ABSTRACT

ORCHARD FACTORS AFFECTING THE INTERNAL BREAKDOWN DISORDER OF 'JONATHAN' APPLES

By

Robert L. Stebbins

The objective of this study was to identify those factors in the orchard environment most closely related to the incidence of internal breakdown of stored 'Jonathan' apple fruit. Special emphasis was given to nutrition since it is one of the environmental factors which can most easily be altered both experimentally and by the fruit grower. This thesis was prepared as a series of chapters each reporting a different subject area investigated in relation to internal breakdown of 'Jonathan' apples. The title and abstract of each chapter follows:

I. Physiological Disorders of 'Jonathan' Apple Fruit Correlated with Nutrition and Other Factors.

Results of mineral analysis of leaves and fruit from each of 4 trees in 12 orchards were correlated with incidence of internal breakdown, water core, lenticel spot, and core browning of 'Jonathan' apples in two seasons.

Fruit K and Ca were negatively correlated with breakdown and water core in both years. Primarily in one orchard, trees with high levels of Mn in leaves in 1969 produced fruit with a high incidence of internal breakdown. Core browning, which developed in CA storage, was negatively correlated with leaf K, B, P, and Ca, and positively with leaf N and fruit size.

II. Internal Breakdown of 'Jonathan' Apple Fruit in Relation to Position on the Tree and Time of Harvest.

Fruit samples were picked from the outer, middle and inner zones of large, mature 'Jonathan' apple trees from the northeast and southwest sectors on three dates. The incidence of internal breakdown of stored fruit increased with later maturity, and from inner to outer zones. No significant differences were observed due to tree sector. Fruit from the outer zone had significantly less K, P, Ca, Mg, Cu, and B than fruit from the innermost zone, but similar amounts of N.

III. The Effect of 2-chloroethylphosphonic Acid (CEPA) on Red Color, Maturity and Internal Breakdown of 'Jonared' Apples.

The application of 250 ppm 2-chloroethylphosphonic acid to 'Jonared' apple trees on August 27, 1969 hastened the development of red skin color. The effect on red color was visible within 1 week of application, but with natural color development treated fruits were indistinguishable

from controls by October 3. Changes in ground color, flesh firmness, weight loss in storage and incidence of internal breakdown indicated that CEPA-treated fruit were more mature. CEPA did not affect fruit size. CEPA fruit could have been harvested commercially a week earlier before it had grown to a size which rendered it more susceptible to internal breakdown.

IV. Calcium-rich Crystals in Apple Trees and Fruit.

Single crystals and clusters of crystals or druses found by polarized light microscopy in tissues of Pyrus malus L. were found to contain Ca, O, and C using the electron microprobe. Crystals, insoluble in 20% acetic acid, were found in cells adjacent to the vascular tissues near the pedicel in mature fruit and in dormant flower buds, stems, petioles, shoot apex, roots and callus tissue. Deposition of Ca as crystals may immobilize Ca and thereby reduce the amount which would otherwise be translocated into cortical cells of apple fruit which may result in an increased incidence of internal breakdown due to low Ca levels in those cells.

V. The Influence of Transpiration and Phloem Transport on Accumulation of ^{45}Ca in Apple Leaves and Tomato Leaves and Fruit of Plants Grown in Solution Culture.

Experiments were conducted to determine whether the accumulation of ^{45}Ca in apple seedlings, rooted layers

of apple or tomato fruit is influenced by: (1) transpiration rate, (2) phloem transport, (3) kinetin applications, (4) age of leaf, and (5) length of one-year-old stem. ^{45}Ca accumulation in leaves increased with increasing rates of transpiration. The rate of ^{45}Ca accumulation in leaves was inversely related to the length of the stem. Accumulation of Ca in tomato fruit increased with increasing transpiration rate of fruit relative to that of leaves as determined in an experiment wherein either the fruit or the entire plant was grown in a polyethylene bag. Although young leaves accumulated ^{45}Ca more rapidly, than old leaves, no difference in the rate of transpiration between young and old leaves was observed. More ^{45}Ca accumulated in mature leaves below rapidly-growing shoot tips than on pruned shoots. Cytokinins had no effect on translocation of ^{45}Ca into old leaves. Girdling experiments showed that translocation of Ca was in the phloem. It is proposed that Ca moves in the phloem and leaks into the xylem at increasing rates as it approaches younger stem and growing apex.

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TABLE OF CONTENTS

LIST OF TABLES

Table Page

Chapter V

Appendix

LIST OF FIGURES

Figure Page

Chapter IV

Chapter V

INTRODUCTION

The objective of this study was to identify those factors in the orchard environment most closely related to the incidence of internal breakdown of stored 'Jonathan' apple fruit. Special emphasis was given to nutrition since it is one of the environmental factors which can most easily be altered both experimentally and by the fruit grower. Calcium was given special attention since physiological disorders of apples in storage have often been associated with low Ca levels in the fruit. Furthermore, there is a considerable amount of evidence in the literature indicating that treatment with Ca often fails to completely prevent disorders which have been associated with Ca deficiency. A need was seen for more fundamental knowledge concerning factors that influence fruit Ca levels.

This thesis was prepared as a series of chapters, each reporting a different subject area investigated in relation to internal breakdown of 'Jonathan' apples. The title of each chapter follows:

I. Physiological Disorders of 'Jonathan' Apple Fruit Correlated with Nutrition and Other Factors.

II. Internal Breakdown of 'Jonathan' Apple Fruit in Relation to Position on the Tree and Time of Harvest.

III. The Effect of 2-chloroethylphosphonic Acid (CEPA) on Red Color, Maturity and Internal Breakdown of 'Jonared' Apples.

IV. Calcium-rich Crystals in Apple Trees and Fruit.

V. The Influence of Transpiration and Phloem Transport on Accumulation of ^{45}Ca in Apple Leaves and Tomato Leaves and Fruit of Plants Grown in Solution Culture.

CHAPTER I

PHYSIOLOGICAL DISORDERS OF 'JONATHAN' APPLE FRUIT CORRELATED WITH NUTRITION AND OTHER FACTORS

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Abstract. Results of mineral analysis of leaves and fruit from each of 4 trees in 12 orchards were correlated with incidence of internal breakdown, water core, lenticel spot, and core browning of 'Jonathan' apples in two seasons. Fruit K and Ca were negatively correlated with breakdown and water core in both years. Primarily in one orchard, trees with high levels of Mn in leaves in 1969 produced fruit with a high incidence of internal breakdown. Core browning, which developed in CA storage, was negatively correlated with leaf K, B, P and Ca, and positively with leaf N and fruit size.

Internal breakdown of fruit in storage occurs with the 'Jonathan' apple variety grown in the USA, Japan, New Zealand, and Europe (6, 7, 21, 34). The disorder appears usually after several months of cold storage followed by a short period at elevated temperatures (5).

The first symptoms are a softening of the flesh near the calyx end and the appearance of brown color in vascular elements and surrounding cortical tissue (7). The browning and softening spreads to the extent that the fruit becomes worthless.

The incidence of breakdown may vary considerably between seasons (22). Within any given season it may vary greatly between orchards and between trees within orchards (22). Due to this large and unpredictable variability, consistent results are difficult to obtain in field experiments with treatments which might reduce the incidence of breakdown.

In a survey of 'Jonathan' orchards in Michigan by Buneman et al. (5), a negative correlation between levels of K and internal breakdown of 'Jonathan' apples was found. Further study of Michigan orchards, which is the substance of this report, was undertaken with the following objectives: (1) to confirm or refute the limited results obtained by Buneman et al.; (2) to determine if nutrients in addition to K are correlated with internal breakdown; (3) to discover correlations which would lead to logical hypotheses regarding the cause of breakdown and, consequently, remedial treatment; and (4) to locate orchards with a history of internal breakdown that could serve for future experimentation on this problem.

MATERIALS AND METHODS

Four orchards in each of 2 central Michigan districts, near Sparta and Belding and 4 in southwest Michigan near Hartford were selected for the survey. Normally, fruit matures about a week earlier in the Hartford district than in the central area. All orchards were non-irrigated with the trees growing in sod on seedling roots. Originally, 10 trees in each orchard were selected for sampling. From the original 10, 4 trees were selected as representative according to leaf analysis in 1968. These trees were marked and used for all subsequent sampling. The trees were 20 to 40 years old and typical of others in the orchard. Terminal growth of the limbs varied from 2-4 inches in some orchards to 2-3 feet in others. Pruning practices and tree spacings also varied considerably.

In addition to the 12 orchards, 12 trees on the Michigan State University Graham Experiment Station, Grand Rapids, were sampled. These trees were smaller than trees employed in the survey, being 10 years old.

Samples for mineral analysis. A leaf sample consisted of 25 leaves including petioles from the middle of current season's terminal shoots at about head height. One sample was collected from each tree at each sampling. Leaves were taken from shoots of average length on all sides of the tree. Five sets of leaf samples were picked from survey trees on the following dates: in 1968, June 14,

July 22 or 23, September 12; in 1968, July 16 or 17 and September 12 or 13. Samples of 25 fruits were picked from similar locations on each tree in 1968 on June 14 and September 12 and in 1969 on July 16 or 17 and September 13 or 14. Trees at the Graham Station were sampled on the same dates as the survey trees in 1968 and on July 17, 1969.

Tissue preparation. Leaves were dried 24 hours in a forced-air oven at 60°C, then ground in a Wiley mill. Whole immature fruits were diced, dried and ground in June and July 1968. In September, 2 sections, including peel and core were cut from opposite sides of the fruit after the method of Perring (27), diced and dried. Since internal breakdown is confined to the cortex, one might expect closer correlations between mineral content of samples consisting only of that tissue. For this reason, in 1968, July fruit samples were cored and September samples were peeled and cored before drying.

Pairs of fruit for mineral analysis of the same size were selected from the same sample in the 1969 survey after storage in which one fruit was sound and the other showed internal breakdown.

Mineral analysis. N was measured by the macro-Kjeldahl method and K, following extraction with water, by flame spectrophotometry. Levels of P, Na, Ca, Mg, Mn, Fe, Cu, B, Zn and Al were determined using

photoelectric spectrometry. Ca was also measured by atomic absorption spectrophotometry. Fruit samples taken in 1969 were concentrated eight-fold for photoelectric spectrometry in order to more accurately measure the small quantities of elements present in fruit flesh. In addition, sensitivity of the Ca scale on the spectrometer read-out console was attenuated to twice normal.

Fruit characteristics. The diameter of 25 fruit per tree was measured on the dates of leaf sampling. The diameter of each stored fruit of the first samples examined in 1968 was measured. Samples were weighed in 1969.

The mean length of current season's terminal shoots was estimated visually at harvest time. A ruler was used to measure a few shoots on each tree to verify estimates.

Crop was estimated visually and noted on a scale, where 1 = very light crop, 2 = moderately light, 3 = full crop, 4 = moderately heavy, and 5 = extremely heavy.

Flesh firmness was measured using a U. S. fruit pressure tester with a 7/16 inch tip and recorded as pounds. Fruits tested were 2.40 to 2.75 inches in diameter. Two measurements were taken from peeled portions on opposite sides of each of 10 fruit. Flesh firmness was measured before and after storage in 1968, and before storage in 1969.

Weight loss in storage was determined for samples consisting of 10 fruits between 2.5 and 2.75 inches

diameter in 1969. The samples were weighed to the nearest gram before and after storage and after two weeks at room temperature. They were stored in paper bags on wire racks to permit good air circulation to all lots.

Ground color was determined by visual comparison with Ditton laboratory green-yellow apple and pear color charts, and numerically rated as 4 = green and 8 = yellow. Red color was estimated on a scale of 1 = none, 2 = very little, 3 = 50% of skin colored, 4 = 75% of skin colored, 5 = 95% of skin colored. Color was determined for each of the 50 fruit in a sample and averaged.

Severity of surface russeting was rated as follows: 1 = none, 2 = moderate, 3 = severe. Presence or absence of lenticel spots was also noted. Internal breakdown, core browning and water core were detected by cutting near the calyx end and at the equator. Number of fruit with these disorders was recorded. Severity of breakdown was rated as 1 = none, 2 = slight, 3 = moderate, 4 = severe or 5 = total. Subsamples of 10 fruit were examined for water core before storage. The number of fruits, in a 50-fruit sample, showing core browning with varying degrees of severity was recorded as follows in 1968: 1 = none, 2 = questionable, and 3 = definite core browning. The incidence of core browning of any severity was recorded in 1969.

Sampling for storage. Fruit was sampled from the outer and middle zones of foliage whenever possible and from all sides of the tree. One bushel per tree was picked on the first harvest date. On the second date 2 bushels were picked, one for storage in air, the other for controlled atmosphere storage. Dates of harvest were as follows: in 1968, Hartford area September 24 and October 3, central areas, September 28 and October 7; in 1969, Hartford area October 3 and October 16, central area October 9 and October 21 and 23. Samples from the Graham Station were picked October 10, 1968 and October 9, 1969.

The crop was too light on trees marked for the survey to obtain a complete sample from one orchard near Hartford in 1969. Fruits were picked from other trees in the block to complete the sample. The second sampling was lost from 2 orchards in 1969 because the grower harvested the plots before the second date of pick.

All samples were collected and stored in wooden field crates of 1 bushel capacity. They were placed in storage on the day of harvest.

Storage conditions. Survey fruit samples were stored in air at 38°F or in 3% O_2 and 5% CO_2 at 32° F in 1968. In 1969, samples were stored in air at 38°F or in 11% O_2 and 10% CO_2 at 32°F. Samples from the Graham Station were stored in 3% O_2 and 5% CO_2 at 38° F in 1968 and in air at 38°F in 1969. Controlled atmospheres were

established within a week after the final harvest. As shown in Table 1, survey samples were removed for examination twice except for the CA samples in 1969. The latter were removed once, examined upon removal and again after 2 weeks at 60 to 70°F. Subsamples of 50 fruit were placed in polyethylene box liners after removal from storage and held at 70°F for 2 weeks before examination. Samples from the Graham Station were examined only once.

Statistical procedure. Simple linear correlations between all variables using the means of 50-fruit samples were calculated on the CDC 3600 computer. For the first harvest in 1968, correlations were obtained between the characteristics other than mineral content, of the 50 individual fruit fruit from each tree. The physiological disorders of fruit were treated as dependent variables. Multiple correlation coefficients were obtained with least squares and least squares delete programs. Mineral analysis values from leaf and fruit samples were transformed to the second, third and fourth power and log base 10. Simple linear correlations and least squares delete problems were computed to select the transformed variables most closely correlated with breakdown. Multiple correlations were selected for presentation which, in general, met the criteria stated below:

1. All partial correlations for independent variables significant at 0.05 or less.

TABLE 1. Cold storage and room temperature periods.

Sample and type of storage	Date Examined	Months of Storage	Days at 70°F
Survey, air	12/28/68	2 1/2	12
Survey, air	2/25 to 3/1/69[a]	4 1/2	12
Survey, CA	3/18/69	5	14
Survey, CA	4/22/69[b]	6 1/2	3
	5/7/69	7	18
Graham Sta.,CA	6/17/69	8	14
Survey, air	12/15, 16, 17/69[a]	2	10
Survey, air	2/19/70	4	14
Survey, CA	3/23/70	5	14
Survey, CA	4/6/70	6	14
Graham Sta., air	2/12/70	4	14

[a]Samples not examined were returned to 32°F until examined.

[b]Twenty-five fruit were examined, 50 fruit on the second date.

2. No more than 5 independent variables involved. Although a larger number of variables may account for more of the variation of the dependent variable, each independent variable accounts for a smaller percent of the variation and is, therefore, less meaningful.
3. All independent variables must logically be candidates for a cause and effect relationship hypothesis with the dependent variable. Of course, no matter how high the R value is, multiple or simple correlations do not establish a cause-and-effect relationship.
4. The R must be above the following minimum values unless it has special interest: 2 independent variables, 0.50; 3 variables, 0.60; 4 variables, 0.66; 5 variables, 0.74. This criterion was used to reduce the number of multiple correlations reported for those most closely associated with the dependent variable.

All correlations reported are significant at the 5% level or higher. Correlations with the opposite sign in either year when compared with the same two factors correlated in the other year, were not reported.

RESULTS

The incidence of internal breakdown in 1968 was unusually low. Of the 9,600 fruits examined after storage, only 5% had breakdown. In 1969, 12.3% of the fruit from the first harvest had breakdown and some samples showed more than 90% breakdown. The trees which produced fruit with a high incidence of breakdown in 1969 were not the same ones with the most breakdown in 1968. Fruit from light-cropping trees were prone to breakdown and water core (Table 2). Estimated shoot length was also negatively correlated with water core. A strong positive correlation was found between internal breakdown and water core in the 1969 survey (Fig. 1). The incidence of core browning increased with the incidence of water core (Table 3) but the relationship was not as close as that between water core and breakdown (Fig. 1). The number of fruit with lenticel spot was correlated with core browning in 1969, $r = 0.49^{**}$.

Fruit size, color and finish vs disorder. The incidence of internal breakdown, water core and internal browning increased with increasing fruit size (Fig. 2 and 3; Table 4). In fruit from the first harvest in 1969, the incidence of breakdown increased with increasing fruit size, firmness at harvest and weight loss during the room temperature period after storage ($R = 0.86^{**}$). Among samples from the Graham Station in 1969, the incidence of breakdown

TABLE 2. Linear correlations of breakdown, water core and core browning with crop load and growth estimates in 1969 (r values).

Disorder	Crop load	Shoot length
	Survey (d.f. 41)	
Internal breakdown	n.s.	n.s.
Water core	-0.31*	-0.67**
Core browning	n.s.	0.51**
	Graham Station (d.f. 10)	
Internal breakdown	-0.63*	n.s.
Water core	-0.74**	-0.64*
Core browning	n.s.	n.s.

*Significant at the 5% level.
**Significant at the 1% level.

Fig. 1. Correlation between water core and breakdown, 1969 survey, second harvest. Each point represents the mean of 50 fruit. [**significant at the 1% level.]

