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Investigations of Radioactive Calcium Uptake and Translocation after Branch Injection, and Painting on the Surface of Fruits of Pear Trees under Orchard Conditions

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ABSTRACT. Experiments were conducted on 60 years old 'Anjou' pear trees under orchard conditions to study the absorbtion of ^{45}Ca by the fruit skin during the growing season, the mobility of ^{45}Ca between the shoots and the fruits.

When ^{45}Ca (Carrier Free) was injected into the branch, 20 cm below the attachment of the fruit spur, the second week of August, 2% of the total ^{45}Ca moved into the fruit within 40 days in both the sun side and the shaded side of the tree. When ^{45}Ca was injected in the branch of 'Bartlett' pear on June 25, after removing the terminal meristem, about 28% of the total Ca moved out of the application zone during three days compared with only 17.5% of the total ^{45}Ca moved out of the application zone if the growing point was left intact. Within three days the activity of ^{45}Ca in the fruits on branches with the growing point removed was three times higher than in fruits on branches with the growing point left intact.

When ^{45}Ca was injected in the fruit flesh or in the carpel cavity, only a negligible amount moved out of the fruit, while 41% of the toal ^{45}Ca absorbed by the fruit from painting on the fruit surface, moved out of the fruit to the adjacent leaves and stems within 40 days.

Interest in Ca translocation has increased after the discovery that many physiological disorders in fruits and vegetables are associated with localized Ca deficiency, including bitter pit in apples and cork spot in 'Anjou' pears.

Calcium translocation has become a controversial topic. Some of the literature (Biddulph *et al.* 1959), (Biddulph *et al.* 1961) has suggested that Ca is immobile in the phloem, while others (Ringoet *et al.* 1968), (Stebbins and Dewey 1972), (Wieneke 1979) (Wallace *et al.* 1978) have suggested that Ca is mobile in both phloem and xylem. The immobility of Ca in the phloem is based on the observation that Ca deficiency appears first in the young leaves and growing points. Calcium concentration in the abscised leaves was about four times higher than Ca concentration in the new leaves of valenceia orange (Wallace *et al.* 1951), while in

avocado trees most of Ca accumulation was in the leaves and the bark of the twigs and shoots (Cameron *et al.* 1951).

Calcium in the older tissues is part of the structure of the cell. This fraction of Ca apparently can not be retranslocated as long as the tissue remains intact. The second fraction of Ca is the physiologicaly active Ca which may be more mobile (Himelrick *et al.* 1982), (Himelrick *et al.* 1983).

The mechanism of Ca translocation in the xylem is described as via an exchange mechanism by many authors (Bell and Biddulph 1963), (Biddulph *et al.* 1961) and Ca can be replaced by all divalent cations in the xylem (Ferguson and Bollard 1976). But Ca has no effect on transport of cations to the xylem exudate of tobacco (Wallace *et al.* 1973), while excess trace metal elements depressed Ca levels in some or all plant parts (Wallace 1979).

The non exchangeable ^{45}Ca in the stem of bean plant was found to be mostly in Ca oxalate crystals (Biddulph *et al.* 1961), while organic acids concentration was very low in the xylem of tobacco roots and Ca concentration was high in the xylem exudate of the root (Wallace *et al.* 1971).

Calcium translocation is proportional to water uptake and transpiration (Wiersum 1979). Transpiration rate in the fruit is very low, so that only a little water reaches them *via* the transpiration stream. Xylem translocated Ca generally bypass the fruits and accumulate in the leaves (Wiersum 1979).

The objectives of this study are to investigate: (1) the absorbtion of ^{45}Ca by 'Anjou' pear fruit skin, which may be used as measure for the effectiveness of Ca sprays; (2) the competition between the leaves and the fruits for Ca moving up the stem by injecting ^{45}Ca in the stem, which may explain the deficiency of Ca in the fruit.

Experimental

The experiments were conducted at the Oregon State University Experiment Station, in Medford. Sixty year old 'Anjou' (*Pyrus communis*, L.) pear trees were used and they were disposed of at the end of the experiment.

