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Role of Transpiration and Phloem Transport in Accumulation of ^{45}Calcium in Leaves of Young Apple Trees[1]

R. L. Stebbins[2] and D. H. Dewey
Michigan State University
East Lansing

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}Calcium 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}Ca than old leaves even though the water losses for plants bearing only young leaves or only old leaves were similar. ^{45}Calcium 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 reates 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}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 NH_4 and 1/2 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}Calcium as $CaCl_2$ was added to the solution at 40μc per 3 liters. After 7 days the entire shoot from each seedling was harvested, placed in a plastic film bag, and held at 2°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°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 suing 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}Ca accumulation.

Since older leaves absorb less ^{45}Ca than younger leaves (3), the transpiration and ^{45}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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[2]Present address: Department of Horticulture, Oregon State University Corvallis

Fig. 1. Dry air was passed through the bag on the left and humidified air through the bag on the right. Silica gel absorbed moisture in the "dry" bag while a wet paper towel assisted in maintenance of humidity in the "wet" bag.

Effect of girdling. A section of phloem 1.5 cm in length was carefully removed with min damage to the xylem from the 1-year-old stem and the girdled area was covered with black plastic tape. Initially, 8 apple seedlings with new growth 20-30 cm in length were used. Four seedlings were girdled on 2 August; the leaves were harvested after 6 days, then dried for 6 days and autoradiograms prepared. The leaf samples were then divided into those from lower and upper parts of the seedlings and examined for radioactivity.

^{45}Calcium was added to the root medium of 8 seedlings 16 hr after girdling of the stems. These plus 8 control plants were harvested 8 days later and the upper leaves dried in a plant press for 12 days. Autoradiograms were prepared by exposure of the plant specimens for 2 weeks. Tissue segments of phloem and xylem from above and below the girdle were included in the autoradiographs. The leaves used for the autoradiographs were ground and 500 mg ashed and radioactivity determined.

A group of 1-year-old rooted layers of the apple rootstock 'MM 106' was used for a third test. When the stocks had developed single shoots 20 to 25 cm in length they were selected for uniformity and assigned to 5 replicate groups of 4 each, with each replicate grown in a single jar. Treatments consisted of non-girdled plants as the control, girdled plants, girdled plants with partial root pruning by means of a fresh cut through the 1-year rooted layer about 1.5 cm above the lower end, and plants girdled between a lower and one or more upper shoots to provide leaves to feed the root system (Fig. 2). Radioactivity was determined on samples of 500 mg of ash of leaf or of stem phloem from girdled and non-girdled plants.

Effect of shoot tip. The effect of the growing tip upon accumulation of ^{45}Ca by the lower leaves was examined with 6 seedlings each with 2 shoots. The stem above the uppermost fully-expanded leaf was marked and both shoots of each plant were adjusted to an equal number of mature leaves. The entire tip above the mark was removed from one of the shoots selected randomly (Fig. 3). After 8 days, the leaves below and above the mark were harvested, dried, weighed, and counted.

Results

Effect of transpiration. The static enclosure of the upper leaves of seedling trees within plastic bags resulted in 1.5 cpm of ^{45}Ca/100 mg dry wt as compared with 104.3 cpm/100 mg for upper leaves of plants in the open (freely transpiring). Leaves harvested below the enclosed plant parts yielded 26.5 cpm. Although no transpiration data were obtained, the differences