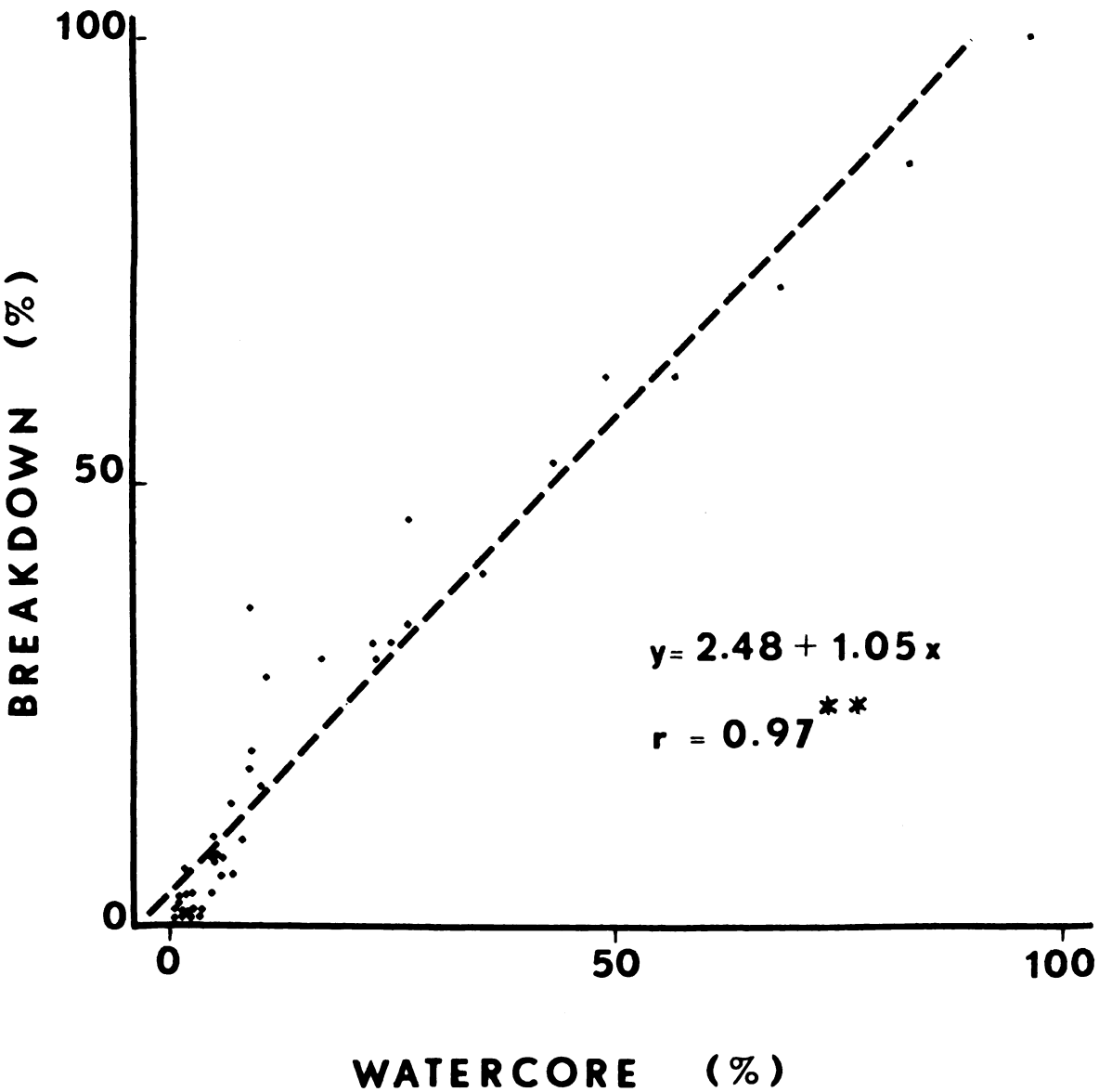

100
50
0
0
50
100
BREAKDOWN (%)
WATERCORE (%)
y= 2.48 + 1.05x
r = 0.97**

TABLE 3. Linear correlations of breakdown and water core with core browning (r values).

Disorder	Core Browning	
	Survey 1968 (d.f.46)	Survey 1969 (d.f. 38)
Internal breakdown	0.48**	0.34*
Water core	n.s.	n.s.
	Graham Station (d.f. 10)	
Internal breakdown	n.s.	n.s.
Water core	0.68*	a

*Significant at the 5% level.
**Significant at the 1% level.

[a]No core browning occurred in 1969 samples.

Fig. 2. Correlation between mean fruit weight and breakdown, 1969 survey. [**significant at the 1% level.]

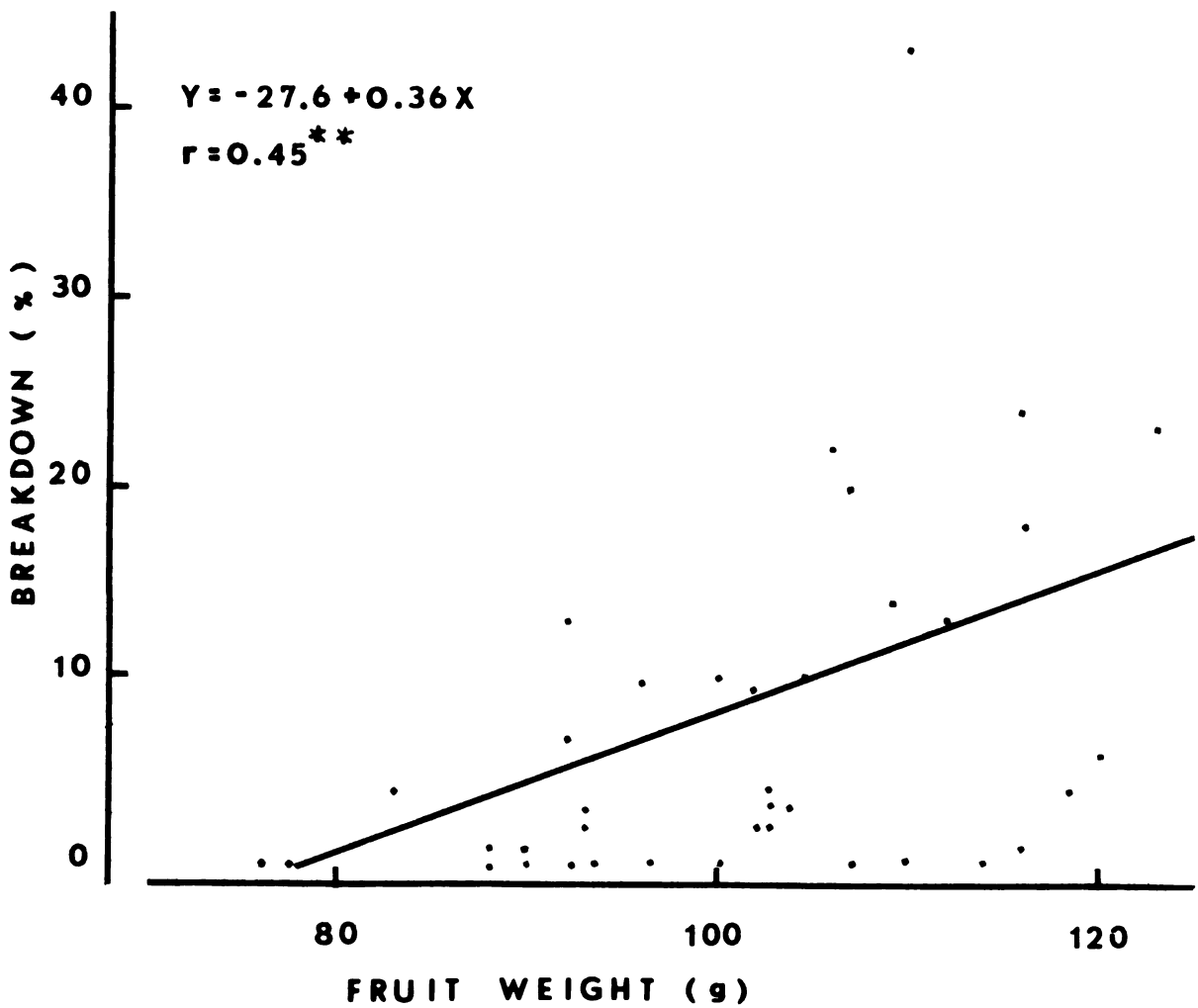

Y = -27.6 +0.36X
r = 0.45**
BREAKDOWN (%)
40
30
20
10
0
80
100
120
FRUIT WEIGHT (g)

Fig. 3. Correlation between fruit diameter and core browning, 1969 survey, second harvest from CA storage. Each point represents the mean of 50 fruit. [**significant at the 1% level.]

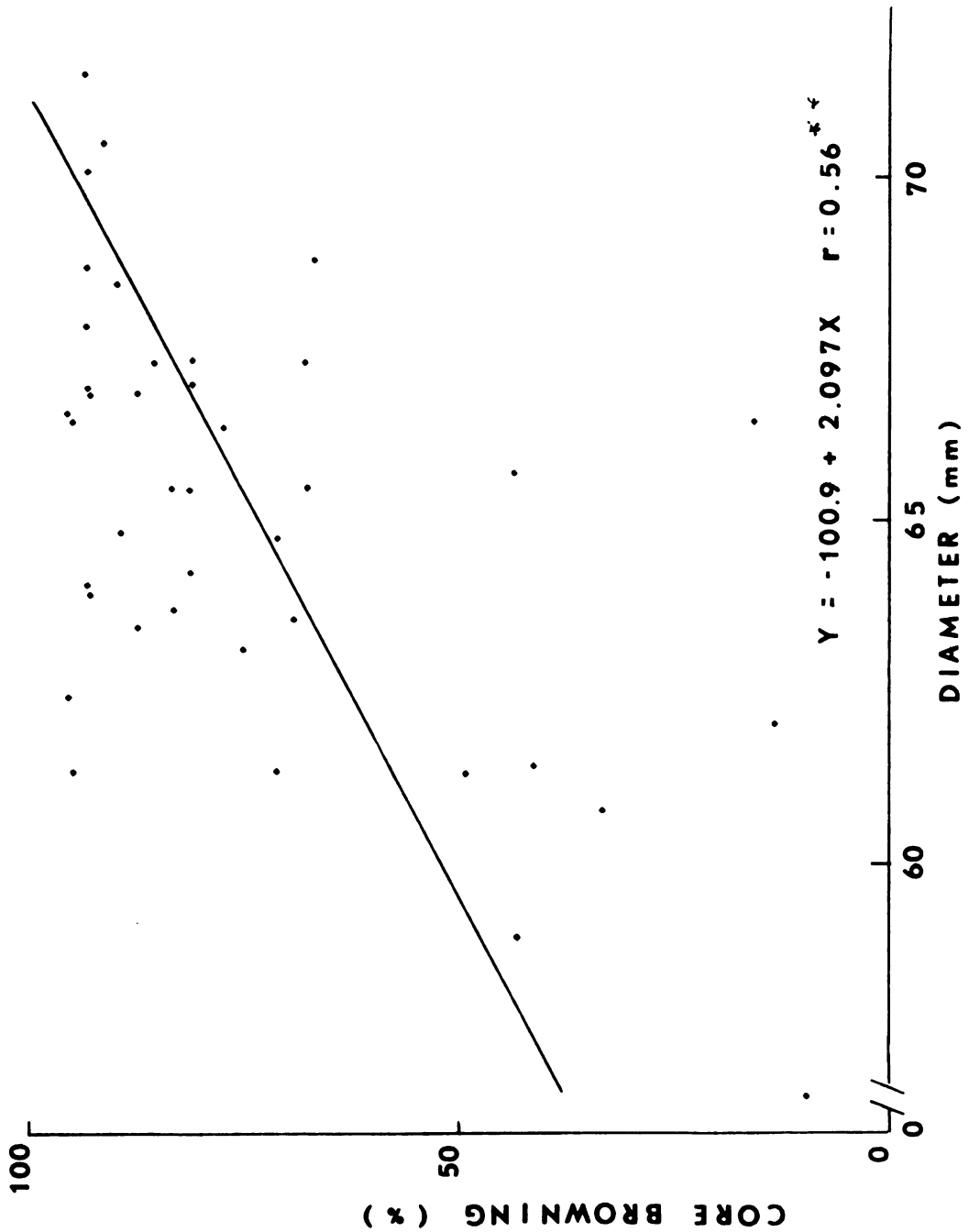

CORE BROWNING (%)
100
50
0
0
60
65
70
DIAMETER (mm)
Y = -100.9 + 2.097X r = 0.56**

TABLE 4. Linear correlations between fruit size and incidence of water core, breakdown and core browning (r values).

Disorder	Fruit size	
	Survey 1968 (d.f. 46)	Survey 1969 (d.f. 38)
Internal breakdown	0.46**	0.34*
Core browning	0.40**	0.56**
	Graham Station (d.f. 10)	
Internal breakdown	0.58*	n.s.
Water core	0.63*	n.s.

*Significant at the 5% level.
**Significant at the 1% level.

decreased with increasing amounts of surface russeting and weight loss during the room temperature period after storage (R = 0.86**). Among samples from the Graham Station in 1969, the incidence of breakdown decreased with increasing amounts of surface russeting and weight loss in cold storage (R = 0.82**). In another multiple correlation, the incidence of breakdown decreased with heavy cropping and skin russeting but increased with fruit size and a high percent moisture in fruit samples in July (R = 0.92**). When cutting the fruit, it was noted that the area of breakdown was associated with the non-russeted side of a russeted fruit. In 1968, russeting was negatively correlated (r = -0.38**) with increase in fruit diameter between mid-August and mid-September. Within fruit samples from individual trees, russeting was negatively associated with yellow ground color (r = -0.59**), and red over-color (r = -0.48**). Russeting was usually associated with small fruit size, but in one orchard the opposite relationship occurred. The incidence of internal breakdown and water core increased with increasing red and yellow skin color.

Among the samples from the 1969 survey, the incidence of lenticel spots decreased with increasing yellow ground color but increased with heavy cropping and weight loss in storage (R = 0.77**). Weight loss in storage increased with increasing incidence of lenticel spots (r = 0.60**).

Fruit size, color and firmness vs. mineral content.

In general, fruit size increased and red color decreased with increased leaf mineral content (Table 5). The trend was opposite with respect to leaf P. Increasing fruit size was associated with decreasing fruit P, Ca, Mg, and Mn but with increasing with fruit Cu, Zn and Al. Yellow ground color increased with increasing fruit P, Ca, Mg and Mn. In 1968, fruit K was negatively correlated with red color, r = -0.34*. In general, however, K levels were not correlated with fruit size or color.

In 1968, but not in 1969, fruit firmness at harvest was negatively correlated with leaf N, Ca, Zn and Al (r = -0.77**, -0.50**, -0.42**, -0.41**, respectively). In 1968, firmness increased with increasing fruit Ca levels (r = -.30*). Samples of fruit with more yellow ground color and more red color tended to lose firmness in storage more rapidly than less well colored fruit in 1968 (r = 0.47**, 0.52**, respectively). Core browning in 1968 decreased with increasing fruit firmness (r = -0.30*). In 1969, firmness at harvest was negatively correlated with internal breakdown, water core, fruit size, and leaf Mn (r = -0.36*, -0.30*, -0.41**, -0.35*, respectively). Firmness increased with increasing fruit K and leaf Zn and Al (r = 0.38**, 0.30*, and 0.35*, respectively).

TABLE 5. Highest significant correlations between fruit size, color in September and mineral content in June, 1968 survey (r values, d.f. 46).

Element	Fruit Characteristic					
	Diameter	Red Color	Ground Color	Diameter	Red Color	Ground Color
	June leaves			June fruit		
N	0.62**	-0.59**	-0.64**	n.s.	n.s.	n.s.
P	-0.78**	n.s.	0.60**	-0.68**	n.s.	0.47**
Na	0.74**	n.s.	n.s.	n.s.	n.s.	n.s.
Ca	0.59**	-0.58**	-0.57**	-0.39**	n.s.	0.43**
Mg	0.48**	n.s.	-0.51**	-0.56**	n.s.	0.34*
Cu	0.54**	-0.56**	-0.55**	0.34*	-0.47**	-0.31*
Al	0.60**	n.s.	-0.43**	0.61**	-0.66**	-0.66**
K	n.s.	n.s.	n.s.	-0.32*	n.s.	n.s.
Mn	n.s.	n.s.	n.s.	-0.36*	n.s.	0.34*
Zn	n.s.	n.s.	n.s.	0.65**	-0.37*	-0.46**
	July leaves			September fruit		
N	n.s.	n.s.	n.s.	0.34*	-0.40**	-0.49**
K	n.s.	n.s.	0.31*	n.s.	-0.29*	n.s.
	September leaves					
Cu	0.40**	-0.66**	-0.64**	n.s.	n.s.	n.s.

*Significant at the 5% level.
**Significant at the 1% level.

Breakdown and water core related to mineral elements.

Analysis of the cortical tissue of pairs of individual fruits of approximately equal size but one sound and the other with breakdown shows a tendency for fruit with breakdown to be lower in Ca, Fe, and Zn and higher in P, Mg, and B than sound fruit (Table 6).

TABLE 6. Mineral content of individual sound and breakdown fruit of similar size, each pair from the same tree.[a]

Element (%)	Sound	Breakdown	Element (ppm)	Sound	Breakdown
P	0.099	0.112	Fe	22.5	13.8
Ca	0.0381	0.0284	B	30.4	36.2
Mg	0.0249	0.0282	Zn	13.39	9.25

[a]Levels of N, K, Na, Mn, and Al showed no trend related to breakdown or sound fruit.

The highest correlation found between a mineral element and a physiological disorder was leaf manganese with water core in 1969 (Fig. 4). This relationship was primarily a result of high, 234-317 ppm, Mn levels in one orchard in 1969. The most consistent relationships were the negative correlations between fruit or leaf K and breakdown (Table 7) and water core (Fig. 5). In 1968, however, leaf K at the Graham Station was positively correlated with breakdown

Fig. 4. Correlation between water core and leaf Mn (ppm) from 1969 survey first harvest, September leaves. [**significant at the 1% level.]