Branch injection

Five similar branches with single fruit on each were selected on the sun-side of the tree (south side) and similarly five fruiting branches were selected on the shaded side (north side). Radioactive Ca was applied by injecting 0.5 ml. (37.33 μc) of $^{45}CaCl_2$ carrier free, using one ml hypodermic syringe with a fine needle inserted through the bark (Martin 1967).

Fruit injection in the flresh

Five branches with single fruit were selected for each treatment as described above. The radioactive Ca was applied by injecting 0.5 ml $^{45}CaCl_2$ (37.35 μc), using one ml hypodermic syringe with a fine needle inserted deep in the fruit flesh.

Fruit injection in the carpel cavity

Five branches with one fruit on each were selected for each treatment as described above, except the needle was inserted in to the carpel cavity through the calyx.

Painting on the fruit surface

Five branches with single fruit on each were selected on the north side of the tree. Radioactive Ca was applied by transferring 0.5 ml $^{45}CaCl_2$ (37.35 μc) in a small vial, and then the solution was painted with care on the fruit surface using a camel hair brush.

All the above treatments were applied on August 11, 1967 and the branches were harvested on September 20, 1976, for a treatment duration of 40 days.

Branch injection treatments were tried earlier on June 25, 1976 with same procedures as described above. Branch injection after removing the growing point below the third leaf from the top was also tried as described above. The branches were harvested after three days to study the short term movement of ^{45}Ca. The treatments were applied to 'Bartlett' pear trees at the Lewis Brown farm (Oregon State University, Corvallis).

Sampling

The branches were cut and divided into subsamples immediately. The subsamples for each treatment are shown in Table 1. In the branch injection treatments, the application zone is the part of the branch where the ^{45}Ca was injected including 3 cm below and 3 cm above the injection location. The part of the branch below the application zone, which was not used in sampling was removed as a potential (but trivial) radioactive waste.

Each subsample was dried in a forced air oven at 80°C, weighed and then ground in a Wiley Laboratory mill to pass 20 mesh (O' Brien and Wardlow 1961).

Fruit samples and leaf samples from the painting experiments, were washed twice with water before drying to remove the remaining unadsorbed ^{45}Ca, and the fruits were cut into quarters (Wang *et al.* 1975). The powder from each subsample was throughly mixed for uniformity.

Counting the radioactivity

A portion of the powder from each composite sub-sample was transferred to a pre-weighed planchet (5 cm diameter), weighed, and the total dry weight calculated. All the planchets were filled to a fixed height by leveling the surface of the ground sample with the edges of the planchets (O' Brien and Wardlow 1961). The dried plant powder was then covered with a collodion liquid which upon drying forms a very thin plastic film to fix the powder into planchets. The collodion liquid was prepared by mixing 30 mg collodion (U.S. Products) with 20 ml diethyl ether and 20 ml of 100% ethanol. Untreated branches were harvested from the same orchard and handled similarly as controls.

Radioactivity in the dried plant material was assayed directly in the planchets at infinite thickness (O' Brien and Wardlow 1961). Counting was done with NMC gas-flow proportional counter interfaced with an automatic scaler. Samples were counted to a total of 10,000 counts or for ten minutes. Background counts were counted for one hour before and after counting the samples, and were averagd and subtracted from the average gross count (Wang *et al.* 1975). All counts less than five per minute above the control were reported as zero. Counting data were corrected for instruments efficiency, by using a ^{36}Cl standard (Wang *et al.* 1975), (two instruments were used and their efficiencies were 0.59 and 0.57). The results are expressed as: Activity (the activity of ^{45}Ca per gram dry weight = dpm/g of dry weight); RTA (Relative Total Activity), which is the activity times the total dry weight in grams; and as % Tdpm (percentage of grand total dpm). Grand total dpm is obtained by adding the total dpm in each subsample of a branch (O' Brien and Wardlow 1961).