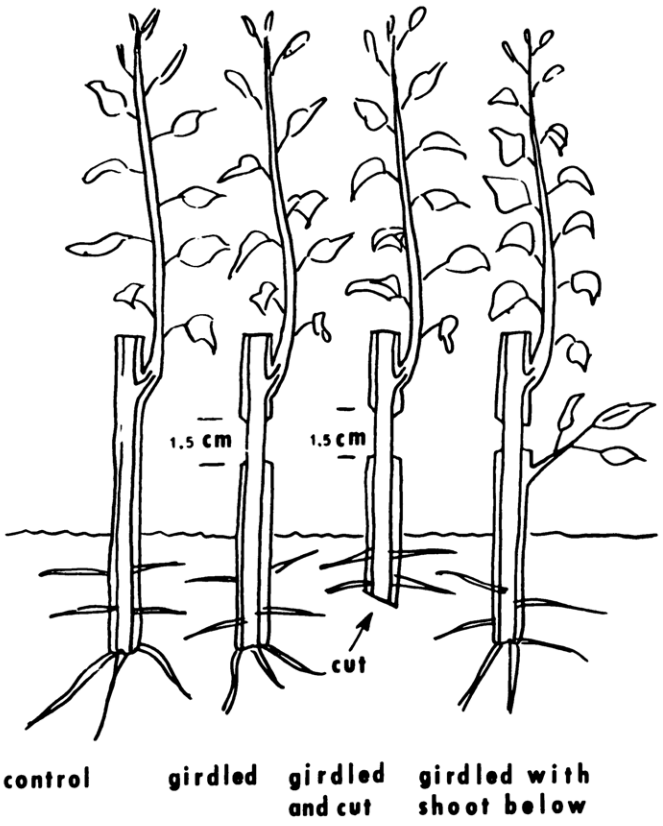

Fig. 2. Illustration of the treatment of rooted apple layers in an experiment to determine 1) if Ca would pass a girdle if it has access to the xylem and 2) if lack of translocation past a girdle was due to root starvation.

were apparently due to the reduced transpiration within the enclosures. This was substantiated by a subsequent test in which moving air of low or high humidity was passed over the leaves. Mean water losses were 101.2 and 170.8 ml/g fresh wt, respectively, for humid vs. dry air. There was a significant

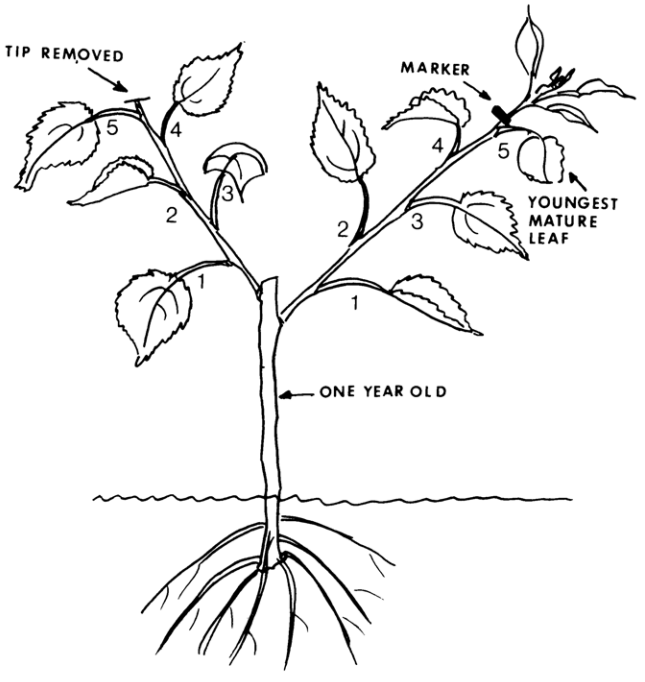

Fig. 3. Illustration of the treatment of seedlings in an experiment to determine the effect of the growing tip upon accumulation of ^{45}Ca by the lower leaves.

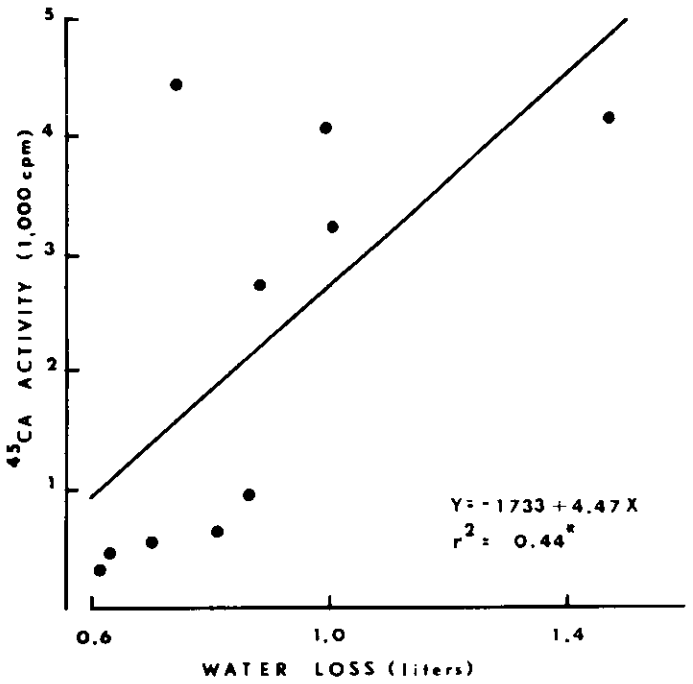

Fig. 4. Regression of ^{45}Ca accumulation in leaves with water loss per plant for apple seedlings growing in solution culture.

increase in leaf accumulation of ^{45}Ca with increases in plant water loss (Fig. 4). Daytime leaf temp were usually 1 to 3°C higher for plants in moving humid air than in moving dry air; even so, the calculated mean vapor pressure deficits between leaves and air were significantly lower in the humid air (5.1 mm Hg) than in the dry air (11.0 mm Hg).