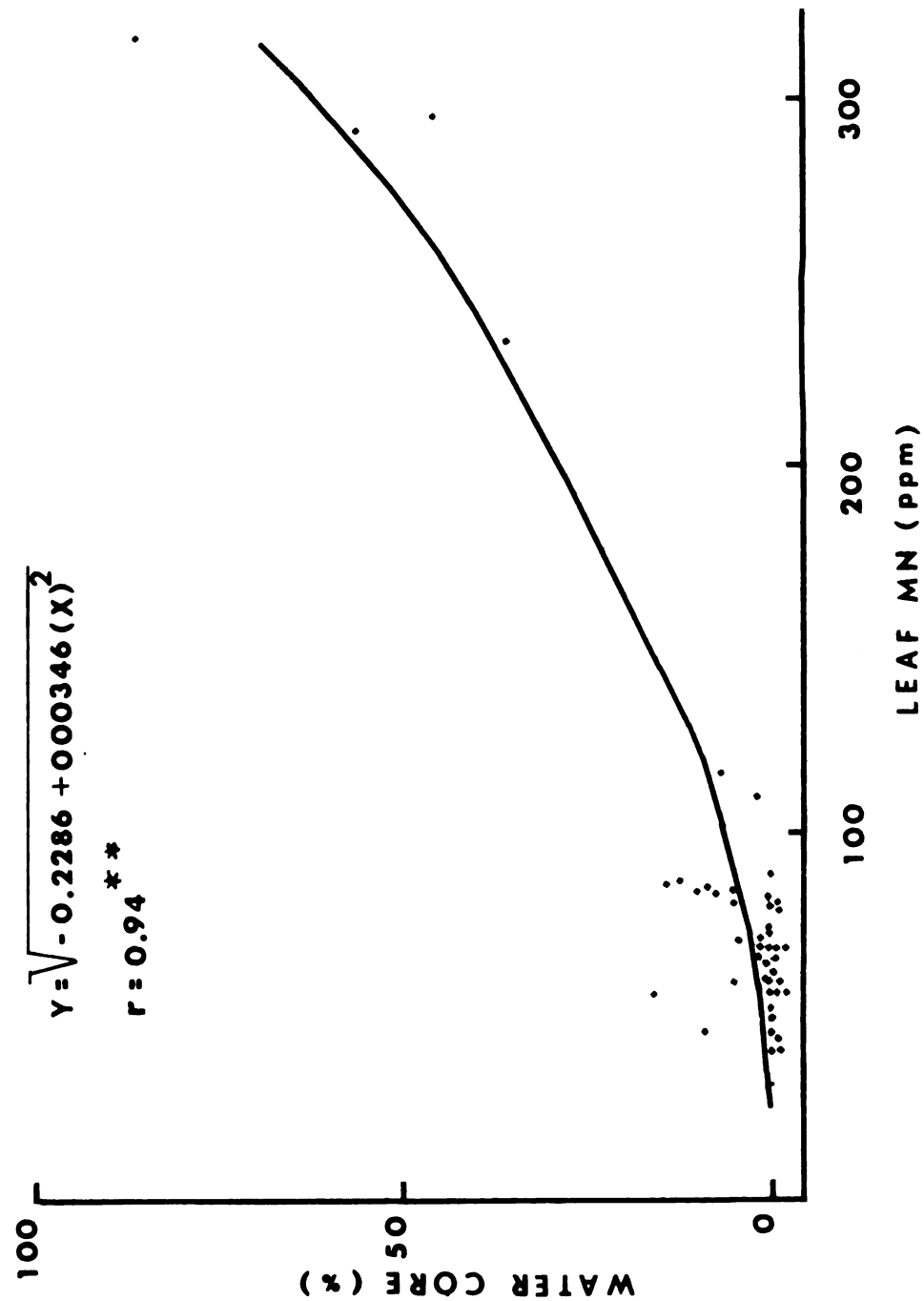

$Y=\sqrt{-0.2286+0.000346(X)^2}$
r = 0.94**
WATER CORE (%)
100
50
0
100
200
300
LEAF MN (ppm)

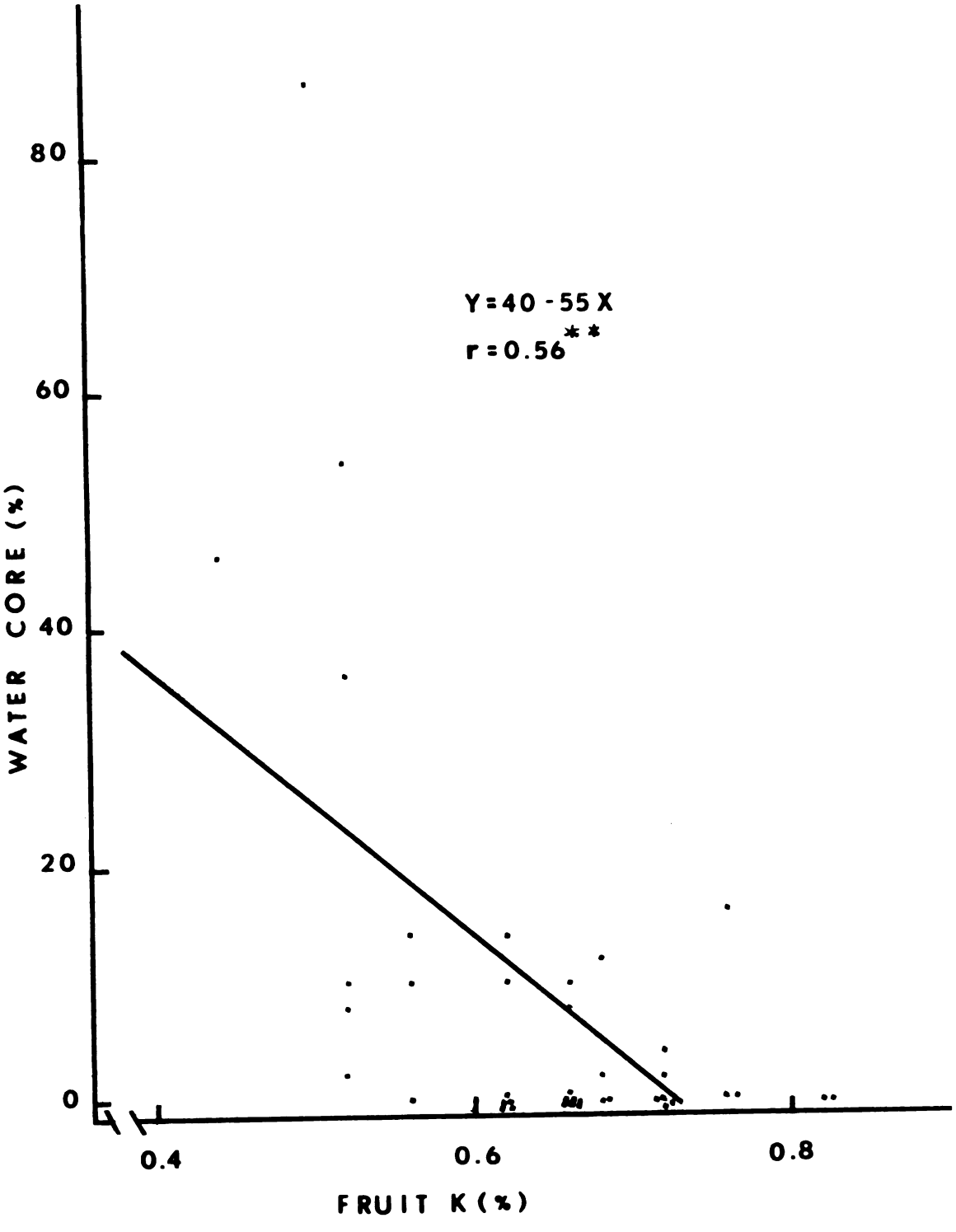
TABLE 7. Highest significant correlations between breakdown, water, core, and mineral elements, (r values).

Element	Survey 1968 d.f. 46			Survey 1969 d.f. 41		
	breakdown	water core	core browning	breakdown	water core	core browning
		Fruit			Fruit	
N	0.39**	n.s.	0.39**	0.36	n.s.	0.43
P	-0.36*	n.s.	-0.45**	-0.33	n.s.	n.s.
Na	n.s.	0.32*	n.s.	n.s.	n.s.	n.s.
K	-0.46**	-0.30*	-0.50**	-0.50**	-0.56**	n.s.
Ca	-0.39**	n.s.	-0.34*	-0.35*	n.s.	-0.40**
Mn	n.s.	n.s.	n.s.	0.34*	0.31*	n.s.
		Leaves			Leaves	
N	a	-0.37**	0.40**	a	n.s.	0.53**
P	-0.31*	n.s.	-0.46**	n.s.	n.s.	-0.40**
K	-0.37**	n.s.	-0.56**	-0.46**	-0.51**	-0.37*
Ca	-0.32*	-0.32*	n.s.	-0.32*	n.s.	n.s.
Mg	0.54**	n.s.	a	n.s.	n.s.	a
Cu	-0.31*	n.s.	n.s.	-0.34	n.s.	n.s.
Fe	-0.36*	n.s.	0.41**	n.s.	n.s.	n.s.
Zn	-0.31*	n.s.	n.s.	n.s.	n.s.	-0.31*
Mn	n.s.	n.s.	n.s.	0.80**	0.94**	n.s.
B	n.s.	n.s.	-0.29*	-0.49**	-0.46**	n.s.

*Significant at the 5% level.
**Significant at the 1% level.

[a]Correlations significant but with opposite signs in the two years.

Fig. 5. Correlation between water core from 1969 survey of first harvest, and K in September fruit. [**significant at the 1% level.]


Y=40 - 55 X
r = 0.56**
80
60
40
20
0
WATER CORE (%)
0.4
0.6
0.8
FRUIT K (%)

(Table 8). Both fruit and leaf Ca had a consistently negative correlation with breakdown in the fruit from the survey and the Graham Station. Correlations with other elements were less consistent, sometimes being significant with the opposite sign in the second year. At the Graham Station, both fruit and leaf Ca were negatively correlated with breakdown and water core (Table 8). Leaf Ca, but not fruit Ca, was negatively correlated with breakdown in 1969 (Table 8). Water core and breakdown increased with increasing leaf B but decreased with increasing fruit B. Multiple correlations between breakdown or water core and B and Ca (Table 9, 10) also show the opposite sign for leaf B, as opposed to fruit B.

In both 1968 and 1969, multiple correlations obtained using the least squares delete program showed fruit K and Ca to be the most closely negatively related elements to internal breakdown (Tables 10 and 11). The The incidence of breakdown (weighted for severity) in larger-than-average sized apples increased more rapidly with decreasing fruit Ca levels than did the incidence of breakdown (weighted for severity) in smaller-than-average sized fruit (Fig. 6). In 1968, fruit Mg and P were most closely positively associated with breakdown. The incidence of breakdown in fruit with higher-than-average Mg levels decreased more rapidly with increasing

TABLE 8. Highest significant correlations between breakdown, water core, core browning and mineral elements for fruit from the Graham Station (r values, d.f. 10).

Element	1968			1969	
	breakdown	water core	core browning	breakdown	water core
		Leaf			
N	n.s.	-0.81**	n.s.	n.s.	n.s.
P	n.s.	n.s.	n.s.	n.s.	0.62*
K	0.67*	n.s.	n.s.	n.s.	n.s.
Ca	-0.69*	-0.59*	-0.70**	-0.63*	n.s.
Mg	n.s.	n.s.	-0.70**	-0.70**	n.s.
B	0.66*	n.s.	n.s.	n.s.	0.70**
Fe	n.s.	n.s.	-0.61*	-0.61*	n.s.
Al	0.64*	n.s.	n.s.	n.s.	n.s.
Cu	n.s.	n.s.	n.s.	n.s.	-0.67*
		Fruit			
Ca	-0.60*	-0.71**	-0.65*	n.s.	n.s.
Mg	n.s.	-0.72**	-0.72**	-0.69*	n.s.
B	n.s.	-0.60*	n.s.	n.s.	n.s.
Fe	n.s.	n.s.	n.s.	-0.67*	n.s.
Al	n.s.	n.s.	n.s.	-0.69*	n.s.
Cu	n.s.	n.s.	n.s.	n.s.	-0.77**
Zn	n.s.	n.s.	-0.63*	n.s.	-0.63*

*Significant at the 5% level.
**Significant at the 1% level.

TABLE 9. Highly significant multiple correlations between water core and mineral content.

Variables		R Value
Dependent	Independents[a]	
	Fruit analysis, survey, 1969	
water core	- K + Cu	0.55
" "	- (log) K - (log) Ca + (log) Mn	0.71
" "	- (log) K - Ca + (log) Mn + (log) Cu	0.69
" "	- K - Ca - Al + Mn + Cu	0.80
" "	- (log) Ca - (log) Zn + (log) Mn	0.63
	Leaf analysis, survey, 1969	
water core	- N + Mn^2	0.95
	Graham Station 1968	
water core	- fruit B - leaf Ca	0.88

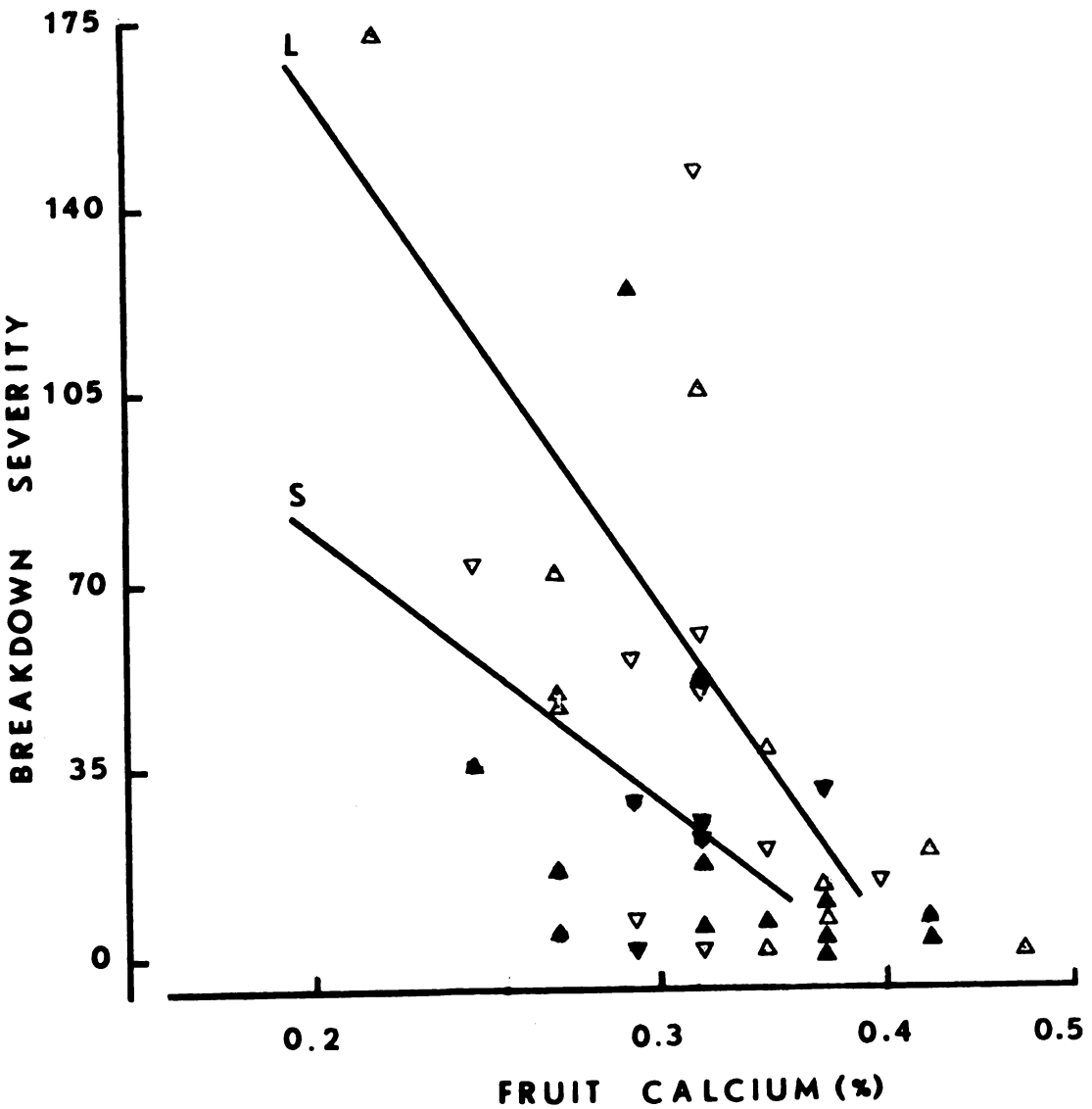
[a](-) or (+) indicates sign of partial correlation.

TABLE 10. Highly significant multiple correlations between breakdown (BD) and mineral content.

Variables		R Value
Dependent	Independents[a]	
	Survey, fruit, 1968	
BD severity	- K^2 + P^4	0.51
BD total	- (log) Ca + Mg^4	0.54
BD severity	- (log) Ca - crop	0.56
BD total	- (log) Ca - K^2 + Mg^4	0.60
BD total	- (log) Ca - K^2 + P^4	0.59
BD total	- (log) Ca - K^2 + Mg^4 + (log) B	0.65
BD CA storage	+ Mg - Cu + diameter	0.59
	Survey, fruit, 1969	
BD 1 st.harvest	- (log) Ca + (log) Mn	0.61
BD " "	+ Mn - B	0.58
BD " "	- (log) K - (log) Ca + (log) Mn	0.70
BD " "	- (log) K - (log) Ca + (log) Mn + (log) Cu	0.75
BD " "	- K - Ca - B + Mn	0.69
BD " "	- K - Ca - Al + Mn + Cu	0.80
BD second "	- Ca + Zn	0.54
	Survey, leaf, 1969	
BD 1st harvest	- N + Mn^2	0.84
BD " "	- (log) N - Mg^4 + Mn^2	0.89
BD " "	- N - Mg^3 - B + Mn^2	0.91
BD 2nd "	- (log) N - Mg^4 + Mn^2	0.58

*(-) or (+) indicate sign of partial correlation.

Fig. 6. Severity of internal breakdown as related to the Ca level of smaller-than-average (S, ▲) and larger than average (L, Δ) fruit in the 1968 survey, June samples.


175
140
105
70
35
0
BREAKDOWN SEVERITY
L
S
0.2
0.3
0.4
0.5
FRUIT CALCIUM (%)

fruit Ca levels than fruit with lower-than-average Mg levels (Fig. 7). Leaf Mn was most closely positively associated with breakdown in 1969. In 1968, there were no multiple correlations between breakdown and leaf analysis which met the selection criteria. In 1969, leaf N and Mg were most closely related with a negative sign (Table 10). The results with water core were similar except that fruit Zn and Al were negatively associated and fruit Mn and Cu were positively associated with water core (Table 9).

Core browning vs mineral elements.

While only negligible amounts of core browning developed in air storage, core browning in fruit stored in controlled atmospheres was extensive. The incidence of core browning decreased with increasing levels of fruit Ca, K, P, Zn and Mn (Table 8 and 12). Core browning decreased with increasing leaf K, B, P and Ca and increased with increasing leaf N, (Tables 7 and 8).

DISCUSSION

The large variations in the incidence of internal breakdown of 'Jonathan' between the seasons of 1968 and 1969 were anticipated since similar results were reported by Martin et al. (22).