Results

Branch injection

When ^{45}Ca was injected into the branch, 20 cm below the attachment of the fruit spure the second week of August, it distributed in an irregular pattern in all parts of the branch including the fruit (Table 1). There was no significant difference in the distribution pattern of ^{45}Ca between the branch injection in the sun side and shaded side of the tree, perhaps because of the unusually wet and humid summer in the Medford area during the time of the treatments. About 2% of the total ^{45}Ca moved into the fruit from the branch injection treatments in both the sun side and the shaded side of the tree. About 89% of the ^{45}Ca moved out of the application zone after 40 days in the branch injection in the sun side and about 92% of ^{45}Ca moved out of the application zone for the same period in the branch injection treatment in the shaded side of the tree. Twenty-two percent of the activity was detected in the leaves between the application zone and the fruit in both the sun side and the shaded side of the tree. The highest activity (28.2%) was detected in the leaves above the fruit in the branch injection in the sun side, while only 17.7%

of the activity was detected in the leaves above the fruit in the shaded side of the tree. Lower activity (36.5%) remained in the stem above the application zone in the sun side than the shaded side (49.4%).

Branch injection of 'Bartlett' par tree early in the season (June 25, 1976), after removing the terminal meristem showed a different distribution of ^{45}Ca compared to branches with the growing point intact (Table 2). About 28% of the ^{45}Ca moved out of the application zone during three days if the growing point was removed compared with only 17.5% if the growing point was left intact. After three days of translocation 20.9% of the ^{45}Ca was detected in the stem of the branch with the growing point removed, compared with 13.3% in the stem of the branch with the growing point intact. The leaves in the branch with the growing point removed accumulated 8.6% of ^{45}Ca after three days, compared with 1.9% in the leaves of the branch with the growing point intact. The Activity of ^{45}Ca in the fruit on the branch with the growing point removed is three times higher than the Activity of the fruit on the branch with the growing point left intact (Table 2). In general, the short term translocation (3 days) of ^{45}Ca showed that most of the activity was present in the stem while the long term (40 days) translocation of ^{45}Ca showed that most of the activity had moved to the leaves.

Fruit injection

When ^{45}Ca was injected in the fruit flesh on August 11, 1976 in the shaded side of the tree, only 0.06% of the ^{45}Ca moved out of the fruit, while in the sun side of the tree, about 0.3% of the ^{45}Ca moved out of the fruit (Table 1). Injection of ^{45}Ca in the carpel cavity showed that 2.9% moved out of the fruit in the shade and 0.14% moved out of the fruit in the sun side of the tree. Most of ^{45}Ca that moved out of the fruit tends to accumulate in the leaves above and below the fruit.

Painting on the fruit surface

Radioactive Ca was absorbed by the fruit after painting on the fruit surface and moved out of the fruit to the stem and redistributed in an irregular pattern in all parts of the branch within 40 days (Table 1). About 41% of the ^{45}Ca that penetrated the fruit surface, moved out of the fruit to the adjacent tissues, mainly to the leaves above the fruit. The ^{45}Ca that accumulated in the leaves was twice as high as that accumulated by the stem.

Discussion

Branch injection

Translocation of ^{45}Ca after 40 days from branch injection treatment showed that most of the activity accumulated in the leaves (50.4%), and only 2% of the activity was detected in the fruit. These results showed that the leaves are the

major competing organs for the ^{45}Ca that is moving up the stem, as suggested by many authors (Biddulph *et al.* 1961), (Martin 1967), (Wiersum 1979). Accumulation of ^{45}Ca in leaves in the shaded side of the tree (40.3%) was lower than that accumulated by the leaves on the sun side (50.4%). That difference may be due to the higher transpiration rate of the sun side leaves as suggested by some workers (Biddulph *et al.* 1959), (Stebbins and Dewey 1972), (Wiersum 1979). After 40 days of translocation, 36.5% of the ^{45}Ca was retained by the stems in the sun side while 49.4% was retained by the shaded side stems and 11% and 8% was retained by the respective application zones (Table 1). These results may agree with the suggestions of many authors (Wieneke 1979), (Wiersum 1979), (Himelrick and Mcduffie 1983), that Ca is immobilized on exchange sites in the xylem. The slow movement of ^{45}Ca upward in the stem, as shown in our data adds further evidence to the concept that Ca translocation in the xylem is by an exchange mechanism, as suggested by many researchers (Bell and Biddulph 1963), (Faust and Shear 1973), (Fergauson and bollard 1976). The result in table 1 show that ^{45}Ca is still moving into the fruit late in the season (August 11, 1976 to September 20, 1976) but at a slow rate, which disagrees with some results from some work with apple, that Ca movement into the fruit ceased late in the season (Wiersum 1979).

