

If GA<sub>4</sub>+7 and cytokinins are developed for commercial use to alter apple shape, their expense will probably limit the feasible concn to 50 ppm or less of each. Our study indicates that fruit set, firmness, size, and return bloom will not be affected at these concn. Multiple sprays at low concn have promise as a method to increase fruit shape response from a limited amount of applied gibberellin and cytokinin.

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## Effect of Gypsum and Dolomite on Pythium Diseases of Seedlings<sup>1</sup>

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**Abstract.** The addition of gypsum (10, 5 or 2½ lb./yd<sup>3</sup>) or gypsum plus dolomite (each 5 lb./yd<sup>3</sup>) to soils before planting with 7 kinds of ornamental and vegetable seeds did not result in more useful seedlings in uninfested soil, but in most instances, significantly increased percent of good seedlings in soil infested with *Pythium myriotylum* or *P. aphanidermatum* over the control. In uninfested soils the addition of 5 lb./yd<sup>3</sup> dolomite did not increase the number of useable seedlings compared with unamended, uninfested soil. The dolomite resulted in significantly more useful, healthy seedlings than the control only with tomato and calendula in *P. myriotylum*-infested soil and in stock with *P. aphanidermatum*-infested soil.

Seedling disease losses in ornamental and vegetable plants are often high although preventive measures are taken. Fulton (7) recently stated that seedling loss is one of the principal hazards in growing cotton. In tests with fungicides sprayed over-the-top of rows after planting, no other treatment was significantly better than a lime [Ca(OH)<sub>2</sub>] water spray. Other workers have found seedling or *Pythium* diseases affected by Ca. Ranney and Bird (15) reported Ca(NO<sub>3</sub>)<sub>2</sub> or CaCl<sub>2</sub>, 10 lb./acre, mixed with soil covering cotton seed reduced losses in Lufkin fine sandy loam. They suggest Ca contributes to a more healthy or resistant plant.

Agricultural gypsum, 8000 lb. per acre (on 6-inch deep acre equals 9.9 lb./yd<sup>3</sup>), greatly reduced peanut pod rot (9) caused mostly by *P. myriotylum*, or an indistinguishable breakdown which may be caused by *Rhizoctonia solani* (8). Vanterpool (22) found browning root rot of wheat caused by *Pythium* spp. reduced by gypsum. A combination of gypsum and triple superphosphate produced better results than either material

alone. Smaller amounts of NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub> alone gave a greater reduction in the disease, but P was necessary to obtain a response from N. Gypsum was thought to make more P available.

I have added gypsum and dolomite but not Ca(OH)<sub>2</sub> to soil naturally and artificially infested with *Pythium* spp. and to steamed uninfested soil. Seven kinds of flower and vegetable seeds were planted in these soils. The results are reported here.

#### Materials and Methods

**General.** Soils in the tests were sandy loam either mixed with Canadian peat moss or used as they came from the field. Sterilization was by steam at 15 lb. pressure for 2 hr. After sterilization Ca was added as gypsum (CaSO<sub>4</sub> 73%) or dolomite (mixture of not less than 49% CaCO<sub>3</sub> and not less than 20% MgCO<sub>3</sub>), or a mixture of gypsum and dolomite. The Ca source was added to tumbling soil in a concrete mixer and mixed 5 min. Fiber trays, 25x15x6½ cm, were half filled with a soil mixture, inoculum added, and each tray filled with soil. Inoculum was prepared by growing *Pythium aphanidermatum* (Edson) Fitz. or *P. myriotylum* Drechs. on V-8 juice agar plates for 7 days at 24°C and comminuting 2 plates in 100 ml water. Ten ml were added to infest the soil in a tray. Appropriate

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Table 1. Effect of Ca additives to 2/3 Dothan loamy sand and 1/3 peat infested with *Pythium* spp. or uninfested upon mean percent of healthy 'Campbell 17' tomato plants.