Fig. 7. Percent breakdown in samples from the 1968 survey as related to the Ca level of fruit with (A, O) less than average and (B, ●) more than average Mg content.

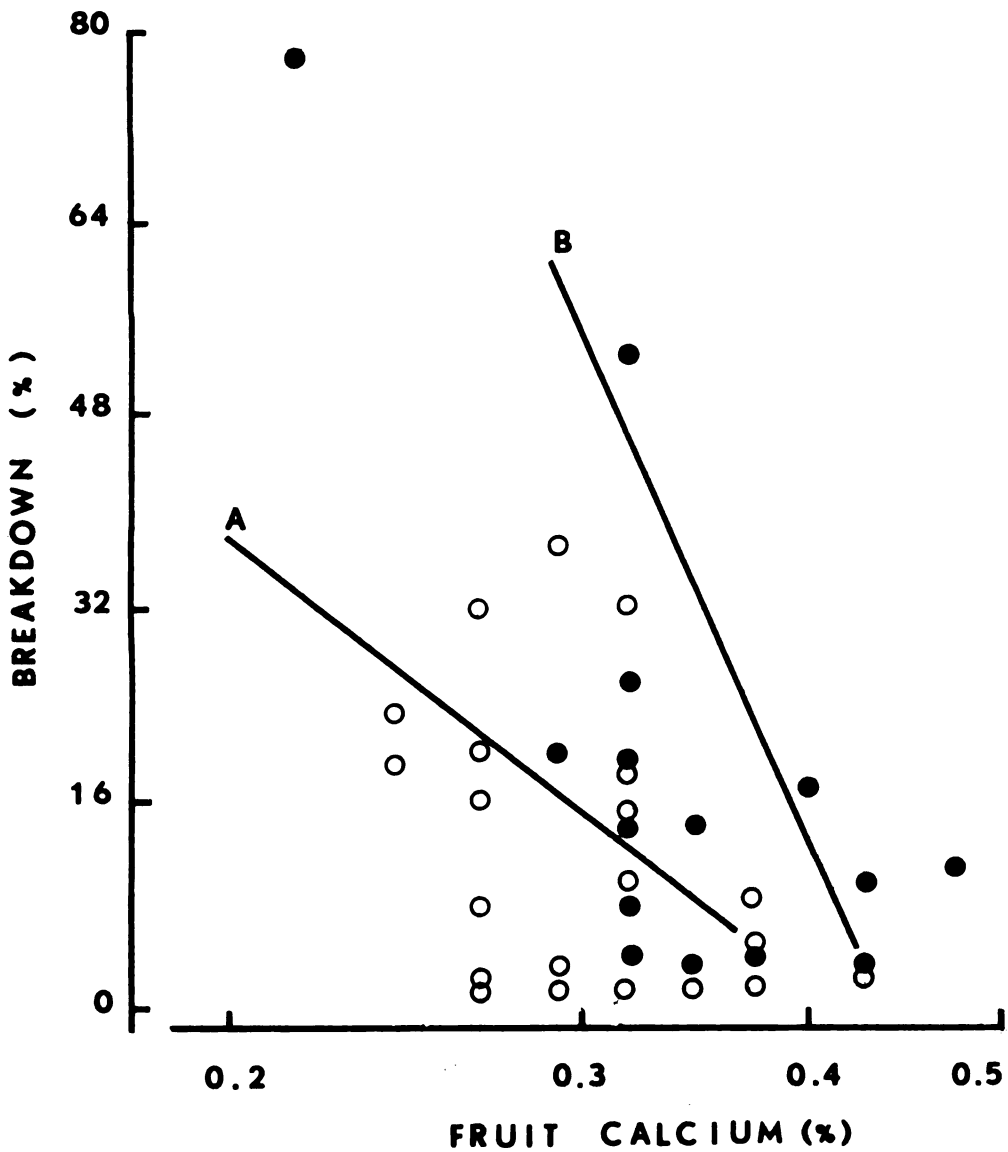

80
64
48
32
16
0
BREAKDOWN (%)
A
B
0.2
0.3
0.4
0.5
FRUIT CALCIUM (%)

TABLE 11. Highly significant multiple correlations between breakdown and mineral elements, Graham Station samples.

Variables		R Value
Dependent	Independents[a]	
1968 breakdown	+ fruit K - fruit Ca	0.73
1968 breakdown	+ leaf B - fruit Ca	0.86
1969 breakdown	- fruit Fe - russeting	0.82

[a]First order, - or + indicate sign of partial correlation.

TABLE 12. Highly significant multiple correlations between core browning and mineral elements.

Variables		R Value
Dependent	Independents[a]	
	1968 survey, fruit from CA storage	
core browning	- K + Fe + diameter	0.72
	1968 leaves from Graham Station	
core browning	- Ca - Fe	
	1968 survey, fruit from CA storage	
core browning	+ N = Mg + Mn	0.68

[a]First order, - or + indicate sign of partial correlation.

The negative correlation found between crop load and breakdown (Table 2) was in agreement with the results of Daley (7) and others (19, 38). Sharples (38) manipulated cropping levels of 'Cox's Orange Pippin' by thinning and found that senescent breakdown developed only in fruit from thinned trees. Cell number was increased slightly, whereas cell size was increased considerably. Also, he found that respiration rate was higher and climacteric advanced in thinned fruit.

The consistent relationship (Fig. 2, Table 4) between breakdown and large sized fruit has been reported by numerous workers (1, 20, 25). Martin (19) found that although breakdown of 'Jonathan' fruit was associated with large cell size, this was not due to lower protein content since protein synthesis tended to keep pace with cell enlargement.

The high correlation between water core and breakdown (Fig. 1) agreed with the results reported by Palmer (26) in 1931. He concluded that breakdown seldom, if ever, develops in 'Jonathan' which do not show water core at the time of harvest. Bramladge and Shipway (3) sorted intact Delicious apples spectrophotometrically using light transmittance measurements into 4 classes of water core intensity. Water core disappeared in storage. Internal breakdown developed only after disappearance of water core and to a greater extent than in fruits that never had water core.

The negative relationship between water loss and breakdown in fruit from the Graham Station was in agreement with the results of Scott and Roberts (37). Wills (45) further showed a relationship between water loss and loss of volatiles. The water loss was considered by Wills to be enhancing the production of acetate esters as well as providing a carrier for their removal from the fruit. In contrast to these results, breakdown incidence in fruit from the survey orchards was higher for fruit which lost the most weight in the room-temperature poststorage period, furthermore it was not significantly correlated to weight loss in storage.

Pieniazek (32) found that lenticular transpiration accounted for only 8-25% of total transpiration from apples, and that the layer of waxy coating on the surface of the skin was of greatest importance in diminishing water losses from apple fruits. Russeting, which tends to cause shriveling, may have reduced breakdown (Table 4) by favoring an increase in water loss. The fact that fruit from the Graham Station was much more russeted than fruit from the survey in 1969, may account for the greater percent weight loss in storage for Graham Station fruit (av. of 4.73%) than for fruit from the survey (av. of 3.08%). The relationship between weight loss in cold storage and breakdown seen in the Graham Station fruit was not observed in the survey fruit probably because the latter lost less weight.

Pieniazek (31) found that overmature apples transpire at a higher rate. This may account for the positive correlation between breakdown and weight loss during the room temperature period after storage since overmature fruit were also most subject to internal breakdown.

The negative correlations found between Ca and internal breakdown and water core (Tables 7, 8, 9, and 10) were consistent with many reports in the literature (24, 27, 29, 30, 39).

Ca sprays have been reported to reduce the incidence of internal breakdown (21, 28, 29, 34) and in other instances, to be of no effect (23). Five or 6 sprays were usually required in order to be effective. With 'Cox's', Schumacher and Fanhauser (35) found that KNO_3 sprays of -0.7%, $CaNO_3$-0.6%, or $CaCl_2$-0.7% reduced or eliminated flesh browning. Leaf injury was severe and occurred with all materials. Schumacher et al. (36) found that Ca sprays increased Ca in the central cortical zone relatively more than in the outer zones.

Perring (3) described internal watery patches in 'Cox's'. The Ca concentration in every apple affected by the disorder was low. With other elements there was no consistent trend. At these low levels of Ca, high concentrations of other elements appeared to be a contributing factor in rendering apples liable to disorders. Perring (27, 29) concluded that, with

'Cox's' fruit, Ca less than 3 mg/100 gm fresh weight lead to senescent breakdown. Less than 8 mg P/100 g had the same result even if the Ca level was high. Sharples (39) reported that breakdown was more severe in fruit with lower contents of Mg, P and Ca. Mason (24) grew Spartan apples in pots with silica sand and varied the Ca supply from 1/8 to full. Percent breakdown decreased markedly with increased Ca supply.

Kohl (14) found that Ca was high in the skin and core of apples, including 'Jonathan', but that it was much lower in cortical tissue. The tissue nearest the calyx end, which was usually the first area to show breakdown, had the lowest Ca content.

The negative relationship between fruit size and Ca content found in the 1968 survey (Table 5) agreed with the results of Martin et al. (22). Although the negative correlations (Tables 7, 9 and 10) between Ca in fruit and internal breakdown may in part reflect the negative relationship between breakdown and fruit size, a trend toward lower Ca content in fruit with breakdown was seen when size was held constant (Table 6).

Blossom-end-rot of tomato and internal breakdown of 'Jonathan' apples appear to be related to similar environmental conditions. Both may be induced by Ca deficiency in the fruit (8, 24). The incidence of both disorders has been reported to increase with increasing

leaf N levels (41, 42) and with larger leaf-to-fruit ratios (10, 38). In both disorders, water-soaked, translucent areas may appear in the fruit flesh before symptoms of the disorder become visible (7, 11). Extreme fluctuations in moisture supply may induce blossom-end-rot of tomato (9). By anology with blossom-end-rot of tomato, a hypothesis that fluctuations in moisture supply increase the incidence of internal breakdown in apple appears reasonable. However, no experimental evidence was found in the literature to support or refute this hypothesis.

Goor (11) found that the uptake of water by immersed tomato fruit tissue appeared to be greater for those of high Ca content than those low in Ca. A relationship between Ca content and water relations of a tissue would be expected since Ca has been associated with the functioning of membranes (41). Treatment with ether, according to Bukovac et al. (4), causes ^{45}Ca applied to leaf surfaces of Phaseolus vulgaris to become mobile; without ether treatment the Ca is immobile. Goor (11) found that ether treatment increased the incidence of blossom-end-rot of tomato. He, also, found that leakage of electrolytes, especially K, from chunks of tissue decreased with increasing Ca content. Leakage of electrolytes after removal of Ca ions with EDTA was shown by Steveninck (40) for disks of Beta vulgaris root tissue. The hypothesis that increased Ca would reduce internal breakdown of

'Jonathan' apples by maintaining a condition of normal permeability of cell membranes to water and electrolytes, appears to be reasonable according to the evidence cited above.

The cells become unusually turgid and sap fills the intercellular spaces in the water core disorder (15). This symptom suggests that the cell membranes are functioning abnormally with respect to permeability to water and solutes. The negative correlation between water core and Ca levels in fruit (Table 8) was consistent with the hypothesis that low Ca may be responsible for the loss of normal membrane functioning.

Ca deficiency has been associated with membrane damage in the apical cells of Ca-starved barley shoots by Marinos (18). The first indisputable signs of structural abnormalities appeared when the nuclear envelope and plasma and vacuolar membranes disintegrated and structureless areas appeared in the cells. This was followed by disorganization of other structures including mitochondria and golgi apparatus, while plastids were more persistent, although they eventually disintegrated. Marinos concluded that the effects of Ca deficiency on cell walls were probably secondary.

The positive correlations between leaf B and break-down and water core in the fruit from the Graham Station (Table 11) agreed with the results of Haller and Batjer (12).

They found that soil-applied B increased the incidence of 'Jonathan' breakdown in 3 consecutive years. In their 1941 trials, 4 pickings of 'Jonathan' were made and no appreciable breakdown in the untreated fruit occurred, whereas breakdown was severe in treated fruit and occurred even in fruit of the first picking that was immature from the standpoint of flavor and color. The breakdown they report may have been other than internal breakdown since it was described as springy and firm rather than mealy and soft. Yet Wilcox and Woodbridge (44) reported typical water core and flesh breakdown symptoms were increased by excess B, and Bramladge et al. (2) also found that B sprays induced water core which eventually led to breakdown and tissue softening.

B may be associated with the mobility of Ca. For example, corn plants supplied with ample B and Ca had high soluble Ca levels which were about 30% of total Ca (Marsh and Shive, 17). The addition of B to their treatment of no Ca tripled the amount of soluble Ca in the tissues. The highest B treatment resulted in more than one-half of the Ca being soluble.

Reeve and Shive (33) studied K-B and Ca-B interactions in tomatos grown in sand culture and found that for any given B concentration in the substrate, there was a progressive increase in the B content of the plants as the K concentration in the substrate was increased.

B toxicity decreased with increasing concentrations of Ca so that toxicity became negligible at the highest Ca level. Jones and Scarseth (13) have suggested that an upset of the Ca-B balance by a small intake of Ca, such as may occur on acid soils, will result in the plant having a very low tolerance for B. This suggestion that soil pH may have a role in the development of breakdown may be substantiated by the correlation of high Mn levels with breakdown in 1969 (Fig. 4). The association of high Mn content in tobacco, soybeans and cotton with low soil pH was reported by Tisdale and Nelson (43). High Mn, rather than directly increasing fruit breakdown, may simply reflect a soil condition that may be deleterious to fruit quality for other reasons.

The positive association found between fruit N and breakdown (Table 7) was in agreement with the results of Letham (16) who reported that fruit from 'Sturmer pippin' and 'Cox's' apple trees which received only N had the largest cells and the highest incidence of internal breakdown in storage. Tiller et al. (42) conducted an NPK fertilizer experiment in apples for 27 years. Increased internal breakdown occurred with N alone on 'Cox's', 'Jonathan' and 'Sturmer'. The harmful effects of N were largely offset by addition of PK, but apples from NPK trees did not store as well as fruit from unfertilized trees.

The negative correlation between breakdown, water core and K levels (Tables 7, 9 and 10; Fig. 5) were in agreement with the finding of Buneman et al. (5) in an earlier survey of 'Jonathan' orchards in Michigan. Taken together, the results of the 2 surveys suggest that the hypothesis that low K levels result in increased internal breakdown is sound and should be tested experimentally in Michigan. The negative relationship observed between K and breakdown was in agreement with Tiller et al. (42). Overly and Overholser (25) also reported that K used either alone or in combination with N and P reduced the percentage of breakdown in 'Jonathan'. Treatment with K, according to Perring (28) decreased the incidence of senescent breakdown in one experiment but not in another. The physiological role of K which may be related to internal breakdown is unknown.

CONCLUSIONS

The incidence of internal breakdown during the storage of 'Jonathan' apples is likely to be extensive if one or more of the following conditions exists:

(1) Large-sized fruit are grown on trees with a light crop.

(2) Harvest is delayed past the optimum stage of maturity for long-term storage.

(3) Leaf or fruit K levels are relatively low (or specifically below 1.0% in leaves in July or below 0.6% in fruit in September).

(4) Levels of Ca in fruit are relatively low (or specifically below 0.03% in September).

(5) Leaf Mn levels are unusually high (or specifically over 400 ppm in September).

(6) A high incidence of water core occurring in the fruit at the time of harvest.

Three hypotheses for remedial treatments to reduce the incidence of breakdown are suggested by the correlations which were found: (1) application of K would reduce the incidence of breakdown in an orchard with low levels of K in leaves; (2) applications of Ca would reduce the incidence of breakdown in an orchard with low levels of Ca in fruit; (3) correction of the conditions which resulted in unusually high leaf Mn levels in an orchard would reduce the incidence of breakdown among fruit from that orchard.

Since trees which produced fruit with a high incidence of internal breakdown in 1969 were not the same ones with the most breakdown in 1968, no orchard with a history of internal breakdown was located for future experimentation.

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CHAPTER II

INTERNAL BREAKDOWN OF 'JONATHAN' APPLE FRUIT IN RELATION TO POSITION ON THE TREE AND TIME OF HARVEST

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Abstract. Fruit samples were picked from the outer, middle and inner zones of large, mature 'Jonathan' apple trees from the northeast and southwest sectors on three dates. The incidence of internal breakdown of stored fruit increased with later maturity, and from inner to outer zones. No significant differences were observed due to tree sector. Fruit from the outer zone had significantly less K, P, Ca, Mg, Cu, and B than fruit from the innermost zone, but similar amounts of N.

Mature 'Jonathan' apple trees on seedling roots as typically grown in Michigan have a branch-spread of 30 to 40 feet. The fruit in the interior portions are partially shaded and never develop as much red color as fruit well-exposed to sunlight on the exterior portions

of the tree. It is possible that development and quality differences related to long-term keeping might distinguish the fruit from interior and exterior portions of the tree. Incidence of core flush and superficial scald of Sturmer apples has been reported by Jackson (4) to be higher in internally than on externally grown fruit, but variations in internal breakdown were not consistent. The incidence of internal breakdown of 'Jonathan' apples has been related for some time to advanced maturity (2). Little was found in the literature on the relationship of within-tree factors to internal breakdown of the fruit after harvest.

This experiment was conducted to determine if the tendency for some fruit to develop internal breakdown during or after storage is related to its position on the tree during development and growth.

MATERIALS AND METHODS

Six 40-year-old 'Jonathan' trees with a branch spread of about 35 feet were selected for the experiment. The trees were typical for well-maintained old 'Jonathan' orchards in Michigan. Each tree was divided for sampling purposes into southwest and northeast sectors and into outer, middle and inner zones. The outer zones penetrated no more than 2 feet inward from the outermost fruit. The middle zone extended from the outer zone to 6-8 feet from the vertical center-line of the main trunk. Fruits from this zone were partially shaded. The innermost zone was

in the shaded area near and above the trunk. Ladders were employed to pick the fruit sample approximately midway between lowest and highest parts of the tree whenever possible.

Samples were picked September 27, October 5 and October 12, except from the sixth replication (tree) which had been harvested by the grower prior to October 12. Samples of 25 fruit were picked at random. Ten extra fruit were selected for prestorage measurement of flesh firmness, ground color, water core and mineral analysis.

The samples were placed on molded fiber trays within unsealed polyethylene liners in fiberboard boxes. They were placed in air storage at 32°F immediately after picking. Later a controlled atmosphere of 3% O_2 and 5% CO_2 was established and maintained until April 10, 1969. All samples were removed from storage April 10, 1969 and held at 70° until May 1. Ground color was estimated by visual comparison with Ditton Laboratory colored cards, in which the lower numbers indicate a greenish color and the higher numbers an increase in yellow color. Ground color was estimated for each apple and the values averaged for the sample.

