3: The amount of Ca accumulated in 24 h by the leaves and stem of cut shoots of apple seedlings expressed as μg per g dry weight. The results of the 't' test were based on 6 seedlings from solutions having the same Ca concentration.

Nutrient Solution	μg Ca accumulated in 24 h	
	Leaves	Stems
Ca-160; B-1	7,70	12,22
B-0,1	11,56	16,40
B-0,001	10,50	13,96
Ca-16 B-1	,17	1,00
B-0,1	,28	1,34
B-0,001	,08	0,45

decreasing Ca concentration. A decrease occurred with the B content of the different plant parts at each constant Ca but decreasing B concentration of the nutrient solution.

In the stems and roots, the Ca levels decreased more rapidly with decreasing solution Ca concentrations than did the Ca levels of the leaves. In the leaves B levels decreased with decreasing Ca concentration. Calcium concentration did not affect stem B levels. The effect of Ca on the B levels of the roots was variable (Table 2). The highest concentration of B (1 ppm) in the nutrient solutions was associated with more Ca in the tissues with the exception of the roots at Ca-16. At Ca-160, as the B concentration of the nutrient solutions decreased so did the Ca levels of the leaves, stems and roots. At Ca-16 and Ca-0, the leaves and stems from plants in the B-1 solutions contained more Ca than did those plants at the other concentrations but the levels in the leaves and stems of the B-0,1 plants were lower than those of the B-0,001 plants. The Ca level of the roots of the Ca-16 plants was higher when grown in the B-0,1 solutions.

Calcium concentration had little effect on leaf Mg but in the stems and roots the Mg levels increased as Ca in the solutions decreased. The K level in the roots decreased with decreasing Ca concentrations at all B concentrations in the nutrient solutions. In the leaves and stems, generally, high K levels were in plants grown in low Ca solutions. Boron had no constant effect on Mg levels at the various Ca concentrations except in the roots at the lowest Ca concentration where root Mg increased with low B. In the leaves and stems high B was generally associated with more K.

Due to the similarity of the dry weights of the plant parts there were only small differences in the results for the 24 h accumulation period of ^{45}Ca whether expressed on a dry weight basis or on an individual plant tissue basis.

The distribution of ^{45}Ca in the leaves and stems was closely reflected in the autoradiographs (not shown) which showed for the intact plants that Ca had not been uniformly distributed during the 24 h absorption period, possibly due to the relatively slow movement of this element in woody tissues. This was

confirmed when the amount of Ca accumulated in the various tissues during 24 h was determined (Tables 2). In the leaves and stems more was accumulated at the Ca-160, Ca-16 and B-1,B-0,1 than at either concentration of Ca at B-0,001. In the roots more Ca was retained at the lower B levels.

Because there were only 2 treated shoots without roots in each treatment, statistical analyses were not made on the variables and the results in Table 3 are the average of duplicates. Leaves and stems from shoots placed in solutions Ca-160 and Ca-16 and having the intermediate B concentration contained more Ca than those from plants placed in solutions having either higher or lower B concentrations. At the end of the 24 h absorption period the amount of Ca present in the leaves and stems of the cut shoots was greater than that present in the leaves and stems of the intact plants when grown in the Ca-160 solutions, but when grown in the Ca-16 solutions the leaves and stems contained less.