Ca source	Rate lb./yd <sup>3</sup>	pH	Mean no. healthy plants*		
			Uninfested	<i>P. myriotylum</i> infested	<i>P. aphanidermatum</i> infested
Gypsum	10	4.3	79.3 a	56.8 a	51.3 a
Gypsum	5	4.2	84.3 a	71.8 a	49.8 a
Gypsum + Dolomite	5	5.5	88.3 a	44.3 b	16.5 b
Dolomite	10	6.3	91.5 a	4.0 c	9.8 b
Dolomite	5	6.2	90.8 a	1.0 c	9.5 b
None		4.3	81.0 a	1.0 c	22.8 b

\*Means within a column followed by the same letter are not significantly different at the 0.05 level according to Duncan's Multiple Range Test.

uninfested controls were used. Soil pH was determined shortly after mixing and at the end of a test.

Seeds of at least 5 different plants were sown in rows in separate trays or flats, 56x40x15 cm. These were placed on a greenhouse bench in a randomized block design with 4 replications. Temperatures varied between 24-30°C days and 18-24°C nights. Frequent misting kept relative humidity and soil moisture high. Plants were harvested by pulling or washing the soil from the roots and were graded as satisfactory for

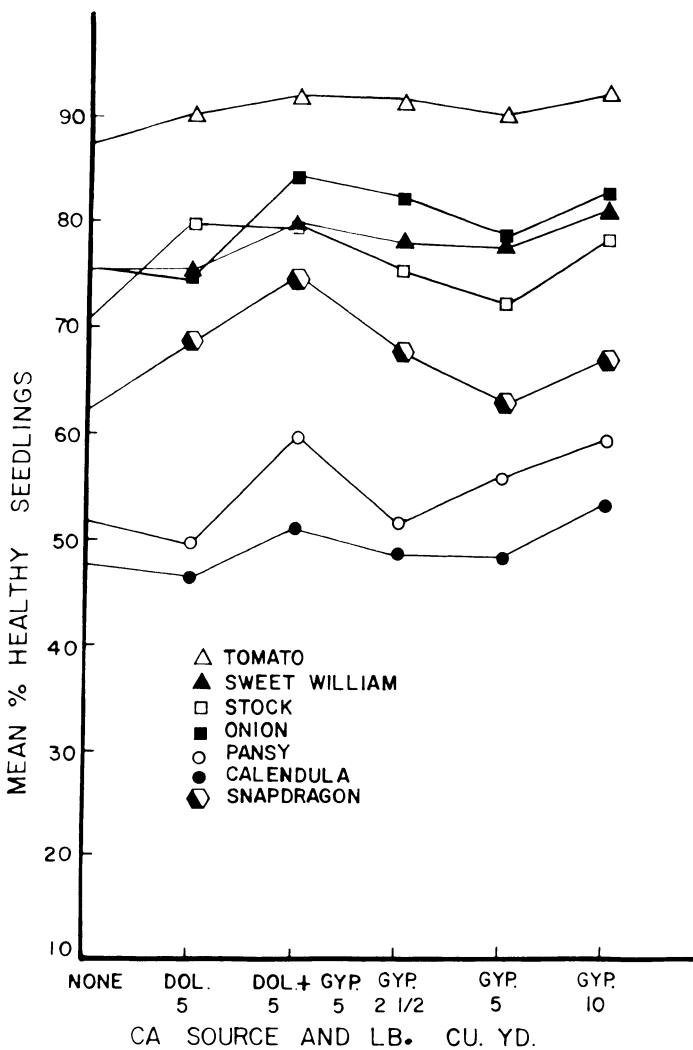


Fig. 1. Effect of Ca additives on mean percent healthy seedlings in steam sterilized uninfested soil.