Flesh firmness was measured on peeled cheek areas 10 fruit, 1 puncture per fruit, using a U. C. pressure tester with a 7/16 inch tip and recorded as pounds. Water

core and breakdown were observed by slicing the fruit perpendicular to its axis at the calyx end and at the equator. Fruit samples collected September 27 were analyzed for P, Na, Ca, Mg, Mn, Fe, Cu, Zn, and Al with a photoelectric spectrometer. Samples were concentrated eight times normal to allow more accurate determination of the small quantities of minerals found in mature fruit. Nitrogen was determined by the Kjeldahl method and potassium by flame spectrophotometry. Fruit samples for mineral analysis included two slices taken from opposite sides of the fruit and including skin and core but without seeds. The slices were diced, mixed together, and a sub-sample of about 150 grams was taken for analysis. Samples were dried in an oven at 60° C, and ground in a Wiley mill.

RESULTS AND DISCUSSION

There were no significant differences between fruits taken from the southwest and northeast tree sectors for any of the variables measured.

The incidence of internal breakdown (Table 1) appeared to increase with later harvests. This observation was in agreement with the findings of several workers (1, 2, 3, 4). Breakdown incidence was higher for fruit of the outer zone and tended to decrease toward the inner zones on all dates, however, a significant difference occurred only for the last picking (October 12). Fruit size, as recorded by

TABLE 1. Mean percentage of 'Jonathan' apples with internal breakdown after storage according to date of harvest and location on the tree, 1968.

	Harvest date		
Zone	9/27	10/5	10/12
Outer	1.5 a	8.0 a	19.2 a
Middle	0.0 a	3.5 a	5.6 b
Inner	0.0 a	0.5 a	3.6 b

Note: Means followed by the same letter are not significantly different (P 0.05, Tukey's test).

TABLE 2. Mean weight (grams) of 'Jonathan' apples according to date of harvest and location on the tree.

	Harvest date		
Zone	9/27	10/5	10/2
Outer	116.3 a	124.3 a	120.2 a
Middle	109.5 a	118.1 ab	115.7 b
Inner	106.3 a	107.8 b	108.5 a

Note: Means followed by the same letter are not significantly different, (P 0.05, Tukey's test).

weight in Table 2, varied inversely to breakdown between zones, but the difference was statistically significant only on October 5. A higher incidence of breakdown with larger fruit has been reported by others (1, 2, 6).

Although red color was not evaluated numerically, it was observed that fruit from the outer zones had much more red coloration, than fruit from the inner zones. Usually less than one-third of the surface of fruit from the innermost zone was red while the outer fruit were more than three-fourths red. The most consistent effect observed was the change from green to yellow color which progressed from earlier to later harvest and outer to inner zones (Table 3). Flesh firmness (Table 4) tended to decrease with later harvest. On the first and second harvest dates, fruit from the inner zone was significantly softer than fruit from one or both other zones.

The larger size and more advanced stage of maturity of fruit from the outer zones indicated by its consistently more yellow ground color probably account for most of the differences in incidence of breakdown. Differences in mineral content may also contribute (Table 5). Perring (5) associated low fruit Ca with increased amounts of internal breakdown in 'Cox's Orange Pippin'. Tiller et al. (7) found that applications of K along with N and P to 'Jonathan' apple trees resulted in a lower incidence of internal breakdown in the fruit than where N was used alone.

TABLE 3. Ground color of 'Jonathan' apples (Ditton laboratory green-yellow color charts, 5 = green, 6 = greenish yellow) at harvest according to date of harvest and location on the tree.

	Harvest date		
Zone	9/27	10/5	10/12
Outer	5.70 a	6.27 a	6.81 a
Middle	5.38 ab	6.05 ab	6.64 ab
Inner	5.14 b	5.82 b	6.08 b

Note: Means followed by the same letter are not significantly different, (P 0.05, Tukey's test).

TABLE 4. Flesh firmness of 'Jonathan' apples (pounds) at harvest according to date of harvest and location on the tree.

	Harvest date		
Zone	9/27	10/5	10/12
Outer	15.5 a	15.4 a	14.3 a
Middle	15.1 b	15.0 ab	14.0 a
Inner	14.7 c	14.6 b	13.9 a

Note: Means followed by the same letter are not significantly different, (P 0.05, Tukey's test).

TABLE 5. Mineral content of 'Jonathan' apple fruits according to location on the tree.

Zone	Element			
	N%	K%	P%	Ca%
Outer	0.21 a	0.76 a	0.0958 a	0.0458 a
Middle	0.23 a	0.80 ab	0.0998 ab	0.0577 b
Inner	0.27 a	0.93 b	0.1133 b	0.0577 b

	Mg%	Cu ppm	B ppm
Outer	0.0404 a	3.400 a	15.45 a
Middle	0.0445 ab	3.825 ab	17.44 ab
Inner	0.0484 b	4.343 b	19.95 b

Note: Means followed by the same letter are not significantly different (P 0.05, Tukey's test).

The results of this study emphasize that in experiments where fruit samples are picked for storage or mineral analysis, sampling should be restricted to defined zones of foliage. It appears that, within the limited scope of this experiment, any sector of the tree may be sampled for these purposes.

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CHAPTER III

THE EFFECT OF 2-CHLOROETHYLPHOSPHONIC ACID (CEPA) ON RED COLOR, MATURITY AND INTERNAL BREAKDOWN OF 'JONARED' APPLES

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Abstract. The application of 250 ppm 2-chloroethylphosphonic acid to 'Jonared' apple trees on August 27, 1969 hastened the development of red skin color. The effect on red color was visible within 1 week of application, but with natural color development, treated fruits were indistinguishable from controls by October 3. Changes in ground color, flesh firmness, weight loss in storage and incidence of internal breakdown indicated that CEPA-treated fruit were more mature. CEPA did not affect fruit size. CEPA fruit could have been harvested commercially a week earlier before it had grown to a size which rendered it more susceptible to internal breakdown.

The incidence of the internal breakdown disorder of 'Jonathan' or 'Jonared' apples usually is greater in fruit of advanced maturity. The demand for considerable red coloration of fresh market fruit often results in the picking of over-mature fruit for storage. Often fruit which remain relatively small even at an advanced stage of maturity do not develop internal breakdown while larger fruit in the same lot may. Especially in years with light crops, when fruit tend to become large and thus susceptible to breakdown, it might be advantageous to stimulate earlier maturity. The fruit could then be harvested earlier before it becomes unusually large. Since ethylene has long been known to advance apple maturity (1), an experiment was conducted to determine if 2-chloroethylphosphonic acid (CEPA) would advance maturity and allow earlier harvest while the fruit was smaller.

MATERIALS AND METHODS

Eight trees of 'Jonared', an early-coloring mutant of 'Jonathan', were selected in a commercial orchard near Laingsburg, Michigan. The trees were 18 years old and bearing a heavy crop. The east half of each of four trees in a randomized-block design was sprayed with 250 ppm CEPA on August 27. The other trees were used as untreated controls. A commercial spreader-sticker, multifilm X77*

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*Colloidal Products Corp.

at 500 ppm, was included. On September 17, 10 ppm of the Na salt of NAA was sprayed on the CEPA-treated parts of trees in two replicates.

Samples of 25 fruit were picked in a random manner from the east half of each tree on September 15. One-bushel samples were picked on September 20, 27 and October 3. Ten-fruit subsamples within a size range of 2 1/2 to 2 3/4 inches diameter were weighed before and after storage to determine weight loss. Flesh firmness was measured on similar samples using a U. C. fruit pressure tester with a 7/16 inch tip and recorded as pounds. Under-color was estimated with the aid of Ditton Laboratory apple and pear color charts in which greenish, 4, to yellowish, 8, shades of color are numerically rated.

Samples were stored in air at 36-38°F, within 4 hours of harvest. They were removed from storage on December 9 and held in air at 70°F until December 22. Eight co-workers served as judges to rate the coded boxes of fruit from each harvest in order from least to most red color. Diameter, ground color and incidence of internal breakdown of 50-fruit subsamples were measured. The respiration rates of 15-fruit subsamples from the September 27 harvest were measured four times daily from September 29 to October 2 as net CO_2 evolution employing an infra-red detector.

RESULTS AND DISCUSSION

The CEPA fruit from the first and second harvests had more red color according to the judges ratings (Table 1). The difference between sprayed and unsprayed half-trees was quite obvious on September 4, one week following application of CEPA. The increase in red color occurred even though there was no influence on fruit size (Table 1). Edgerton and Greenlaugh (2) reported a reduction of fruit size due to CEPA sprays applied to 'Democrat' apples 10 days after full bloom. However, although the size of CEPA-treated fruit was reduced 2 weeks after application, they found no significant difference in fruit size at harvest time.

CEPA-treated fruit were estimated to have sufficient color for commercial harvest by September 27, one week before the control fruit were judged by the grower to have sufficient color for fresh market purposes. During the one week period between Sept. 27 and Oct. 3 the fruit averaged a 5 mm gain in diameter. This continued growth resulted in larger, more mature fruit which were more susceptible to internal breakdown.

All parameters of maturity measured, flesh firmness, under color and anthocyanin development, indicated that CEPA-treated fruit were more mature. Differences in pressure and under color were rather slight in contrast to the quite obvious difference in red over-color. These

TABLE 1. Effect of a 250 ppm foliar spray of CEPA on mean values of several characteristics of harvested 'Jonathan' apples.

Harvest Date	Control	CEPA	Level of Significance
	Size (diameter in mm, 50 fruits)		
9/20	59	59	n.s.
9/27	61	61	n.s.
10/3	66	63	n.s.
	Red color (ranking)[a]		
9/20	2.7	6.6	---
9/27	3.4	5.6	---
10/3	4.5	4.6	---
	Ground color (chart numbers)[b]		
9/15	4.35	5.00	.05
9/20	7.20	7.69	n.s.
9/27	7.57	7.89	n.s.
10/3	7.93	7.91	n.s.
	Flesh firmness (pounds)		
9/15	20.27	19.30	.05
9/20	17.54	17.11	.05
9/27	17.25	16.73	n.s.
10/3	17.19	17.11	n.s.
	Weight loss during storage (%)		
9/20	3.87	4.26	.10
9/27	3.10	3.92	.05
10/3	3.86	4.62	.10

[a] 1 = least, 8 = most.

[b] Low number = green; high = yellow.

findings were in agreement with the results of Allen (1), who treated 'Gravenstein' apples with ethylene after harvest. He observed a change in skin color from light green to greenish yellow, softening of the flesh, and an increase in reducing sugars and decrease in acidity.

Water core at harvest was not apparent until October 3. At this time, 80% of the subsamples from CEPA treatments had water core, whereas the controls had none. Likewise, with the exception of one fruit, internal breakdown was confined to the CEPA-treated fruit picked October 3. The extent of breakdown was small (5%), but all fruits were rather small, being 59-66 mm in diameter, and relatively little breakdown was expected.

Respiration rate ranged from 5.5 to 9.5 mg CO_2/Kg/hr with no indication of a climacteric. There was no significant difference in respiration due to treatment. This was surprising since ethylene applications usually increase the respiration rate and hastens the onset of the climacteric (4). A rise in respiration rate of CEPA-treated fruit may have occurred earlier, before the time of measurement. Later, the respiration rate of the control fruit may have risen to equal that of the treated fruit. This possibility seems likely since other aspects of maturity were not significantly different one week later.

Postharvest transpiration of water by apples has been reported to decrease with advancing maturity until optimum

maturity has been passed, when they again transpire at a more rapid rate (3). CEPA fruit (Table 1) appeared to lose more weight than controls whether picked slightly immature on September 20 or later. Water loss differences for fruit picked September 20 and October 3 were significant only at the 0.1 level. September 27 was considered to be the optimum picking date for storage; it was subsequently found that the treated and control samples followed the reported trend toward increased weight loss after this date.

An average of 9 fruits dropped from control half-trees as compared with 27 fruits from CEPA-sprayed trees between 9/27 and 10/3. Fruit drop was more severe in the week following application than in later weeks. Edgerton and Greenlaugh (2) also observed extensive thinning of apples after application of CEPA. Stimulation of preharvest drop could seriously limit the usefulness of this chemical, especially since there was no apparent counter influence of NAA. The oldest spur leaves also abscised following treatment.

The results suggest that CEPA treatment, especially of light-cropping trees, would allow earlier harvest of the fruit at a smaller size. The treated fruit in this experiment had sufficient red color to permit a one week earlier harvest without adverse effects on storage quality or the development of internal breakdown.

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CHAPTER IV

CALCIUM-RICH CRYSTALS IN APPLE TREES AND FRUIT

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Abstract. Single crystals and clusters of crystals or druses found by polarized light microscopy in tissues of Pyrus malus L. were found to contain Ca, O, and C using the electron microprobe. Crystals insoluble in 20% acetic acid were found in cells adjacent to the vascular tissues near the pedicel in mature fruit and in dormant flower buds, stems, petioles, shoot apex, roots and callus tissue. Deposition of Ca as crystals may restrict translocation of Ca into cortical cells of apple fruit which may result in an increased incidence of internal breakdown due to low Ca levels in those cells.

Molisch (9), in 1913, described precipitates of Ca as carbonate, phosphate, tartrate, sulfate and, most commonly, as oxalate. A positive relationship between oxalic acid concentration in the stem and susceptibility to a Ca-deficiency disorder in Nicotiana tabacum was described by

Brumagen and Hiatt (4). They suggested that high levels of oxalic acid in the upper stalks and young leaves of susceptible varieties apparently induce Ca deficiency by interfering with translocation and utilization of absorbed Ca.

Chang et al. (5) found that symptoms of a Ca-deficiency disorder in young tobacco leaves increased with temperature from 21 to 30°C. The Ca content of the stems increased as temperature increased, suggesting that Ca was immobilized in the stems at higher temperatures. A similar phenomenon with maize seedlings was observed by Walker (15). At 21°C soil temperature, leaves appeared normal. At each one-degree increment higher temperature, symptoms were more severe. The concentration of Ca in the maize shoots did not indicate a Ca deficiency level. Ca concentration in the top half of the blades of the youngest two leaves was in the deficiency range. Whether the immobilization of Ca in maize and tobacco was related to crystal formation was not made clear by either Chang et al. (5) or Walker (15).

Several authors have reported that calcium oxalate was a waste product which was neither dissolved nor re-used by the plant (3, 9). Even with Ca starvation, calcium oxalate crystals did not dissolve, according to Muller (10). Wardowski (16) and Scott (13) reported a tendency for the crystals to disappear in later stages of development of the plant. Scott (13) described, in Ricinus communis,

crystals suspended in protoplasmic strands in vacuolating cells. The larger druses are enclosed in a cellulose sheath and anchored to the cell wall by one or more hollow cellulose stalks. The mature solitary crystal was enclosed within a heavy cellulose sheath. In the mature, leafless lower stem, he reported, a few empty crystal sheaths were present and indicated the disappearance of druses, and starch grains developed within the former crystal idioblast. This disappearance of crystals was said to be very common in the inflorescence and fruiting axis.

Since Ca deficiency has been related to susceptibility to internal breakdown of the apple fruit (14), this study was undertaken to examine the formation of crystals as a possible cause of Ca deficiency in the cortex of this fruit.

METHODS AND MATERIALS

All apple tissues for cryostat sectioning were cut with a razor blade and immediately mounted and frozen in Optimum Cutting Temperature Compound (OCT -15 to -30°C, Fisher Scientific Company), according to the method of Rasmussen (11). Sections were cut with a cryostat at -20°C. They were dried in air and examined under polarized light. Individual crystals, or more often, clusters of crystals, which will be referred to as "druses," exhibited birefringence when the plane of

polarized light was rotated. It was more difficult to observe birefringence in druses because only the individual crystals of which they were composed changed from blue to yellow.

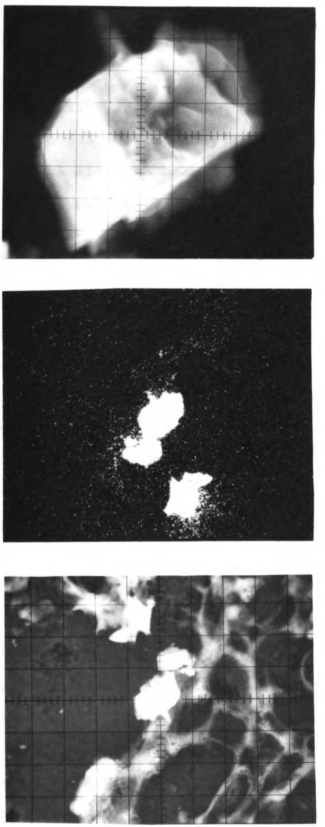
Crystals were extracted from short shoots on year-old seedlings by mascerating in a mortar and pestle with a few ml of water. Druses survived this treatment with no apparent change. Drops of material thus prepared were placed on slides and treated with 20% acetic or 0.1 N HCl.

The procedures employed in examination for crystals by microprobe are described by Rasmussen et al. (11). Sections for microprobe analysis were mounted on polished carbon discs. Identification of elements present in typical crystals was accomplished by scanning the x-ray spectrum emitted during bombardment of the crystal. Line profile analysis showed the relative quantities of various elements in different parts of the section. Oscillograms showed the distribution of a given element over the section.

RESULTS

Ca-rich crystals (Fig. 1) were found near the stem in a mature 'Jonathan' apple which had been in cold storage at 38°F for 4.5 months and exhibited severe internal breakdown. No crystals were found in the large cortical cells or in the epidermis.

Fig. 1. Crystals in small cells adjacent to vascular bundles near the pedicel of a mature apple with internal breakdown: left, reverse sample current oscillogram; middle, Ca x-ray distribution oscillogram; right, reverse sample current oscillogram.

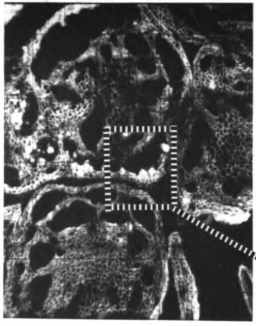

25 kV
500 X
25 kV
500X
25 kV
2,500 X

The x-ray spectrum was scanned while the beam rested on the crystal shown in Fig. 1, right. The crystal was extremely high in Ca. It contained about 8 - 10% O and about 10% C. Exact ratios could not be obtained because the counts fluctuated, probably due to changes caused by the heat of the electron beam. A relatively small amount of K was found, but it is not known whether this was part of the crystal or merely deposited on the surface. Scanning showed none of the following elements to be present: B, Na, Si, Mg, Zn, Al, Fe, Mn, Cu, P, S. Pb, Ni, Cr, V, Ti. Similar crystals found by polarized light microscopy were insoluble in 20% acetic acid.