Trees with either young or old leaves, exclusively, lost similar amounts of water. Water loss was positively correlated with leaf surface area (r = 0.64*).[3] The young leaves were similar in size and age to those which absorbed large quantities of ^{45}Ca in other experiments. This shows that preferential movement of Ca into young leaves is not related to higher transpiration rate of young leaves.

Effect of girdling. Autoradiographs showed that the leaves of the control (non-girdle) seedlings accumulated ^{45}Ca, whereas 3 of the 4 girdled seedlings produced no image and the fourth showed faint traces of ^{45}Ca at the midribs of the leaves. These observations were confirmed by count data of the leaf ash (Table 1, Exp. 1). Similar results were obtained in another experiment (Table 1, Exp. b) using twice as many trees. The data for 3 of the girdled trees of the latter experiment were excluded since the roots appeared damaged or dead. Roots of the other trees appeared normal. These results offer evidence that transport of ^{45}Ca, in the old stem at least, is confined to tissues exterior to the xylem, probably the phloem.

The results of girdling rooted layers (Table 2) show that little ^{45}Ca moved past a girdle unless the base was cut. Presence of a

[3]* r significant at the 5% level.

Table 1. Effect of stem girdling on the accumulation of ^{45}Ca in the leaves of apple seedlings growing in solution culture .

	^{45}Ca (cpm/100 mg dry wt)		
	Exp. a		Exp. b
Treatment	Younger leaves	Older leaves	All leaves
Control	40	30	101
Girdled	1	1	3
Probability	<0.05	<0.05	<0.05

Table 2. Effects of phloem girdling, the presence of leaves below the girdle, and cutting of the base on the accumulation of ^{45}Ca in the upper leaves of rooted leayers of apple growing in solution culture[z].

Treatment	^{45}Ca (cmp/100 mg dry wt)
Nongirdled (control)	1,049 a
Girdled	47 b
Girdled, base cut	4,392 a
Girdled between main lower shoots	36 b

[z]Means followed by the same letter are not significantly different (P < 0.05, Tukey's test).

leafy shoot below the girdle did not change the result, therefore, the reduced uptake of Ca apparently was not due to root starvation.

Possible damage to the xylem of girdled layers was indicated when the air temp in the greenhouse inadvertently increased to 35°C for several hr. Two of the girdled trees of 1 test wilted indicating xylem damage, whereas the other 3 girdled trees did not wilt. Some control trees wilted even though they were not girdled, thereby indicating the severity of the conditions.

Effect of shoot tip. Young leaves below a rapidly growing shoot tip accumulated significantly greater amounts of ^{45}Ca than comparable leaves on an adjacent shoot of the same plant from which the tip had been removed. The total cpm for all leaves were 51,711 for whole shoots and 26,641 for shoots with tips removed. The shoot tips themselves had an average count of 63,979 cpm. Seemingly, there was little competition from the older leaves for the available supply of Ca.

Discussion

The severe restriction by girdling on the accumulation of ^{45}Ca in the leaves showed that ^{45}Ca was translocated primarily in the phloem of 1-year-old apple stems. However, these results do not agree with those of Mason and Maskell (4, 5) with cotton, or of Koontz and Foote (3) with bean who demonstrated acropetal transport of Ca across a girdle. Still, the phloem could be the normal pathway of translocation of Ca in some plants, even though girdling does not severely affect accumulation, because girdling may force a lateral movement into the xylem. In such cases, one would expect the transport of Ca to take place in both the phloem and xylem. Our results show only limited lateral transport from phloem to xylem in 1-year-old apple stems. Layers with freshly cut bases showed rapid ^{45}Ca transport in the xylem, whereas, only small amounts of ^{45}Ca by-passed the phloem girdle via the xylem.

A greater absorption of ^{45}Ca by roots probably accounts for most of the increased ^{45}Ca accumulation in the leaves that occurred with increased transpiration. Since leaves below a plastic enclosure accumulated more ^{45}Ca than leaves within the enclosure, reduced transpiration apparently restricts ^{45}Ca movement as well as absorption in apple.

The increased accumulation of ^{45}Ca with increased water loss appears to contradict the phloem transport hypothesis. This is because transpiration rate is generally thought not to influence the rate of acropetal translocation in the phloem. Yet higher rates of transpiration may increase translocation of ^{45}Ca by increasing its rate of movement from phloem to xylem, particularly in new stem tissue. Lateral transport through living ray cells and possibly through the cambium, with subsequent leakage into tracheids, would be a function of concn. Little lateral transport would take place when the transpiration rate is relatively low, as under conditions of low vapor pressure deficit. As transpiration and water movement increase, the relative concn of Ca in the xylem fluid may decrease so that Ca moves more rapidly into the transpiration stream from the phloem. If this process were more rapid near the shoot tip, it would account for the lesser movement of ^{45}Ca into the more

proximal older leaves.