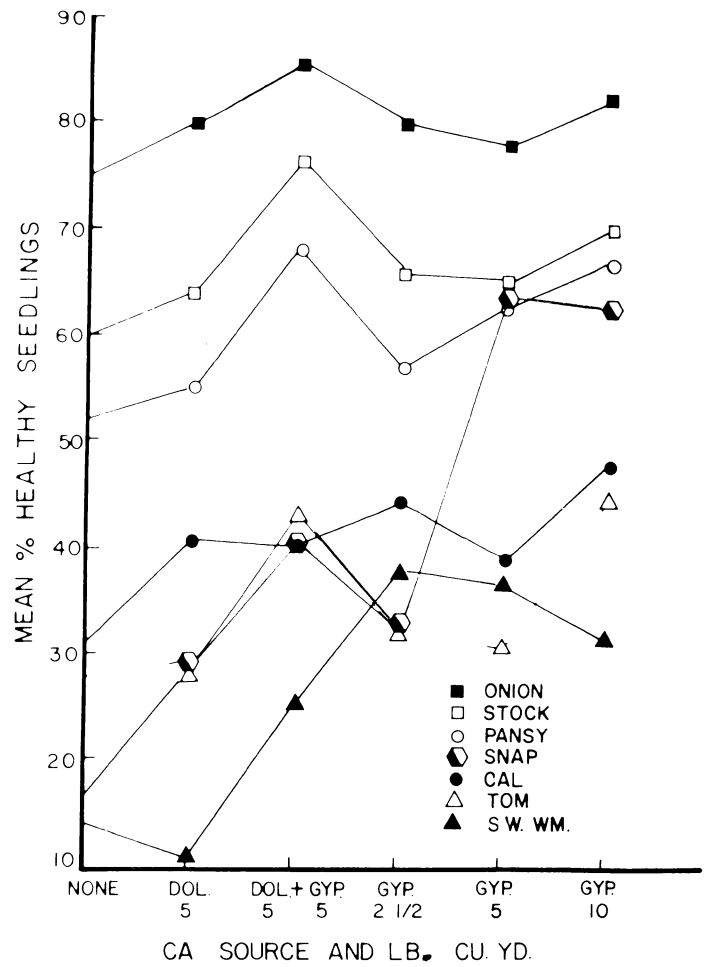


Fig. 2. Effect of Ca additives on mean percent healthy seedlings in steam sterilized soil infested with *P. myriotylum*.

transplanting (healthy) or unsatisfactory (diseased or horticulturally unacceptable).

**1969-1970 tests.** A Tifton loamy sand was used in 1969 and Dothan loamy sand in 1970. Soils were mixed with peat moss 2:1 v/v and steamed. Gypsum or dolomite 10 and 5 lb./yd<sup>3</sup> and gypsum plus dolomite 5 lb. each were used. After infesting the soil 100 seeds per replication of tomato (*Lycopersicon esculentum* Mill. 'Campbell 17'), snapdragon (*Antirrhinum majus* L. 'California Giant'), sweet william (*Dianthus barbatus* L.), calendula (*Calendula officinalis* L. 'Pacific Beauty') and pansy (*Viola tricolor* L. 'Swiss Giant') were planted October 13, 1969, and August 3, 1970. Plants were harvested after 22 and 58 days, respectively.

**1970-1971 tests in artificially infested soils.** I conducted 3 tests using steamed, artificially infested soil, and uninfested soil as in earlier tests but substituted 2½ lb. gypsum for 10 lb. dolomite. The first test using Dothan loamy sand and peat moss 2:1 v/v was planted September 18, 1970, and harvested after 48 days. The second test using Dothan loamy sand from a tomato transplant production field was seeded November 23, 1970, and harvested after 49 days. Tifton sandy loam from a field growing ornamental bedding plant transplants was used in the third test planted February 4, 1971, and harvested 37 days later.

In these tests 100 seeds per replication of stock (*Matthiola incana* (L.) R. Br. 'Giant Imperial') were used in addition to the seeds previously listed, and onion (*Allium cepa* L. 'Crystal Wax') was planted in the last 2 tests.

**1970-1971 tests in naturally infested soils.** The same Ca additives as used in 1970-71 with artificially infested soils were used to amend soil from 3 fields with natural *Pythium*

infestation. A soil lot brought from the field was thoroughly mixed before adding gypsum or dolomite in an effort to get even distribution of the inoculum. In the first 2 tests, soil was placed in flats. Two hundred seeds per treatment of the 7 kinds used in previous tests were planted per replication. In the third test, fiber trays were used with 200 seeds per treatment in each replication.

The soil in the first test was Dothan loamy sand from a tomato transplant production field. Seeds were planted September 20, 1970, and plants harvested after 22 days. The second soil was Tifton loamy sand from a cabbage transplant production field. It was seeded September 25, 1970, and plants harvested 33 days later. The soil in the third test was Dothan loamy sand from another tomato transplant production field. It was planted May 25, 1971, and plants harvested after 28 days.

### Results

**1969-1970 tests.** The results from the 1970 test with tomatoes are shown in Table 1 as representative of the 1969 and 1970 tests. The pH values were determined 3 days after mixing and are similar to those in all other tests where peat was used. In this test pH of soil without additive was 4.3. The addition of gypsum had no effect on soil pH. Dolomite and gypsum in combination raised the pH to 5.5 while dolomite alone raised it to 6.3 and 6.2 for 10 and 5 lb./yd<sup>3</sup>, respectively.