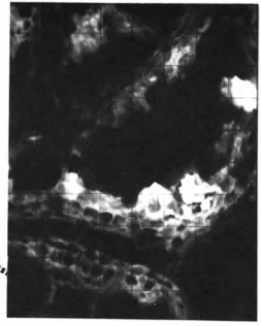
Crystals were found in a dormant apple bud (Fig. 2). Partial scans of these and other crystals corroborated the results of the first complete scan. Ca was the only metal found in large concentration.

Samples of the bark of 'Jonathan' apple trees of bearing age collected in January contained many crystals. Near the apex of the previous season's growth rectangular and druse crystals were found. These were located in rectangular cells between the sieve elements and the periderm, often in longitudinal rows (Fig. 3). The crystals were either druses from 9 to 14μ in diameter or rectangular, about 28 x 14μ. Bark from a limb about 5 years old sampled at the same time appeared to have fewer druses but many more rectangular crystals (Fig. 5). The rectangular

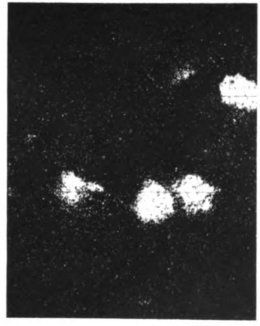
Fig. 2. Crystals in cross section of dormant apple flower bud: left, reverse sample current oscillogram showing part of two florets; middle, enlargement of center portion; right, Ca x-ray distribution.



25kV 128X



25kV 500X

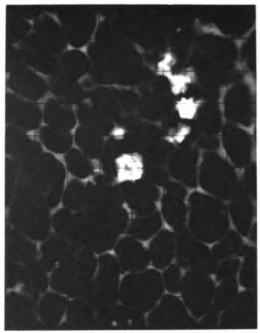


25kV 500X

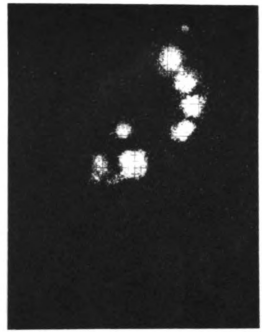
Fig. 3. Ca crystals in bark of apple shoots of the previous season's growth: left, reverse sample current oscillogram, periderm on left (P); middle, enlargement of right center portion; right, Ca x-ray distribution.

25 kV
128 X
25 kV
500X
25 kV
500 X

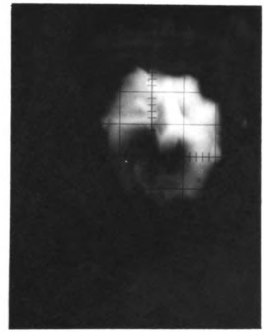
Fig. 4. Ca druses in bark at apex of apple seedling: left, reverse sample current oscillogram; middle, Ca x-ray distribution; right, a typical druse.



25 kV 500 X



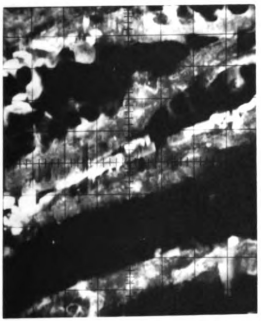
25 kV 500 X



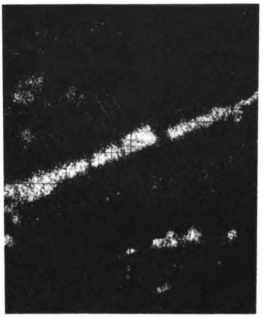
25 kV 2,500 X

Fig. 5. Ca crystals in bark from an old apple limb; left, reverse sample current oscillogram; right, Ca x-ray distribution.

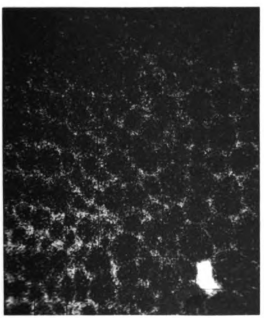
Fig. 6. Ca x-ray distribution in petiolar xylem (left) and phloem (right).



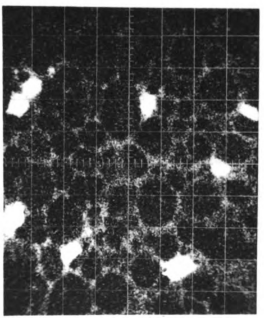
25kV 1,000X



25kV 1,000 X



25kV 500 X



25kV 500X

crystals, which measured about 22 x 6μ, were arranged in longitudinal rows within septate fibers. These were located very close to the cambium where numerous phloem rays were evident.

Druses (Fig. 4) were found in the region of elongation immediately below the apical meristem of a rapidly-growing apple seedling. Similar crystals were found in the petioles of both young and old leaves. Many crystals were found in petiolar phloem, but very few in petiolar xylem (Fig. 6). Druses occurred singularly or in rows in parenchyma cells between the sieve tubes and epidermis. Rectangular crystals apparently deposited in septate fibers were found in petiolar phloem. Petiolar phloem of a senescing leaf appeared to be virtually "choked" with rectangular crystals. Crystals also occurred in callus tissue and in old roots.

DISCUSSION

The much higher Ca content of stems and the core region of apple fruits in comparison with the flesh, as observed by Wilkinson and Perring (17, 18) may be due to the presence of crystals such as those which were observed deposited along the vascular bundles (Fig. 1). Deposition of Ca in this region may deprive the cells of the middle cortex of Ca.

Bark of mature Delicious apple trees, according to Batjer et al. (1) may contain as much as 4.0 to 4.5% Ca on

a dry weight basis. Much of this is probably deposited in septate fibers as described by Fahn (8). These fibers are characterized by the presence of internal septa and usually, of a living protoplast. Evert (7) described them as fiber sclereids with associated crystal-containing cells. Under polarized light, it was difficult to distinguish between fiber sclereids and crystal-containing cells. Probably deposition as crystals in the stem, under most conditions, simply removes excess Ca from the system. However, if environmental conditions stimulated formation of crystals beyond the normal rates, lateral transport and deposition could seriously inhibit acropetal transport of Ca.

Although a close correlation exists, as shown by Chandler (6), between Ca content and total oxalates in the leaves of many species of trees, it is not known whether Ca is absorbed as a result of oxalate formation or oxalate is formed in response to absorption of Ca. Richardson and Tolbert (12) found that the product, oxalic acid, inhibits an enzyme, glyoxylic acid oxidase, which is capable of catalyzing the synthesis of oxalic acid. Precipitation by Ca would remove this product inhibition, in which case oxalate would be formed in response to absorption of Ca. Beevers (2), however, found that oxalate synthesis may occur as a by-product of acetate utilization in the TCA cycle. It is not known which system is responsible for oxalate formation in apple fruits.

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CHAPTER V

THE INFLUENCE OF TRANSPIRATION AND PHLOEM TRANSPORT ON ACCUMULATION OF ^{45}Ca IN APPLE LEAVES AND IN TOMATO LEAVES AND FRUIT OF PLANTS GROWN IN SOLUTION CULTURE

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Abstract. Experiments were conducted to determine whether the accumulation of ^{45}Ca in apple seedlings (or rooted layers of apple) or tomato fruit is influenced by: (1) transpiration rate, (2) phloem transport, (3) kinetin applications, (4) age of leaf, and (5) length of one-year-old stem. ^{45}Ca accumulation in leaves increased with increasing rates of transpiration. The rate of ^{45}Ca accumulation in leaves was inversely related to the length of the stem.

Accumulation of Ca in tomato fruit increased with increasing transpiration rate of fruit relative to that of leaves. Although young leaves accumulated ^{45}Ca more

rapidly than old leaves, no difference in the rate of transpiration between young and old leaves was observed. More ^{45}Ca accumulated in mature leaves below rapidly-growing shoot tips than on pruned shoots. Cytokinins had no effect on translocation of ^{45}Ca into old leaves. Girdling experiments showed that translocation of Ca was in the phloem. It is proposed that Ca moves in the phloem and leaks into the sylem at increasing greater rates as it approaches the younger stem and growing apex.

The physiological disorders of apple fruit known as bitter pit and internal breakdown have been associated with low levels of Ca in the fruit (28). This fact has generated interest in the factors which affect the Ca levels of fruit. In the literature, the effect of transpiration (15) and of metabolic activity (9) on transport of Ca have been reported.

Koontz and Foote (15) found that Ca accumulation by leaves of Phaseolus vulgaris was not influenced by transpiration rate. They enclosed leaves in a plastic container. Transpiration differences were established by varying the air flow and shading the leaf. Air was first dried by passage through a $CaSO_4$ column. Transpiration was determined by trapping water in another $CaSO_4$ column and weighing.

Gerard and Hipp (10) reported that tomatos grown under lower evaporative conditions had a higher Ca content and lower K/Ca ratios than those grown under high evaporative conditions. They concluded that reduction in leaf transpiration enhances Ca movement into the fruit. Wiersum (37) found that ^{45}Ca entered only the extremely young tomato fruits. Since colored dyes failed to enter the older fruit, he concluded that only the young fruits transpired. When fruiting clusters were enclosed in polyethylene bags, the fruit absorbed much less Ca than unbagged fruit. Bagged fruit showed a high incidence of blossom-end rot, a Ca deficiency related disorder. Wiersum also reported experiments with fruiting branches cut from apple trees. These were held 3-4 days in a solution with ^{45}Ca. Some Ca did enter the fruit.

Bledsoe et al. (4) showed that the peanut fruit did not completely develop unless Ca was available in the fruiting medium. The Ca in the rooting medium was not sufficient. Radioactive Ca entered all parts of the top of the plant when applied to the rooting medium but only entered the young gynophore, not the mature or partly mature fruit. Wiersum (36) further showed that fast green dye in the transpiration stream does not enter the gynophores buried in the soil but does enter them when they

are exposed to air. He concluded that the xylem stream does not enter the gynophore when buried in the soil. Use of an artificial medium showed that Ca was the only requirement from the fruiting medium needed for gynophore development.

Ca may also move out of the fruits as shown by Ziegler (38) for Cucurbita maxima. He injected ^{45}Ca into fruit over the vascular bundle and found radioactivity in the stem 13 cm from the fruit 4 hours later. Movement of ^{45}Ca from apple fruits into adjacent leaves was reported by Martin (19).

Several workers (3, 20, 21) have reported that Ca translocation takes place exclusively in the xylem. Mason and Maskell (20) found no evidence that Ca moved either upward or downward via the bark. They girdled cotton plants and calculated the total amount of element above and below the girdle 24 hours later. Accumulation below and a decrease above the girdle would indicate upward movement via the bark. Their results showed a slight decrease in Ca both above and below the girdle. They repeated the experiment using periods of 48 to 52 hours during which they established that uptake had taken place. Again there was no sign of accumulation. In 1936, Mason et al. (21) reported more complicated girdling studies which further suggested a lack of phloem transport of Ca. Their experiment included two girdles with a section of

leafy stem between. The apical region of such plants had significantly less Ca than controls but the total content appeared to have increased slightly during the experiment. The basal region, even though ringed, had more than twice the gain in Ca when compared with non-ringed plants. The difference, however, was not statistically significant.

Heat-killing a centimeter section of the leaf petiole of Phaseolus vulgaris, according to Koontz and Foote (13), induced no change in the amount of water transpired or Ca deposited in the leaf.

Evidence that Ca is either absent from phloem sap or present in only trace amounts has contributed to the conclusion that Ca is phloem immobile (38). Lack of ^{45}Ca in the phloem sap from Cucurbita maxima was reported by Eschrigh et al. (7). After roots were dipped in a ^{45}Ca solution for 18 hours, phloem sap was collected from the apical half of the plant and xylem from the basal half. The xylem sap from the basal half did show activity. Young, functioning sieve cells showed ^{45}Ca only at sieve plates on callose. Only non-functioning cells had ^{45}Ca throughout. No ^{45}Ca was found in the cell walls of young phloem elements. Callose formation occurred in sieve cells after cutting the stem. This newly-formed callose absorbed Ca rapidly.

Sap from the sieve tubes of the willow, Salix viminalis, was obtained by Peel and Weatherly (27) using the mouth

parts of the willow aphid. No Ca was detected by analysis using flame emission spectrophotometry. The xylem of a willow segment 30 cm in length was perfused with a 100 ppm solution of Ca in further work by Hoad and Peel (13). Although uptake occurred, no Ca was found in the honeydew during the course of the experiment. Movement of K from the xylem to aphid style exudate was found.

Lauchli (17), using an electron microprobe, found Ca present in the lumen of sieve cells of the fruit of Pisum sativum. He concluded that Ca, since it was found in the lumen of sieve cells which were apparently functioning, was transported in both xylem and phloem of the fruit stem.

Studies of phloem exudate and parenchyma sap from mature trees of Fraxinus americana, F. pennsylvanica var. lanceolate and Platanus occidentalis by Moose (25) showed Ca in excess of 1 mg per cc. Sap was collected in August and September from a 2-inch slit in the trunk.

Phloem exudate from the inflorescence stalk of Yucca flaccida, according to Tammes and Van Die (34), contained Ca at 0.014 mg per ml. Only phloem sap was included since the xylem was under tension from the leaves below. Presence of Ca in phloem sap or phloem cells may, however, reflect deposition rather than transport.

In brussels sprouts, Millikan and Hanger (24) were able to identify the xylem with methyl blue introduced

through a leaf midrib flap and excise it with the unstained phloem. Appreciable concentrations of ^{45}Ca occurred in both the methyl blue stained xylem and the unstained phloem. Although within the first 10-20 minutes ^{45}Ca occurred in all vascular tissues including the xylem, the latter lost its original content with time, even though the dose was still being taken up by the midrib flap and moving down the petiole. They maintained that it was also apparent that internal moisture tension did not affect the pathway of movement of the isotope. They concluded that movement of ^{45}Ca in the stem occurred in the phloem as well as in the xylem.

A unique system which employed 2 β sensitive semi-conductor detectors was employed by Ringoet, et al. (31) to detect movement of ^{45}Ca applied to the surface of oat leaves. Downward movement occurred at high concentration (0.02 M $CaCl_2$) and was delayed in relation to upward movement. Upward and downward transport velocities of respectively 30-75 and 15-30 cm per hour were recorded. In a later paper (32) he concluded that the slow and quantitatively limited redistribution of Ca is most probably not due to inability to move in the phloem elements but to the great accumulation and absorption capacity for Ca of the various tissues. This conclusion is reinforced by the finding of Millikan and Hanger (22) that chelation enhanced the movement of foliar-applied ^{45}Ca.

Stout and Hoagland (33) studied the upward and lateral movement of radioactive isotopes of K, Na and P. They found that radioactive P passed upward through the xylem. Lateral movement across the cambium through living cells was so rapid that analysis of wood and bark, separated after the salt had moved up, showed the test material in both tissues. Only by separating the bark from the xylem by oiled paper were they able to demonstrate that conduction was taking place in the xylem.

That Ca does not recirculate in plants after its initial deposition has been cited as evidence against phloem transport (3). Circulation patterns for Ca in the bean plant were determined by radioautographic means from single aliquots of tracer administered to the roots during a one-hour period by Biddulph et al. (3). The ^{45}Ca did not recirculate following its initial delivery via the transpiration stream. Later Green (11) found some retranslocation of ^{45}Ca in bean under stress conditions.

Millikan and Hanger (23) reported redistribution of ^{45}Ca in Trifolium subterraneum and Antivihinum majus. For both normal and low Ca plants, 2 weeks growth in tracer-free solution resulted in a reduction of the mean ^{45}Ca concentration in both laminae and petioles of the old leaves, while the isotope appeared in new leaves produced during this period.

Kohl (16) and Wieneke and Benson (35) found that Ca applied to leaves of apple was not translocated to the fruit. Martin (19) reported that ^{45}Ca applied to the leaves after harvest moves back into the tree and reappears in the following season's leaves and fruit. Injected into the branch it tended to travel to developing leaves rather than to mature leaves. He concluded that applied Ca is quite mobile in apple trees. Ghosheh (12) reported that more than 30% of the Ca in woody tissues of apple moved into new growth.

That applied Ca tended to move into younger plant parts has been reported by several authors (9, 4, 23, 36). Norton (26) applied ^{45}Ca to the root system of the strawberry, Fragaria spp. It was absorbed and translocated to the youngest runner plants past older runner plants which absorbed relatively little. These results cannot be explained by differences in transpiration rate.

Faust (9), reported that kinetin and benzyladenine increased the movement of ^{45}Ca into mature leaves of apple seedlings when the isotope was applied to the roots. He suggested that movement of Ca from the vascular system to the parenchyma tissue of the leaf blade may be influenced by the metabolic activity of the expanding leaf. Cytokinins would then increase movement into mature leaves by increasing their rate of metabolism. In contrast with the findings of Faust are those of Enayat and Hofner (6) who found that

kinetin, applied to another part of a tobacco leaf, did not attract $^{86}RbCl$, $^{42}KNO_3$, $KH_2{}^{32}PO_4$, $^{59}FeCl_3$, $^{45}CaCl_2$, $^{65}ZnCl_2$, $Mn_2{}^{35}SO_4$, $^{60}CoCl_2$, or $^{54}MnCl_2$ when the radioactive salts were applied to the surface of the leaf. In addition, Quinlan and Weaver (29) found with Vitis vinifera that N-6 benzyl adenine (BA) was much more effective in stimulating movement of ^{14}C labeled assimilates into darkened leaves or into leaves which were not fully expanded. BA, applied without darkening the leaves, had little effect on ^{14}C imported into older leaves.

Evidence in the literature cited above does not clearly establish the effect of transpiration on Ca accumulation in apple. Furthermore, it does not establish the occurrence or lack of phloem transport of Ca in apple. Therefore, experiments to establish the influence of transpiration in apple and tomato and the occurrence or lack of phloem transport of Ca in apple were conducted.

MATERIALS AND METHODS

The experiments were conducted in a greenhouse using plants growing in solution culture. A nutrient solution used by Faust (9) was used throughout. Aeration was provided by forced air passing through aquarium stones. The nutrient solution was usually changed weekly. Plants were grown in gallon jars. Three liters of nutrient solution were placed in the jars at the start of an experiment. Deionized water was added, as needed, for the remainder of the week.