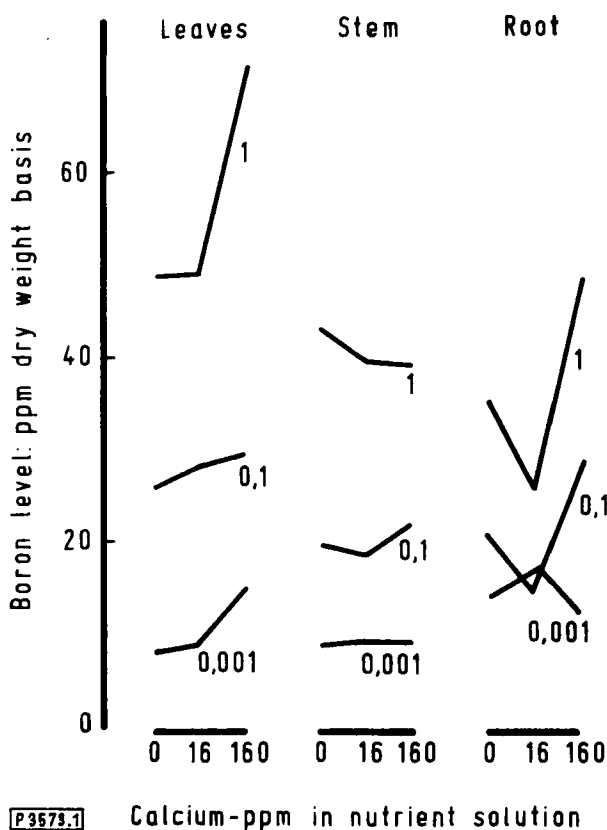


Figure 1: The B level of apple seedling tissues as a function of Ca concentration in nutrient solutions. The numbers on the curves are the B concentration in the nutrient solution.

Discussion

There were no great changes in solution pH (Table 1) with changes in Ca concentration so it was assumed that in this study pH effects were of minor importance in both Ca and B uptake. This is in contrast to the study of *Shear and Faust* (1971) where variations in pH were quite pronounced. However, as noted by *Wallace* (1961) when the concentration of one nutrient is greatly changed the absorption and distribution of others may be greatly affected. This is possibly reflected by the large increase in the Mg level of stems from low Ca solutions.

Calcium levels in all tissues decreased as solution Ca decreased. However, the decrease varied with the tissue – in the leaves the decrease was least, in the roots greatest and in the stems intermediate. These changing rates of change were interpreted to mean that at Ca-160 adequate Ca was absorbed and translocated to satisfy the young apple seedling and that at lesser levels of Ca, insufficient of this element was available for the plant and possibly an incipient deficiency was present. Ca deficiency symptoms were visible in the roots of the Ca-0:B-0,001 seedlings. At Ca-160, the Ca levels in the leaves and stems were similar suggesting that an equilibrium condition had been reached between the Ca levels in these tissues. At lower Ca concentrations, as solution Ca decreased, the ratio of leaf Ca to stem Ca increased, indicating a need for more Ca. Similar results were found by *Woodbridge* (1972).

At Ca-160, as the B concentration decreased, there was a decrease in Ca levels in all tissues (Fig.1). Calcium absorption and translocation were therefore influenced by the B concentration. The differences between 1 and 0,1 ppm B were small. At the lower Ca concentrations the influence of B on Ca absorption and translocation was not as marked but with the exception of the roots at Ca-16,B-1 was associated with more Ca accumulation than was B-0,001. In the review by *Faust and Shear* (1968) it was noted that in young apple seedlings B seemed to be needed for the transport of Ca but its effectiveness depended upon the levels of both elements. The results of the present study confirmed their opinion. The results implied that if there was a Ca:B interaction for apple seedling tissues it was manifested principally at Ca concentrations that were considered adequate for plant growth as in full strength Long Ashton nutrient solution. Generally, high B in the nutrient solutions was associated with high Ca levels in the tissues. These results were similar to those of *Kepka* (1969) but not to those of *Marsch* (1942) and *Shear and Faust* (1971), who studied the Ca:B interrelationships in the leaves of young bearing apple trees but with much higher and varying pH values.

There was also an interaction of Ca on B levels (Fig.2). The highest concentration of Ca in the nutrient solutions was associated with more B in the leaves at all B concentrations. Calcium did not affect the distribution and accumulation of B in the stems. The effect of Ca on the B level of the roots is not clear. At B-1 and B-0,1 concentrations, Ca-160 was associated with higher B levels and whereas the difference between Ca-160 and Ca-16 was significant at the 0,005

and 0,001 levels of significance, respectively, there was no difference ($P > 0,05$) between Ca-160 and Ca-0. At B-0,001 the roots from the Ca-16 solution had the lowest B level.