Branch injection of 'Bartlett' pear early in the season (June 25) showed that ^{45}Ca arrived at the fruit within three days of translocation. The total activity in the fruit was 364 dpm, if the growing point in the branch was not removed, compared with 1525 dpm in the fruit if the growing point was removed. A total of 7729 dpm was accumulated in the growing point after three days, which suggests that the growing points are one to the major organs competing with the fruit for the Ca moving up the stem early in the season. These findings agree with the suggestions of some authors (Martin 1967), (Wiersum 1979) that the young fruits, meristematic tissue, and young leaves act as strong sinks. The growing points may have other effects on the translocation and distribution of Ca due to apical dominance (Faust and Shear 1973). These results may explain the advantage of summer pruning in increasing the Ca content of the fruit and reducing bitter pit in apple (W.J. Bramlage, communication by letter).

Fruit injection

Fruit injection late in the season showed that up to 2.9% of the total activity was detected out of the fruit, and mainly in the leaves. This suggests that the leaves are not only competing with the fruit on the ascending Ca but also are 'Pulling' Ca from the fruit (Wiersum 1979).

Painting on the fruit surface

Table 1 shows that 41% of the ^{45}Ca that was absorbed by the fruit moved out of the fruit, mainly to the leaves above the fruit. These results agree with the

fingings of Martin (1967), that painting ^{45}Ca on the skin of apple fruit showed that ^{45}Ca moved out of the fruit to the adjacent leave. Wilkinson (1968) suggested that Ca uptake by the apple fruit via the vascular systed during August and September is very slow and the total Ca per fruit could decline, means that part of the fruit Ca moved out. Wilkinson, (1968), also suggested that some of the Ca (1 to 1.5 mg) late in the season is in a free mobility between tree and fruit, which is sufficient to affect storage behavior. Our results from 'Anjou' pear fruit confirm these hypotheses. Our data suggests that Ca movement out of the fruit late in the season could be the main cause of cork spot in 'Anjou' pear fruit. Wilkinson (1968) suggested that among the factors which may affect Ca movement into or out of apple fruit late in the season is the competion between shoot and fruit. The results in Table 1, show that most of the ^{45}Ca that moved out of the fruit accumulated in the leaves.

We do not have a good explanation for the low activity of ^{45}Ca found out of the fruit in the fruit injection treatments compared with the high activity out of fruit in the fruit painting treatments (Tables 1 and 2). One possible explanation is that, in the painting treatments, ^{45}Ca is distributed on a larger surface area of the fruit and may have penetrated the skin to the ends of the vascular bundles, and then may have moved out of the fruit with the water that is leaving the fruit in the xylem to the leaves especially under high transpiration conditions. A support to this hypothesis is that the development of cork spot lesions typically appear close to the ends of the vascular bundles. In the fruit injection treatments, the ^{45}Ca was injected either in the carpel cavity or deep in the flesh, which may cause the ^{45}Ca to accumulate in a very small amount of the fruit tissues.

References

Bell, C.W. and **Biddulph, O.** (1963) Translocation of calcium, exchange versus mass flow. *Plant Physiol.* **38:** 610-614.

Biddulph, O., Cory, R. and **Biddulph, S.** (1959) Translocation of calcium in the bean plant. *Plant physiol.* **34:** 512-519.

Biddulph, O., Nakayama, F.S. and **Cory, R.** (1961) Transpiration stream and ascension of calcium. *Plant Physiol.* **36:** 429-436.

Cameron, S.H., Mueller, R.T. and **Wallace, A.** (1952) Nutrient composition and seasonal losses of avocado trees. *Calif. Avoc. Soc. Yearbook*, **37:** 201-209.

Faust, M. and **Shear, C.B.** (1973) Calcium translocation patterns in apples, *Proc. Res. Insit. Pomol. Skierniewiz, Poland.* Nr. **3:** 423-336.

Ferguson, I.B. and **Bollard, E.G.** (1976) The movement of calcium in woody stems. *Ann. Bot.* **40:** 1057-1065.