Our evidence for phloem transport of Ca in young apple seedlings or rooted layers should be verified for older trees. Even though Ca is considered to be immobile in the mature bean plant, it has been shown that there is redistribution of Ca from the cotyledons during germination and early seedling growth (2). The age at which the nutrient distribution pattern is established for apple plants is not known.

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Snapdragon Stem Tip Breakage as Related to Stem Lignification and Flower Color[1]

David G. Adams and Wesley A. Urdahl[2]

South Dakota State University, Brookings

Abstract. Breakage in the floret area of the stem which occurs during harvesting or post-harvest handling in commercially mature snapdragons was investigated. The point of breakage was not influenced by the number of open florets on the stem, provided many unopened buds remain at the apex. Breaking occurred lower on the stem in crops harvested during fall as opposed to summer months. The break point appears to be related to the end of the concentric column of safranin stainable, lignified xylem. Although not without exception, breakage also appears related to flower pigmentation in that anthocyanin (red) containing cultivars tend to break high whereas aurone (yellow) containing cultivars break low in the floret area. These factors suggest a competition for phenyl propanoid precursors which are consumed in both lignification and pigmentation.

Production of weak, succulent-stemmed ornamentals in the greenhouse has been a perpetual problem and most often noticed during months of low light intensity. Breakage of stems in the floret area during harvesting or post-harvest handling in the snapdragon, *Antirrhinum majus L.*, is usually clean, without much tearing of tissues. This suggests that lignification has not taken place. Most problems concerning snapdragon quality have been approached from the standpoint of environmental or cultural manipulation.[3] Such factors as nutrition, withholding water (7, 8), temp and photoperiod manipulation (15, 20, 23), spacing or increasing illumination per plant (2), and cultivar selection have been common areas of investigation.

We studied stem breakage within the area of the florets as it relates to xylem lignification in the stem and to flower color. Lignification and flower pigmentation were investigated because of their mutual dependence on phenyl propanoid precursors. Research and review articles on the biosynthesis of lignin (1, 4, 16, 21, 22, 25) and plant pigments (5, 9, 10, 11, 18) clearly show that precursors such as p-coumaric, ferulic, and caffeic acids, and other phenyl propanoid compounds derived from the shikimic acid pathway are principal components of both processes.

Materials and Methods

Snapdragons were greenhouse-grown in raised beds and were flowered during spring, summer, fall, and winter. Normal spacing and feeding, and temp of 50-55°F, when possible, were maintained for each crop. Within each cropping period, several cultivars were grown. Cultivars were planted in single rows across the bench and were randomly mixed. Guard rows were used only on bench ends. Samples of 10 stems each were pulled from the bed when individual stems had 6 or more, (often 15-20) fully open florets. Stems were taken randomly from all positions within the row and bench as flowering commenced.

In order to determine if stem breakage was correlated with stem lignification, individual stems were physically broken by hand, often in several places. The recorded point of breakage (hereafter referred to as "break-point") was determined as that point on the stem furthest from the apex which broke cleanly and without visible tearing of the xylem tissues.

Free hand cross sections of the same turgid stems were immediately cut and stained with a dilute safranin solution. Sections were made from internodal tissue approx half way between individual florets. Usually, 5-8 sections were made from each stem. The point on the stem where a continuous ring of stainable xylem ceased and individually stainable bundles continued was designated as the "stain-point." Above this point stainable xylem was found only in isolated bundles and below, it was a continuous ring. Records based on hand breakage and stain acceptability were taken on each of the 10 stems in a sample. Samples were replicated 2-4 times depending on quantity of material.

Locations of break- and stain-points were also recorded in relation to floret size and openness to establish an external index for xylem lignification and point of breakage. The system used for characterizing size and degree of floret openness was modified from Schmidt (21); fully open floret "6," floret more than half open "5B," floret beginning to open "5A," floret full size but prior to opening "5," sizes "4, 3, 2, 1" as per Schmidt (Fig. 1).

Floret development was compared with break- and stain-points on an arbitrary scale (Fig. 1). A numerical value of 13 was assigned to those samples which broke or stained at the internode between the last fully open floret (size 6) and the first

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[2]Associate Professor of Horticulture and Assistant-in-Horticulture, respectively.

[3]Grower Circle News. April, 1967 and Jan., 1968. Yoder Brother Inc., Barberton, Ohio.