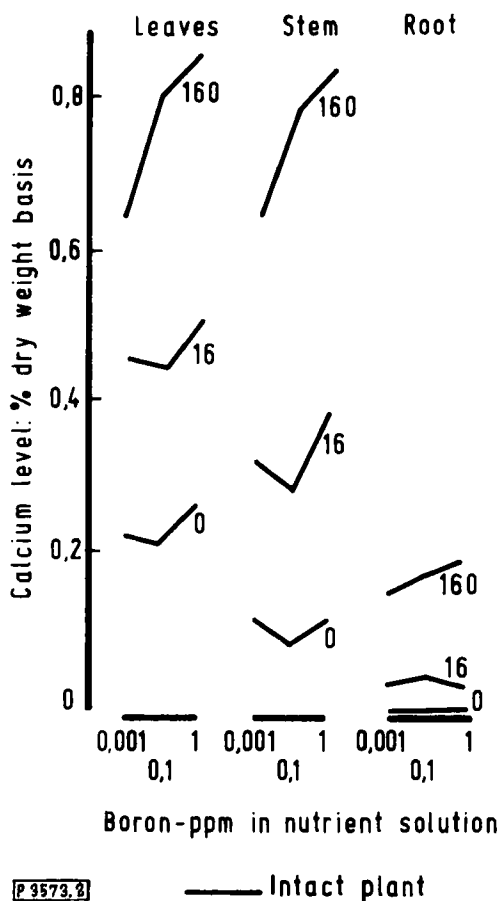


Figure 2: The Ca level of apple seedling tissues as a function of the B concentration in nutrient solutions. The numbers on the curves are the Ca concentration in the nutrient solution.

In comparison to the results of the nutrient status inside the plants after the long term treatment the ^{45}Ca uptake experiment reflects the Ca absorption and distribution during a relative short time after the long term treated plants had adapted to the different nutrient levels.

In the leaves of intact plants the ratio between the amount of Ca absorbed by those growing in Ca-160 solutions and Ca-16 solutions at the 3 concentrations of B (Table 2) was about 3 but in the shoots the ratio was about 40 (Table 3). Less pronounced differences were present in the stems and roots. Also in the intact plants at Ca-160 the amount of Ca accumulated in 24 h in the leaves was about

one fourth that in leaves from shoots but at Ca-16 the level of Ca in the leaves from intact plants was about 3 to 4 times as much as that from shoots (Fig.3). This observation is surprising but the lower Ca translocation at Ca-16 level of Ca by the shoots was not due to a depletion of the nutrient solution (Table 1).

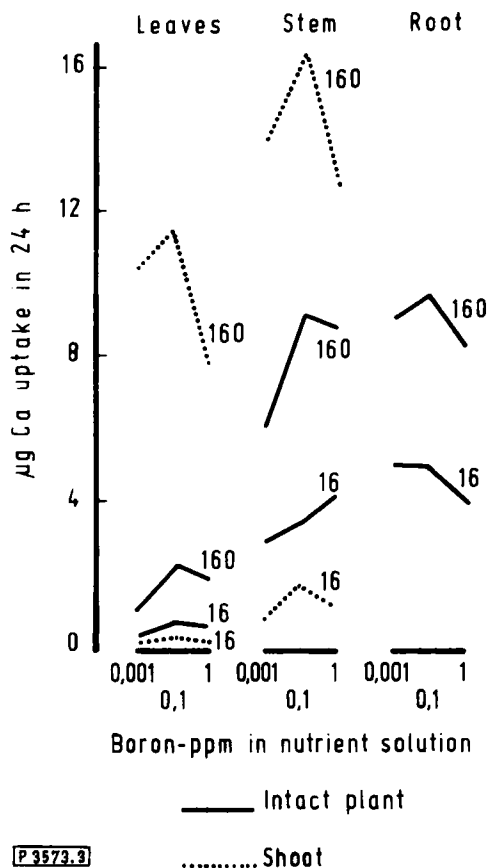


Figure 3: The $\mu\text{g Ca uptake per gram}$ of dried tissue in 24 h at various Ca and B concentrations in the nutrient solutions. The numbers on the curves are the Ca concentration in the nutrient solution.

The differences in the distribution pattern of 24 h accumulated Ca between intact plants and cut shoots may be due to different absorption and movement mechanisms and to the amounts of nutrients already in the seedlings. In intact plants Ca moves by an exchange mechanism (Bell and Biddulph, 1963) whereas in shoots it would appear that its movement was more closely associated with mass flow in the transpiration stream.

The distribution patterns of the level of total Ca and of the 24 h accumulation of Ca in the intact plants were quite different. The accumulation pattern indicated that the movement of Ca was slow since in a 24 h period only a small

quantity of Ca absorbed moved through the plant to the leaves. These different patterns also indicate that absorption and movement of Ca were not quantitatively related physiological processes. Neither the levels of Ca in the tissues nor the amount accumulated in 24 h were closely proportional to the solution concentration. There was an apparent more rapid absorption and distribution of Ca at its lowest concentration. The ratio between the specific activities does not account for the great differences in the μg Ca accumulated by the leaves and stems nor for the differences observed in the radiographs. It is possible that the plants accumulated more Mg and that this phenomenon decreased the Ca uptake.