Either one-year-old apple seedlings, or rooted layers of 'Malling Merton 106' apple rootstock clone purchased from nurseries were used. One-year-old pear seedlings were used in one experiment. One experiment involved the use of tomato plants, 'Farthest North'.

Nursery trees 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 three or four days prior to use.

In all experiments, ^{45}Ca as $CaCl_2$ was applied to the solution at 40 μ Ci per 3 liters.

At the end of an experiment, the entire shoot from each seedling was harvested. For autoradiography it was placed in a plastic bag and held in a refrigerator up to several hours before the leaves were separated and taped to a blotter. Leaves were dried in a plant press for 5-7 days. Occasionally after autoradiography, the samples were ashed and counted. In other instances the leaves were harvested directly into paper bags and dried in a forced air oven. They were ground in a Wiley mill or by mortar and pestle, ashed in a muffle furnace and transferred to stainless steel planchets. The samples were counted with a TGC-14 Gieger tube and automatic scaler. Counts were corrected for self-absorbance using a curve obtained with apple leaf ash.

Trees were grouped in replicates by plant size. Randomized complete block designs were used throughout. Plots consisted of single trees or single branches. Whenever practical, all trees in a replicate were grown in the same solution culture jar.

Transpiration experiments. In a preliminary experiment, plastic bags were placed over the tips of apple seedlings. Wet paper towels were enclosed and the bag was tied tightly around the stem. Slits were cut near the top of the bags to facilitate gas exchange. Treatments were replicated 3 times. The experiment was conducted for 1 week in September. Samples including leaves in the bag, below the bag and lower and upper leaves on control plants were counted.

The effect of conditions in a polyethylene bag on Ca levels in tomato fruits and leaves was studied. The treatments were: (1) control in open air, (2) branch girdled by hot wax, (3) fruiting cluster in polyethylene bag, and (4) entire plant in polyethylene bag. A wet towel was placed in the bag and ventilation holes were cut. A single cluster with 1 or 2 fruit 6 mm or less in diameter was selected on each plant. All other fruiting clusters were removed. Fruits were nearly ripe after 20 days and were harvested for analysis. One leaf from each of 10 shoots constituted a sample. The first fully expanded leaf below the terminal was chosen. Leaves from plants with wax

girdles were not sampled. Ca content of fruit was determined by atomic absorption spectrophotometry. Leaf samples were analyzed spectrophotometrically.

In an attempt to vary only the vapor pressure deficit, another experiment using plastic bags was conducted. Rooted layers of 'MM 106' with new shoots 14-16 inches in length were used. An air flow through each bag of 2,000 ml per minute was maintained by means of a system combining capillary tubes and water barostats. Since the bags held approximately 6,000 ml, the air in the bags was exchanged every 3 minutes. Five plants were in bags through which humid air was passed. Dry air was passed through 5 more. Each plant was in a separate jar. Solution use during the course of the experiment was measured. The tops of the jars were covered with plastic to reduce evaporation and no water was added during the experiment. Air was humidified by first heating it on the greenhouse heating pipes and then passing it through two different water chambers. Air was dried by passing it over silica gel and then through a small refrigeration unit specifically designed for drying air. Temperature and relative humidity of the air in the bags was determined by pumping air out of the bags past dry and wet thermocouples using a small electric powered pump. Fine wire thermocouples were used to measure leaf temperature.

One potted plant in a polyethylene bag without air flow was maintained for comparison. Temperature and relative humidity were measured 3 or 4 times daily. Vapor pressure deficit calculations were based on air temperature rather than leaf temperature. After 8 days the leaves from each tree were harvested, weighed fresh, dried at 60°C, weighed and counted.

Girdling experiments. In all girdling experiments, a section of phloem 1.5 cm in length was cut from the one-year-old stem with a sharp knife, care being taken to do minimum damage to xylem tissues. The girdled area was covered with black plastic tape. Plants thus girdled usually appeared to grow normally. After 2 weeks, if the shoot above was not harvested, the girdles were usually callused over.

In Experiment 1, 8 apple seedlings with new growth 8-12 inches long were used. Four seedlings were girdled on August 2. The leaves were harvested 6 days later and dried for 6 days. X-ray film was then exposed to the leaves for 11 days. The leaf samples were then divided into those from lower and upper parts of the seedlings. Radioactivity was counted with a Geiger tube for 50 minutes per planchet on October 6.

In Experiment 2, 8 apple seedlings were girdled at 9 pm. Sixteen hours later the ^{45}Ca was added to the medium. All 8 control and 8 girdled trees were harvested

8 days later and the upper leaves prepared for autoradiography. After 12 days drying in a plant press, x-ray films were exposed to the specimens for 2 weeks. Slices of phloem and xylem from both above and below the girdle were included in the autoradiographs. The leaves used for the autoradiographs were ground and 500 mg were ashed and counted for 1 hour.

For Experiment 3, a group of one-year-old rooted layers of the apple rootstock clone 'MM 106' were used. When each stock had developed, a single shoot 8-10 inches long, they were sorted by size into 5 replicate groups of 4 trees each. Each replicate was grown in a single jar. Treatments were established as follows: (1) control, undisturbed plants; (2) girdled; (3) girdled and a fresh cut through the one-year rooted layer about 1/2 inch above the lower end, usually with removal of some roots; and (4) girdled between a lower and one or more upper shoots to provide leaves to feed the root system.

Samples of 500 mg of leaf ash were counted for 1 hour. Samples of phloem from treatments 1 and 2 were counted, also.

Cytokinin experiments. The first experiment was to determine if girdling would reduce or eliminate the accumulation of ^{45}Ca in a cytokinin-treated leaf spot. Treatments were: (1) none, (2) cytokinin spot, (3) girdle 0.5 cm long, (4) girdle plus cytokinin spot. Plots consisted of 1 shoot

on a plant with 2 shoots of equal length in the first 2 replicates. In the third replicate, plots consisted of single trees. Girdling consisted of severing and carefully removing a section of bark about 0.5 cm. long. In the first 2 replicates, the current season's shoots were girdled. A synthetic cytokinin from Shell Development Co., 6'-benzylamine-9-(tetrahydropyran 2-yl)9H - purine was mixed at the rate of 1 mg cytokinin per gram of lanolin. An area of about a cm^2 on the upper surface of mature leaves near the base of apple seedlings was covered. The experiment was started May 23rd and the tissues were harvested May 30.

Since lack of response to cytokinin in the first experiment may have been due to lack of penetration of the cuticle, a second experiment was undertaken in which an attempt was made to eliminate this difficulty. Mature leaves were taped to a board, underside up. Small plastic rings were attached with silicon rubber glue to form a well. A solution of kinetin (6-furfuryl amino purine), from Cal Biochem, in aqueous solution at 2.5×10^{-4}M, was poured into the wells and allowed to dry. Water was applied in the same manner to control plants. There were 3 replications. The results were evaluated by autoradiography.

The effect of stem length. By pruning the apple seedlings at different heights, trees with: (1) short [1-1 1/2 inches]; (2) medium [4-5 inches]; (3) long [7-8 inches], lengths of one-year-old wood were grown.

New growth was 10-12 inches long at the start of the experiment. A knife score through the phloem was made just below the new growth on half of the seedlings. A split-plot design was employed with scoring as the main plots and stem length as sub-plots. There were 8 replications, 18 trees in all, each in a separate jar of solution. Trees were grouped in replications according to size. The shortest trees had fewer, shorter roots than the taller trees. One-year-old and current xylem and phloem of 3 tall trees were dissected, ashed and counted separately. After 1 week, leaves were harvested and prepared for counting.

Influence of leaf age. That older leaves absorb less ^{45}Ca than upper leaves has been established (9). If transpiration is important in the distribution of ^{45}Ca one would, therefore, expect the younger leaves to transpire more rapidly. An experiment to determine whether young leaves transpire more rapidly than old leaves was conducted. Five pairs of trees of equal size were selected. Several of the oldest, small leaves at the bottom of the 'MM 106' rooted layers were removed. The shoot was then pruned just 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. Solution use over the next 3 days was measured.

Then one-half of the leaves were pruned off of each plant by either (a) removing upper leaves or (b) removing lower leaves. Water use was measured again after 3 days. Fresh weight, dry weight and surface area of leaves was measured. Water use per cm^2 leaf area, per fresh weight and per g dry weight was compared.

Effect of the growing shoot tip. If the growing tip of the shoot is competing with lower leaves for Ca, removing it should increase ^{45}Ca uptake in the lower leaves. An experiment to test this hypothesis was conducted using 4 pear and 2 apple trees. The stem above the highest fully-expanded leaf was marked. Then the shoots were adjusted to an equal number of leaves. The entire tip above the mark was pruned off of one of the shoots selected randomly. After 8 days, leaves below and above the mark were harvested, dried, weighed and counted.

RESULTS

Transpiration experiments. Conditions within the polyethylene bags strongly suppressed translocation of Ca in apple seedlings (Table 1, Fig. 1). The vapor pressure deficit between water in the leaf and air was probably the environmental factor most significantly altered by the treatment. Leaf temperature was probably several degrees higher during the day as was found in a later experiment (Fig. 2). Even though slits had been cut in the bags, CO_2 content of the air may have been reduced also.

Fig. 1. An autoradiograph showing restricted translocation of ^{45}Ca by conditions within a polyethylene bag compared with lower leaves outside the bag.


below
bag
within bag

Fig. 2. Leaf temperatures of trees in humid vs. dry air compared with air temperature; (a) air temperature in bag, (b) leaves in humid air, (c) leaves in dry air.

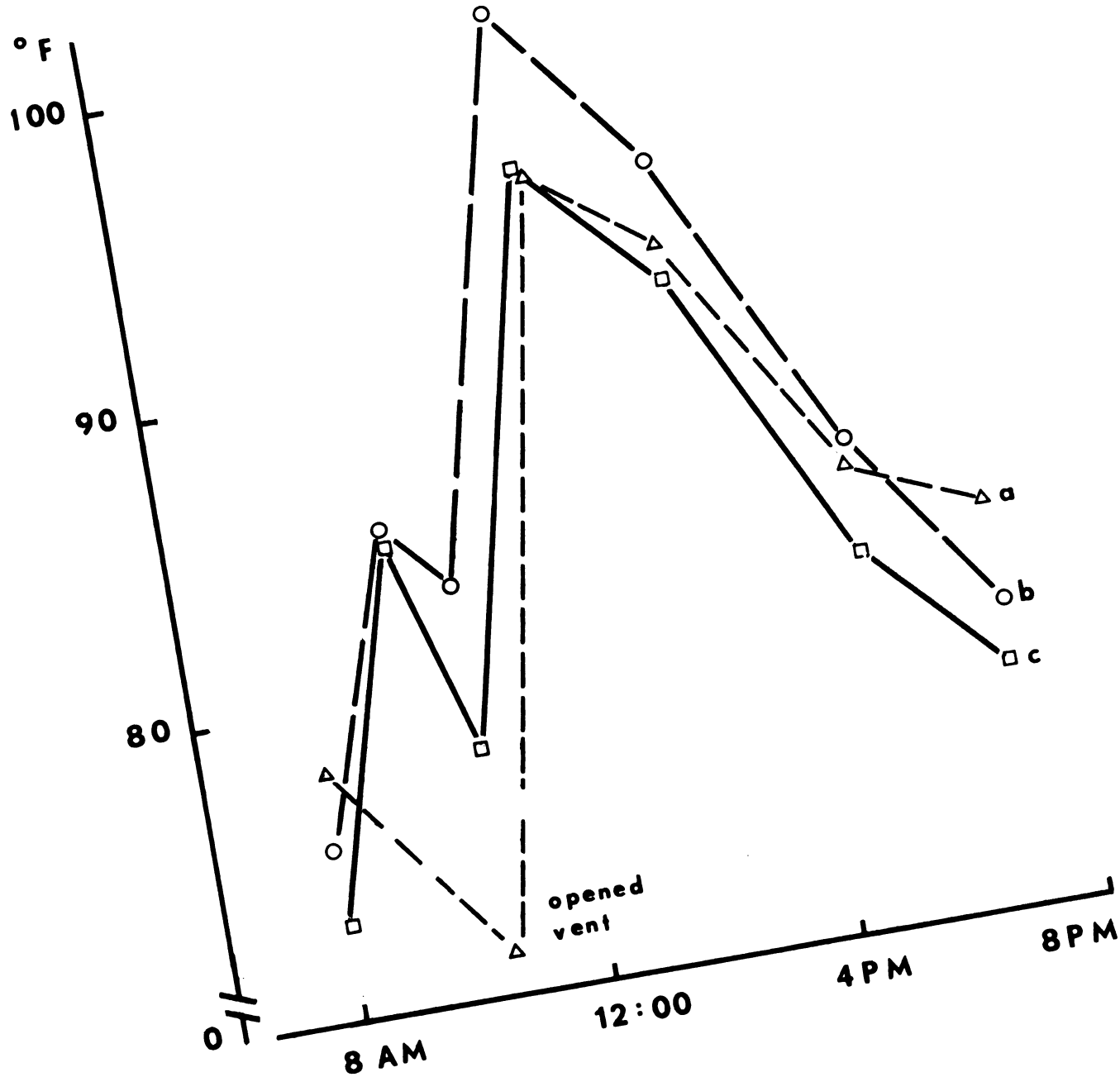

°F
100
90
80
0
8 AM
12:00
4PM
8PM
opened vent
a
b
c

TABLE 1. Effect of conditions within polyethylene bags on translocation of ^{45}Ca to leaves of apple seedlings (counts per minute per 100 mg dry weight of leaf).

Bagged Trees		Non-bagged controls
Within bag[a]	Below bag[b]	Upper leaves[a]
1.5	26.5	104.3

[a]Mean of 3 trees
[b]2 trees

A similar experiment with tomatos (Tables 2 and 3) showed that Ca accumulation by fruits was reduced by placing the fruiting cluster in a polyethylene bag. When the entire plant was in a polyethylene bag (Table 2), fruit Ca was not reduced in comparison with fruit from non-bagged control trees. Leaf Ca, on the other hand, was reduced by conditions in a polyethylene bag (Table 3). The leaves also contained less Mg, Fe, Zn, and Al.

TABLE 2. Effect of girdling and conditions in a polyethylene bag on Ca content (percent dry weight) of tomato fruits.

Treatment	Ca content
Entire plant in a plastic bag	0.2856 a
Untreated control	0.2102 ab
Fruiting cluster in a plastic bag	0.1192 bc
Wax girdle of branch	0.0704 c

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

These results suggest that a reduced transpiration rate of the fruit relative to that of leaves was a factor which, at least in part, reduced the Ca content of fruit.

The results of an experiment in which apple trees were grown in moving humid or dry air clearly indicated that accumulation of Ca in leaves increased with increasing transpiration rate of leaves (Fig. 3). Leaves in dry air transpired significantly more water than leaves in humid air (Table 4).

TABLE 3. Effect of conditions in a polyethylene bag on mineral element content of tomato leaves.

Treatment	Element				
	Ca(%)	Mg(%)	Fe(ppm)	Zn(ppm)	Al(ppm)
Control	4.11 a	1.19 a	205 a	47.5 a	325 a
Fruit in poly bag	3.21 ab	0.93 ab	136 ab	32.0 ab	221 ab
Entire plant in poly bag	2.71 b	0.70 b	127 b	29.9 b	193 b

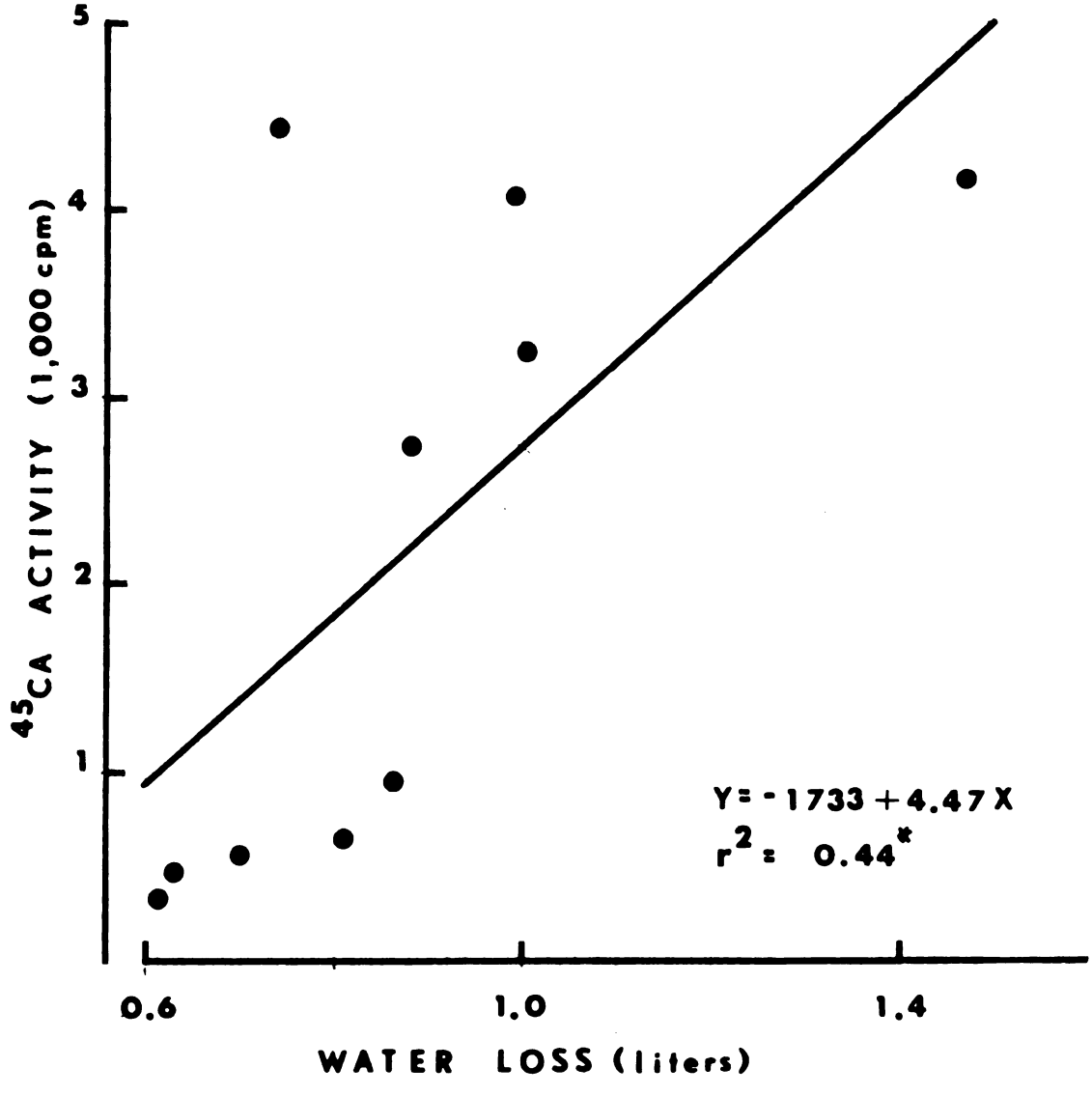
Means followed by the same letter are not significantly different (P 0.05, Tukey's test).