The number of satisfactory plants in the uninfested steamed soil was comparable in all mixtures. In *P. myriotylum*-infested steamed soil the gypsum and gypsum plus dolomite resulted in significantly more healthy seedlings than dolomite alone or the control (Table 1). In *P. aphanidermatum*-infested soil the 2 gypsum treatments produced significantly more healthy plants than the dolomite plus gypsum, and the dolomite alone or the control.

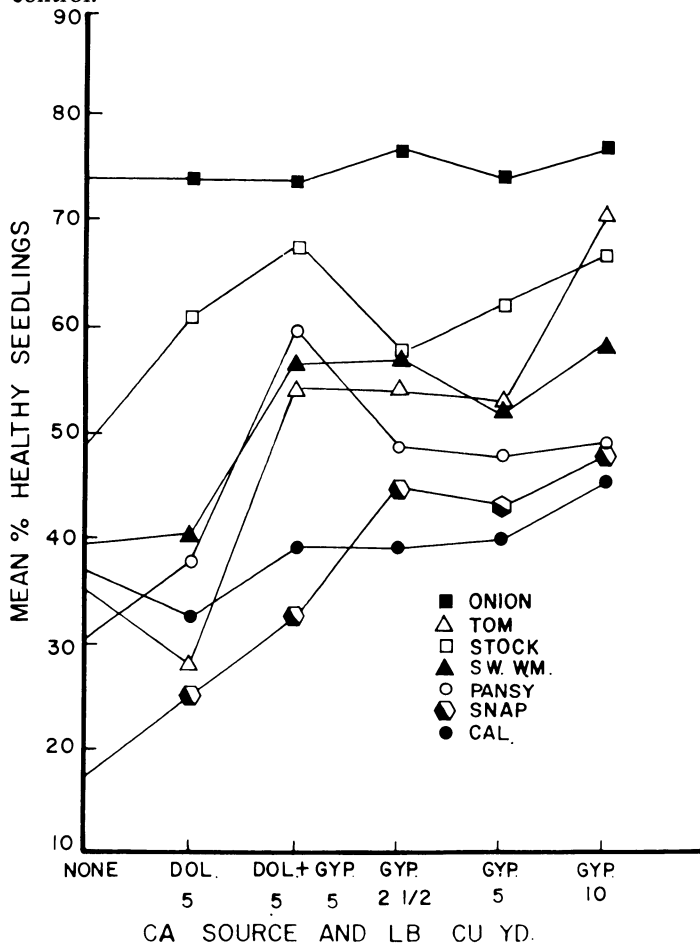


Fig. 3. Effect of Ca additives on mean percent healthy seedlings in steam sterilized soil infested with *P. aphanidermatum*.