Himelrick, D.G. and **Mcduffie, R.F.** (1983) The calcium cycle, uptake and distribution in apple trees. *Hortscience* **18:** 147-151.

Himelrick, D.G. and **Walker, C.E.** (1982) Seasonal trends of calcium, magnesium and potassium fractions in apple leaf and fruit tissues. *J. Am. Soc. Hort. Sci.* **107:** 1078-1080.

Martin, D. (1967) ^{45}Ca movement in apple trees. *Field station record* **6:** 49-55.

O'Brien, T.P. and **Wardlow, L.F.** (1961) The direct assay of ^{14}C in dired plant materials. *Aust. J. Biol. Sci.* **14:** 361-367.

Ringoet, A., Sauer, G. and **Gielink, A.J.** (1968) Phloem transport of calcium in oat leaves. *Planta (Berl.)* **80:** 15-20.

Stebbins, R.L. and **Dewey, D.H.** (1972) Role of transpiration and phleom transport in accumulation of ^{45}Ca in leaves of young apple trees. *J. Amer. Soc. Hort. Sci.* **97:** 471-474.

Wallace, A. (1979) Excess trace metal effects on calcium distribution in plants. *Communications in soil science and plant Analysis,* **10:** 473-480.

Wallace, A., Abou-Zamzam, A.M. and **Cha, J.W.** (1973) Effect of calcium on transport of cations to the xylem exudate of tobacco. *Plant and soil,* **38:** 41-48.

Wallace, A., Abou-Zamzam, A.M. and **Motoyama, E.** (1971) Cation and anion blance in the xylem exudate of tobacco roots. *Plant and soils,* **35:** 433-438.

Wallace, A., Cameron, S.H. and **Mueller, R.T.** (1951) Seasonal use and loss of nutrients and dry matter in orange trees. *Proc. Am. Soc. Hort. Sci.* **58:** 5-10.

Wallace, A., Romney, E.M. and **Alexander, G.V.** (1978) Mineral composition of cuscuta nevadensis Johnston (dodder) in relationship to its hosts. *Plant and soil,* **50:** 227-231.

Wang, C.H., Willis, D.L. and **Loveland, W.D.** (1975) *Radiotracer Methodology in the Biological, Environmental, and Physical Sciences.* Ch. 13, pp. 310-232. Prentic-Hall, Inc., New Jersey.

Wieneke, J. (1979) Calcium transport and its microautoradiographic localization in the tissue. *Communications in Soil Science and plant Analysis,* **10:** 237-250.

Wiersum, L.K. (1979) Effects of environment and cultural Practices on calcium nutrition. *Communications in soil Science and plant Analysis,* **10:** 259-278.

Wilkinson, B.G. 1968. Mineral composition of apples. IX. Uptake of Calcium by the fruit. *J. Sci. Fd. Agric.*, **19:** 646-648.

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Table. 1. Distribution of activity after translocation for 40 days in parts of branches of 'Anjou' pear tree, treated with ^{45}Ca. The activity is expressed as: SA (specific activity in dpm/g of dry weight); RTA (relative total activity, which is the SA times the total dry weight in grams; and as % Tdpm (percentage of grand total dpm). Each value is the mean of five subsamples.