Summary

1. Using ^{45}Ca , at 3 concentrations of Ca (160, 16 and 0 ppm) each at 3 concentrations of B (1, 0,1 and 0,001 ppm) in nutrient solutions, the interactions between Ca and B in apple seedlings were studied,.
2. The Ca and B levels in leaves from intact apple seedlings indicated a greater mutually beneficial effect on the absorption and accumulation of these elements when their concentrations in the nutrient solution was 160 and 1 ppm, respectively, than when their concentrations in the nutrient solutions were lower. The mutual beneficial effects were less pronounced in the stems and roots.
3. In a 24 h uptake period B at either 1 ppm or 0,1 ppm solution concentration was associated with a greater Ca translocation and subsequent accumulation in leaves and stems when the nutrient solution contained Ca at either 160 or 16 ppm. In the roots more Ca was present from plants grown in solutions having lower B concentrations.
4. The interaction of Ca and Mg was pronounced in the stems and roots at all concentrations of B but in the leaves only from plants growing in the B-0,1 solutions.
5. In intact plants, the rate of Ca absorption and distribution followed the exchange concept but the levels of Ca in the tissues as well as the amount of Ca accumulated in 24 h were not proportional to the concentrations in the solution.

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Zusammenfassung

1. In einem nach faktoriellen Gesichtspunkten angelegten Versuch wurden bei 3 verschiedenen Ca-Konzentrationen (160, 16 und 0 ppm) sowie jeweils 3 verschiedenen B-Konzentrationen (1,0, 0,1 und 0,001 ppm) in der Nährlösung die Wechselwirkungen zwischen Ca und B am intakten Apfelsämlingen untersucht.
2. Bei einer Konzentration von 160 ppm Ca und 1 ppm B in der Nährlösung deuteten die in den Blättern gefundenen Ca- und B-Gehalte auf eine größere wechselseitig fördernde Wirkung auf Absorption und Akkumulation dieser

Elemente hin als bei niedrigeren Konzentrationen. Die positiven Wechselwirkungen waren in Stamm und Wurzeln weniger ausgeprägt.

3. Wurden die Apfelsämlinge am Ende der Anpassung an die verschiedenen Nährlösungskonzentrationen in einer 24 h Periode mit ^{45}Ca markiert, so war die Ca-Translokation und Akkumulation in den Blättern und im Stamm bei einer Konzentration von 160 oder 16 ppm Ca und 1 oder 0,1 ppm B größer als bei einer niedrigeren B-Konzentration. In den Wurzeln war dagegen bei einer B-Konzentration $< 0,1$ ppm in der Nährlösung mehr Ca vorhanden.
4. Wechselwirkungen zwischen Ca und Mg waren im Stamm und in den Wurzeln bei allen B-Konzentrationen in der Nährlösung, bei den Blättern jedoch nur bei einer B-Konzentration von 0,1 ppm ausgeprägt.
5. In den intakten Pflanzen folgte die Ca-Aufnahmerate und -Verteilung der Austauschkonzeption. Die Ca-Gehalte in den Geweben wie auch die ^{45}Ca -Aufnahme innerhalb von 24 h waren jedoch nicht den jeweiligen Konzentrationen in der Nährlösung proportional. [3573]

Gehalt, Formen und Fixierung von Arsenat im Vergleich zu Phosphat in Waldböden

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Problemstellung

Während in der Bundesrepublik Deutschland die Anwendung von arsenhaltigen Pestiziden nicht erlaubt ist, nimmt die Anwendung von Arsenpräparaten in der Pharmakologie und der Einsatz des As in der Metallindustrie zu, so daß man eine Zunahme der As-Gefährdung erwarten kann. Zum anderen ist die Beseitigung der arsenhaltigen Lösungen, welche als Abfallprodukt bestimmter Industrien entstehen, ein schwieriges Umweltproblem und führt mancherorts durch unsachgemäße Behandlung zu verbreiteter Kontamination. Die langfristige Anwendung von As-haltigen Düngemitteln (nach Tremearne und Jacob) sind in Düngerproben bis 170 mg As/kg festzustellen) kann ebenfalls zur Arsenanreicherung im Boden führen.

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