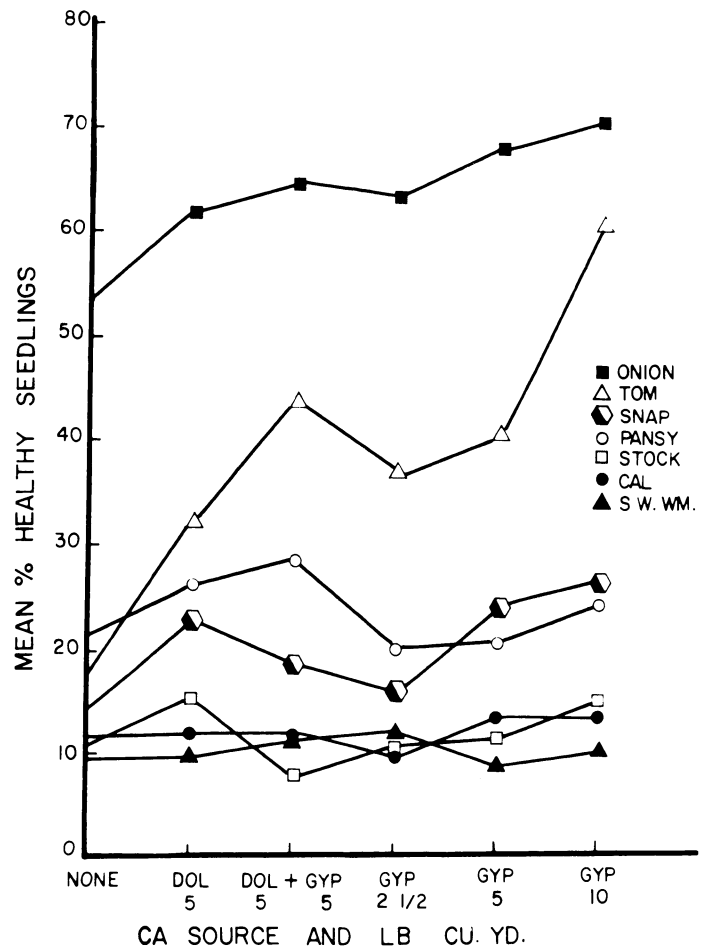


Fig. 4. Effect of Ca additives on mean percent healthy seedlings grown in greenhouse in field soil naturally infested with *Pythium aphanidermatum*.

**1970-1971 tests in artificially infested soil.** In all soils without peat, pH was approx 5.3 for the untreated and 5.3, 5.9, and 6.3 for the gypsum, gypsum plus dolomite, and dolomite soils, respectively. Results for onion and snapdragon in *P. myriotylum*-infested soil were from 2 tests only.

In uninfested soil a significant difference in means occurred only in onion and pansy (Fig. 1). In onions differences were slight but dolomite and the control produced a lower percent of healthy seedlings than gypsum except the 5 lb. rate. With pansies 10 lb. gypsum and gypsum plus dolomite were significantly more effective in producing healthy seedlings than the other treatments except 5 lb. gypsum.

In *P. myriotylum*-infested soils, mean percent of healthy tomato seedlings was significantly increased with 10 lb. gypsum and gypsum plus dolomite additives, and soil without additive produced significantly fewer plants than soil with additive (Fig. 2). With *P. aphanidermatum* infestation, 10 lb. gypsum was significantly better than other treatments and dolomite and no treatment were poorer than all others (Fig. 3). With calendula a significant difference occurred only in the *P. myriotylum*-infested soil where all additives were significantly more effective than no additive. With stock a significant difference occurred only in *P. aphanidermatum*-infested soil where the control produced fewer seedlings than all other treatments except 2½ lb. gypsum. With onions in *P. myriotylum*-infested soil (2 tests only) the gypsum plus dolomite was the only additive yielding significantly more healthy seedlings than the control. With sweet william in *P. myriotylum*-infested soil, dolomite and the control produced significantly fewer healthy seedlings than the other treatments. These 2 treatments also gave significantly fewer healthy

seedlings than the other treatments except the 5 lb. gypsum in *P. aphanidermatum*-infested soil. With *P. myriotylum* the control produced significantly fewer healthy pansy plants than the other treatments except the dolomite and 2½ lb. gypsum. With *P. aphanidermatum* the control was significantly inferior in the percent of satisfactory pansy plants to all additives except dolomite. In *P. myriotylum*-infested soil (2 tests), gypsum at 10 and 5 lb. yielded significantly more healthy snapdragon seedlings than the other treatments. In *P. aphanidermatum*-infested soil the 3 rates of gypsum additives gave significantly more healthy snapdragon plants than the dolomite and control but were not significantly better than the dolomite and gypsum mixture.

**1970-1971 tests in naturally infested soils.** Calcium additives produced significant results in unsteamed soils only with onion and tomato (Fig. 4). With these crops the control yielded a significantly lower percent of healthy seedlings than the others except the dolomite additive. In each of the 3 soils the *Pythium* present was *P. aphanidermatum*. *Rhizoctonia solani* Kuehn, however, was also present and was a factor in the results obtained. In a second planting in the first 2 soils, little differences occurred and losses were caused almost entirely by *R. solani*.

### Discussion and Conclusion

Gypsum and dolomite had no apparent direct effect in these tests upon germination and growth of seedlings in soil not infested with *Pythium* spp. except with onions and pansies. With these plants significantly fewer useful seedlings were produced in untreated soil and soil with dolomite than in soil with gypsum except at the 5 lb./yard<sup>3</sup> rate. In soil infested with *P. myriotylum* or *P. aphanidermatum* some variation in results occurred, but in general the addition of gypsum resulted in more healthy plants. The addition of dolomite resulted in significantly more healthy plants only in *P. myriotylum*-infested soil with tomato and calendula and in *P. aphanidermatum*-infested soil with stock. A mixture of dolomite and gypsum was more comparable to gypsum used alone than to the mixture.