Treatments and subsamples	Activity	S D*	RTA	% Tdpm
1a. Branch Injection in Sun				
Fruit	393	24	10967	2.067
Application Zone	43864	2068	58896	11.102
Leaves between Application Zone & Fruit	70259	9613	117586	22.166
Leaves above Fruit	31581	1648	149600	28.201
Stem btween Application Zone & Fruit	25214	1311	122034	23.004
Stem above Fruit	15307	1410	71396	13.459
1b. Branch Injection in Shade				
Fruit	307	26	9558	1.873
Application Zone	40878	2189	43565	8.538
Leaves between Application Zone & Fruit	58744	2048	115182	22.574
Leaves above Fruit	24960	1204	90149	17.668
Stem btween Application Zone & Fruit	42722	2989	142966	28.019
Stem above Fruit	47083	2125	108826	21.328
1c. Fruit Injection under Skin (in the flesh) in Shade				
Fruit	22976	1406	692838	99.940
Leaves 20 cm below Fruit	28	13	63	0.009
Leaves above Fruit	16	6	39	0.006
Stem 20 cm below Fruit	0	0	0	0.000
Stem above Fruit	189	15	310	0.045
1d. Fruit Injection in Calyx End (Carpel Cavity) in Shade				
Fruit	17161	1618	364191	97.134
Leaves 20 cm below Fruit	1581	179	3790	1.011
Leaves above Fruit	1351	131	4073	1.086
Stem 20 cm below Fruit	386	85	826	0.220
Stem above Fruit	1933	358	2056	0.549
1e. Fruit Injection under Skin (in the flesh) in Sun				
Fruit	11181	1928	350737	99.707
Leaves 20 cm below Fruit	21	12	89	0.025
Leaves above Fruit	65	22	612	0.174
Stem 20 cm below Fruit	105	36	328	0.093
Stem above Fruit	0	0	0	0.000
1f. Fruit Injection in Calyx End (Carpel Cavity) in Sun				
Fruit	18247	1874	671097	99.864
Leaves 20 cm below Fruit	60	18	133	0.020
Leaves above Fruit	74	19	542	0.081
Stem 20 cm below Fruit	30	15	110	0.016
Stem above Fruit	35	14	126	0.019
1g. Painting on Fruit Surface				
Fruit	4988	384	198931	59.403
Leaves 20 cm below Fruit	4307	204	31535	9.417
Leaves above Fruit	7470	911	59760	17.845
Stem 20 cm below Fruit	960	46	8344	2.492
Stem above Fruit	6579	628	36316	10.844

* S D is the standard deviation of the A.

Table. 2. Distribution of activity after translocation for three days in parts of branches of 'Bartlett' pear tree, treated with ^{45}Ca. The activity is expressed the same as Table 1. The treatments were applied June 25, 1976.

Treatments and subsamples	Activity	S D*	RTA	% Tdpm
2a. Branch Injection with Growing Point Removed				
Stem 20 cm below Application	51	12	310	0.064
Application Zone	37836	2262	350501	72.331
Leaves between Application Zone & Fruit	4429	385	17937	3.702
Stem between Application Zone & Fruit	10468	1084	77905	16.077
Leaves btween Fruit & Growing Point	826	43	12931	2.668
Stem between Fruit & Growing Point	1504	246	23472	4.844
Fruits with Stems	75	18	1525	0.315
2b. Branch Injection with Growing Point not Removed				
Stem 20 cm below Application	0	0	0	0.000
Application Zone	38704	1747	301209	82.525
Leaves between Application Zone & Fruit	406	34	2516	0.689
Stem between Application Zone & Fruit	4591	154	16759	4.592
Leaves btween Fruit & Growing Point	324	32	4528	1.241
Stem between Fruit & Growing Point	1943	179	31888	8.737
Fruit with Stem	21	7	364	0.100
Growing Points with Folded Leaves	1755	181	7729	2.118

* S D is the standard deviation of the A.

دراسات لمعرفة امتصاص وانتقال عنصر الكالسيوم المشع بعد زرقه في الأغصان أو طلائه على سطح ثمار الكمثرى في ظروف البستان

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أثبتت نتائج هذه الدراسة أن زرق عنصر الكالسيوم المشع في غصن شجرة الكمثرى على مسافة ٢٠ سم تحت محل اتصال الثمرة بالمهماز في الأسبوع الثاني من شهر آب يؤدي إلى انتقال ٢٪ فقط من مجموع الإشعاع إلى الثمرة خلال ٤٠ يوم من المعاملة سواء كانت الثمرة في الجانب المعرض للشمس أو في الجانب المظلل من الشجرة. أن إزالة القمم النامية للأغصان يؤدي إلى زيادة انتقال الكالسيوم المشع إلى الثمرة بمقدار ثلاثة أضعاف. أن طلاء محلول كلوريد الكالسيوم المشع على سطح الثمرة أدى إلى انتقال ٤١٪ من الكالسيوم المشع خارج الثمرة خلال ٤٠ يوم. أما عند زرق عنصر الكالسيوم داخل الثمرة فإن الانتقال إلى خارج الثمرة يكون قليلاً ولا يتأثر باختلاف موقع الثمرة على الشجرة.

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