Although gypsum increased the number of useful healthy seedlings under greenhouse conditions, it is not a satisfactory control for *Pythium*-incited seedling diseases. It may have use however, as a tool to reduce losses in a loamy sand soil and possibly other soils.

Soil pH is not a factor because pH values of the soil prior to and after gypsum was added were practically the same.

Calcium as CaCl<sub>2</sub> (1, 14, 15, 21), CaCO<sub>3</sub> (21), Ca(NO<sub>3</sub>)<sub>2</sub> (15, 21), and CaSO<sub>4</sub> (14, 19, 21, 23) is necessary for optimum seedling development of several species. Albrecht (1) reported CaCl<sub>2</sub> soil treatment increased the number of tomato seedlings. Seedling number was greater proportionally to the CaCl<sub>2</sub> treatment as seedling rate increased. Albrecht believed this was a nutritional effect of Ca. A hypocotyl necrosis of snap bean was thought to be an expression of Ca deficiency by Shannon et al. (17). Wiles (23) showed that cotton seedlings in Ca deficient soil, without pathogenic organisms, developed symptoms closely resembling those attributed to attack by soil-borne fungi. Rolling seed in CaSO<sub>4</sub> prevented these symptoms. Seedlings emerged faster and were more vigorous. Wiles suggested (a) a possible Ca deficit in the area around developing roots, (b) healthy seedlings have more resistance to disease, and (c) Ca in the diluent may affect results with some fungicides.

Seeding rates in my tests were heavy and a Ca deficiency may have been present around the roots (23), or could have been increased by the high seeding rate (1). If a Ca deficit were a factor, however, some differences should have occurred in the number of seedlings in the uninfested soil. Further, dolomite should have produced better results. Since more differences occurred in the percent of healthy seedlings in the infested soils,

it is likely that seedling resistance was increased as suggested by Wiles (23) or that pathogen virulence was decreased (4).

Calcium has been reported to reduce losses from *Sclerotium rolfsii* (3, 10, 13, 18), *R. solani* (2), *Fusarium oxysporum* f. sp. *lycopersici* (4, 5, 6, 11), and *Botrytis cinerea* (12). Causes for these effects of Ca have been suggested as (a) a micronutrient imbalance in tomato wilt (11), (b) and increase in the difficulty of cell penetration for *S. rolfsii* (13) and *B. cinerea* (12), and (c) an inhibition of polygalacturonase activity in attacks by *S. rolfsii* (3), *R. solani* (2), and *F. oxysporum* f. sp. *lycopersici* (4). Reen (16) also found polygalacturonase and other pectic enzymes produced by *Pythium* spp. (including *P. aphanidermatum*) but maceration of potato tubers could occur only after mycelial penetration of the cells.

In the present study no effort was made to determine the cause of the effect of gypsum. Enzyme activity or greater resistance of cells to penetration by the fungus may be involved. Why dolomite did not produce better results is unknown. Other forms of Ca may be as effective as gypsum against *Pythium*. Gypsum is usually considered less soluble in soils than other forms of Ca but Tiedjens and Schermerhorn (20) found solubility of gypsum increased in well watered soils. Soils in the present tests were kept well watered.

These tests indicate a need for more studies on the beneficial effects of Ca on ornamental and vegetable seedlings as recently suggested by Fulton (7) for cotton.

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## Role of Transpiration and Phloem Transport in Accumulation of $^{45}\text{Ca}$ in Leaves of Young Apple Trees<sup>1</sup>

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**Abstract.** Apple (*Pyrus malus* L.) seedlings or rooted layers growing in nutrient solution in the greenhouse were used to determine the role of xylem and phloem in the accumulation of Ca in the leaves.  $^{45}\text{Ca}$  accumulation increased with increasing rates of transpiration as measured by water losses. Girdling experiments demonstrated that the phloem was the primary route of translocation. Young leaves accumulated more  $^{45}\text{Ca}$  than old leaves even though the water losses for plants bearing only young leaves or only old leaves were similar.  $^{45}\text{Ca}$  accumulation in mature leaves was decreased when the shoot tips were removed. Apparently, in young apple trees Ca moves primarily in the phloem, but leaks into the xylem at increasing rates in the younger stem and near the growing apex.

Factors affecting the translocation of Ca in the apple are of interest because several physiological disorders of the fruit have been associated with low levels of Ca (6). Calcium movement in the xylem should be influenced by transpiration, whereas phloem transport would be affected primarily by metabolic activity. Even recent researches (1, 7) have not established definitely the role of either tissue in the transport of Ca. Our studies were undertaken to ascertain the effect of transpiration and girdling on the accumulation of root-supplied  $^{45}\text{Ca}$  in the leaves of apple seedlings and layered plants grown in nutrient solutions in the greenhouse.

### Materials and Methods

Either 1-year-old apple seedlings or rooted layers of 'Malling Merton 106' apple rootstock were held in cold storage and started in sand as needed. When well-rooted, the sand was washed from the roots and the plants were transferred to solution culture, usually at the initiation of an experiment. Trees were assigned according to size in a randomized complete block design. Plots consisted of single trees or single layers. Whenever practical, all trees in a replicate were grown in the same solution culture jar.

All experiments were conducted in the greenhouse using a solution culture containing an intermediate level of Ca (7) and N as  $1/2 \text{NH}_4$  and  $1/2 \text{NO}_3$ . Aeration was provided by forcing air through aquarium stones. Three liters of nutrient solution were used for culture at the start of an experiment, thereafter only deionized water was added.  $^{45}\text{Ca}$  as  $\text{CaCl}_2$  was added to the solution at  $40\mu\text{c}$  per 3 liters. After 7 days the entire shoot from each seedling was harvested, placed in a plastic film bag, and held at  $2^\circ\text{C}$  up to several hr before the leaves were separated, taped to a blotter, and dried in a plant press for autoradiography. Autoradiographs were prepared using Kodak no-screen x-ray film which was exposed for 2 weeks.

Leaves were dried at  $60^\circ\text{C}$  for 6 to 8 days, ground in a Wiley

mill or by mortar and pestle, ashed in a muffle furnace, transferred to stainless steel planchets, and radioactivity determined. The samples were counted with a TGC-14 G-M tube and an automatic sealing circuit. Counts were corrected for self-absorption using a curve obtained with apple leaf ash.

**Effect of transpiration.** To test the effect of transpiration on Ca accumulation, the upper halves of apple seedlings were covered in plastic bags. Wet paper towels were enclosed and each bag was tied tightly around the stem. Slits were cut near the tops of the bags to permit gas exchange. After 1 week, the leaves within or below the bag, and the lower and upper leaves of control plants were harvested, dried, ashed, and counted.

Better control of temp and humidity was attempted in a subsequent experiment in which positive air flows of 2000 ml or 3 changes per min were supplied to individual plants within polyethylene film enclosures. Air which had been heated, humidified, and cooled was supplied to 5 plants. Another 5 plants received air which had been dried by passage over silica gel and through a refrigerated dryer. Each plant was grown in a separate jar which was closed at the top with plastic film to minimize evaporation (Fig. 1). No water was added during the course of the experiment and total solution used was determined at the conclusion. Temperature and relative humidity of the air within the enclosures were determined 3 or 4 times daily by pumping air over dry and wet thermocouples. Fine wire thermocouples were used to measure leaf temp. Vapor pressure deficit calculations were based on air temp exclusively since leaf temp were extremely variable. After 8 days the leaves from each plant were evaluated for  $^{45}\text{Ca}$  accumulation.

Since older leaves absorb less  $^{45}\text{Ca}$  than younger leaves (3), the transpiration and  $^{45}\text{Ca}$  accumulation of young leaves was compared with those of old leaves. Five pairs of trees of equal size were selected and several of the oldest, small leaves at the bottom of the 'MM 106' rooted layers were removed. The shoot was then pruned immediately above the youngest fully-expanded leaf. Pairs of trees were adjusted to equal numbers of leaves by removal of 1 or 2 lower leaves as required. Nutrient solution used during the next 3 days was measured. Then, half of the leaves were pruned from each plant by removing either the upper or lower leaves. Water use, fresh wt, dry wt, and surface area of the leaves were measured.

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