TABLE 4. Effect of vapor pressure deficit (VPD) on transpiration of apple trees in solution culture.

	Large VPD	Small VPD
Water loss (ml per g fresh weight of leaves	170.6	101.2*

Means significantly different (P 0.05, Tukey's test).

Fig. 3. Regression between ^{45}Ca accumulated in leaves and water loss by apple trees. [*significant at 5% level.]


5
4
3
2
1
^{45}CA ACTIVITY (1,000 cpm)
Y = -1733 + 4.47X
r^2 = 0.44*
0.6
1.0
1.4
WATER LOSS (liters)

That the contrast in humidity was probably not as great as in the bagging experiments without air flow was illustrated by the much higher vapor pressure differences in a tree bagged without air flow (Fig. 4) and the greenhouse air. The calculated difference in vapor pressure deficit between humid and dry air (Fig. 5) was statistically significant at each measurement period. The largest contrast occurred at midday. Even though the air flow was high, 2,000 ml per minute, and the air was thoroughly dried prior to entering the bag, transpiration by the plants was so rapid that the high VPD in the greenhouse could not be matched in the bags supplied with dry air. Vapor pressure deficits in the bags with humid air flow were usually as low as in the bag with no air flow.

Girdling experiments. In Experiment 1, 3 of the 4 apple seedlings with girdles on the old stem produced no autoradiograph image. A fourth showed faint traces of ^{45}Ca in the midribs of leaves. Counts of the leaf ash (Table 5) confirmed the results seen in the autoradiographs.

One of the girdled seedlings wilted during the course of the experiment indicating that the xylem had been damaged. Such damage undoubtedly would have caused wilting since daytime greenhouse temperatures often exceeded 80°F and reached a maximum of 94°F. Since the intensity of radioactivity in control leaves was rather low, another

Fig. 4. Difference in vapor pressure deficit between (a) bags with moving humid or dry air and (b) plant in still air in polyethylene bag and greenhouse.

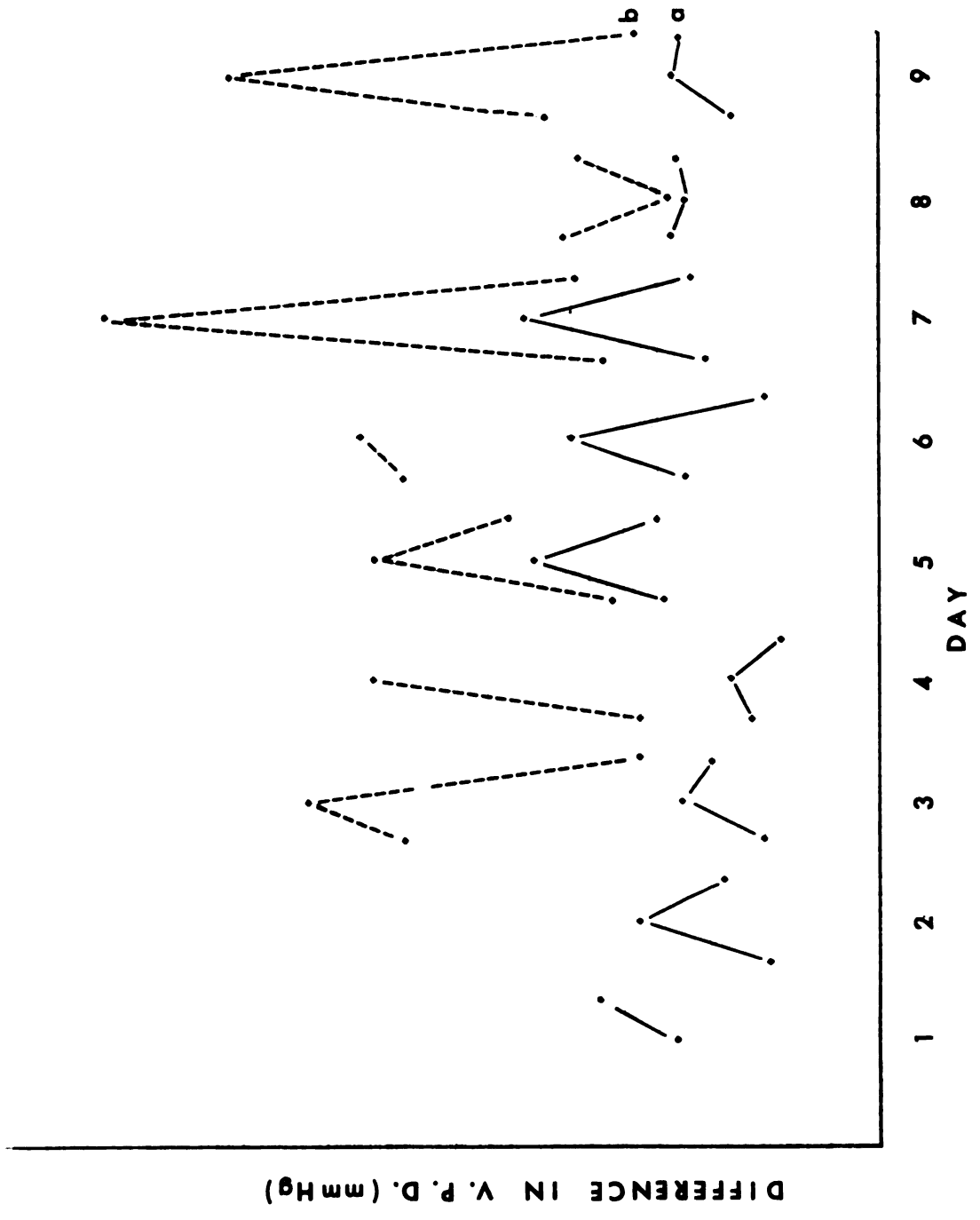
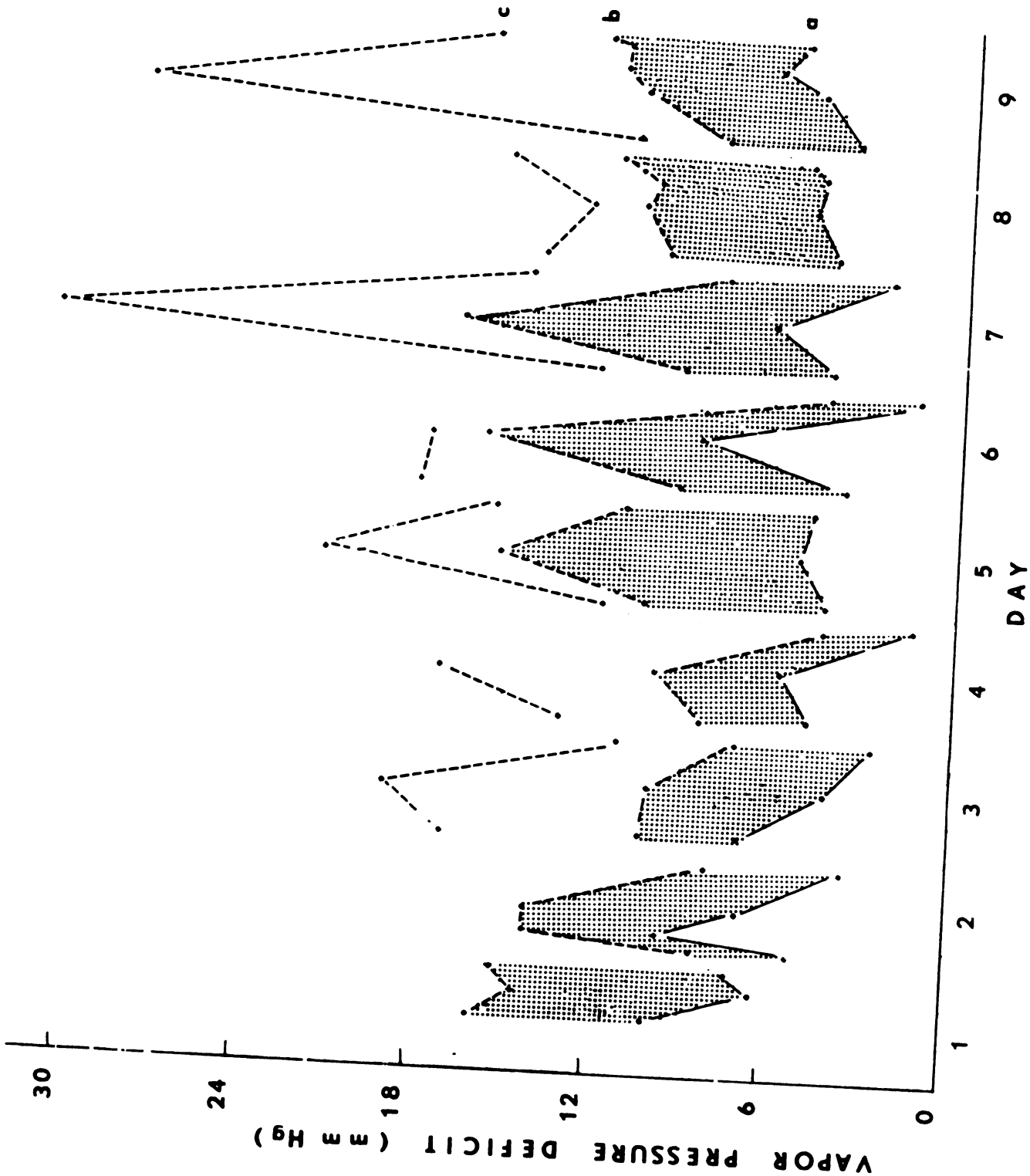

DIFFERENCE IN V. P. D. (mm Hg)
DAY
1
2
3
4
5
6
7
8
9
a
b

Fig. 5. Vapor pressure deficits in polyethylene bags with moving (a) humid air, (b) dry air and (c) in the greenhouse.


VAPOR PRESSURE DEFICIT (mm Hg)
0
6
12
18
24
30
DAY
1
2
3
4
5
6
7
8
9
a
b
c

experiment, which included twice as many trees, was conducted. The results (Table 5) were substantially the same.

TABLE 5. Effect of stem girdling on assimilation of ^{45}Ca by apple seedlings in solution culture (counts per minute per 100 mg dry weight of leaf).

Experiment 1 treatment	Leaves upper	lower	Experiment 2 leaves
Control	40*	30*	101*
Girdled	1	1	3

*Means significantly different (P 0.05, Tukey's test).

The autoradiographs from Experiment 2 (Fig. 6) show that the phloem below, and to a much more limited extent, above the girdle had absorbed ^{45}Ca. The xylem appeared to have little or no labeled Ca except along the outer edges. The roots of 3 of the girdled trees appeared damaged or dead at the conclusion of the experiment so these data were discarded. Roots on the other trees appeared to be healthy.

These experiments offer evidence that, in the old stem at least, transport of ^{45}Ca may be limited to tissues outside of the xylem, probably the phloem.

The questions of root starvation and damage to the xylem were answered in Experiment 3. In order to demonstrate that Ca would pass a girdle if it had access to the xylem, a fresh cut was made across the base of a

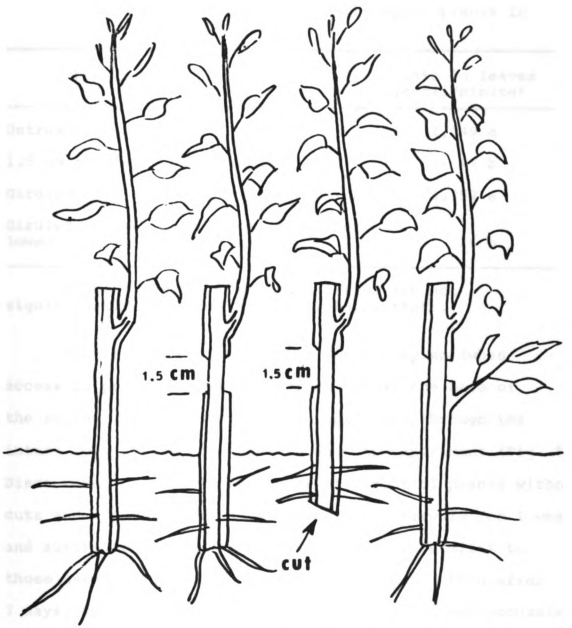
Fig. 6. Blockage of ^{45}Ca movement by a girdle in the one-year-old stem of an apple seedling as shown by the lack of an autoradiographic image of leaves from a girdled tree. Autoradiographs of slices of wood and bark from the one-year-old stem above and below the girdle show a lack of ^{45}Ca in the center of the xylem.

#10 GIRDLED UP GD
ABOVE
BELOW
#14 CONTROL UP GD

rooted layer under water. The layer was then quickly transferred to the nutrient solution. Strong radioactivity in the leaves would indicate that the girdle did not directly impede translocation of Ca in the xylem. The question of root starvation was answered by girdling between a lower and an upper shoot. The leaves on the lower shoot provided photosynthates for the roots (Fig. 7).

The question of damage to the xylem was answered through a fortuitous accident in Experment 3. On the fourth day, the temperature in the greenhouse suddenly increased to 95°F and possibly higher where it remained for several hours until the vents were opened. In replicates 1 and 3, all girdled trees wilted, indicating that the xylem had been damaged. The girdled and root-pruned trees in 4 of the 5 replications also wilted. Some other non-girdled trees which were not part of the experiment also wilted, thereby indicating the severity of the conditions. Since 3 of the 5 girdled trees showed no sign of wilting it would seem evident that the xylem was not seriously damaged. The data of this experiment (Table 6) show that ^{45}Ca was translocated in the xylem past the girdle in large amounts when access was provided through a fresh cut and that the reduced accumulation of ^{45}Ca in leaves above a girdle was not due to root starvation.

Fig. 7. Illustration of the treatment of rooted apple layers in an experiment to determine (1) if Ca would pass a girdle if it had access to the xylem and (2) if lack of translocation past a girdle was due to root starvation.



control girdled girdled and cut girdled with shoot below

TABLE 6. Effect of girdling, leaves below the girdle and cutting the rooted layer, on absorption and translocation of ^{45}Ca by apple plants in solution culture.

Treatment	Counts in leaves (total/minute)
Untreated control	1,049 a
1.5 cm girdle on past season's wood	47 b
Girdled, cut end in nutrient solution	4,392 a
Girdled between main shoot and a short lower shoot with a few leaves	36 b

Means followed by the same letter are not significantly different (P 0.05 Tukey's test).

That translocation of ^{45}Ca via the xylem (when access is provided through a fresh cut at the base of the rooted layer) was much more rapid than through the intact root system was shown in another experiment (Fig. 8). Discs were cut from lower leaves of 3 control plants without cuts and 3 plants with cuts at 24-hour intervals for 1 week and autoradiographed. The plants used were similar to those used in the third girdling experiment. Even after 7 days, the lower leaves of control trees did not accumulate as much ^{45}Ca as trees with a fresh cut.

Counts of phloem samples (Table 7) show that Ca did not accumulate in large amounts in phloem below the girdle. That Ca did move in the xylem at a reduced rate was indicated by the low counts in the phloem of the old stem

Fig. 8. Accumulation of ^{45}Ca in lower leaves of rooted apple layers: (a) intact control plants and (b) with a fresh cut at the base of the layer allowing access of the nutrient solution to the xylem. Leaf discs were punched at 24 hour intervals, ph = phloem sample.

a

b

a

b

a

b

above the girdle. These data suggest that girdling also reduced root absorption of ^{45}Ca.

TABLE 7. Effect of girdling on accumulation of ^{45}Ca in phloem of apple (counts per minute per 100 mg dry bark).

Treatment	Counts in phloem
Non-girdled controls	316
Below girdle	60
Old stem above girdle	29

Results of an experiment in which cellulase acetate film was slipped between phloem and xylem strips on either side of the one-year-old stem of 4 'MM106' rooted layers also indicated the occurrence of phloem transport of ^{45}Ca. Since the treatment could not be performed without damage to the phloem, 11 days were allowed for callus formation before the plants were transferred to the medium with ^{45}Ca for one week. In each of the 4 plants involved, one of the 2 phloem strips produced a strong autoradiograph (Fig. 9). However, the treatment induced callus formation which in itself might attract Ca.

That ^{45}Ca in the vascular system of the one-year-old stem of apple seedlings remained in a form which allowed subsequent movement into new growth after the seedling was transferred to non-radioactive medium and the main shoot was removed was shown by autoradiography (Fig. 10).

Fig. 9. Translocation of ^{45}Ca into strips of phloem of 'MM 106' apple layers separated from the xylem by cellulose film. The highest fully-expanded leaf was also autoradiographed to indicate that absorption and translocation had taken place in the plant. Left: leaves and phloem strips. Right: autoradiograph.

Harvested
3-19-70
Plant #1
Plant #2
Plant #3
Plant #4
Plant 1
2
3
4
Phloem strip

Fig. 10. Movement of ^{45}Ca from vascular tissues of the old stem of an apple seedling into regrowth after removal of the current season's shoot and transferral to non-radioactive medium.

The seedlings were grown in medium with ^{45}Ca for one week. The current-season shoot was completely removed and the one-year-old stem and root system were washed with non-radioactive medium before completing the transfer to non-radioactive medium.

In order to investigate retranslocation of foliar-applied ^{45}Ca, leaves were treated with approximately 2 μ Ci of $45Cacl_2$ on November 8. Subsequent leaf fall in the greenhouse continued for a period of almost 2 months. Autoradiographs of strips of bark collected after leaf fall (Fig. 11) show that some of the ^{45}Ca applied to leaf laminae moved out of the leaves during senescence. The phloem was not strongly radioactive, however. New growth in spring was not radioactive as determined by autoradiography.

Effect of stem length. An inverse relationship of the rate of accumulation of ^{45}Ca in leaves to the length of one-year-old stem is illustrated by the experiment which involved plants with short, medium and long lengths of old stem (Table 8).

Fig. 11. Autoradiograph of strips of phloem from an apple seedling showing the presence of ^{45}Ca which was translocated from the leaf lamina during leaf senescence.

TABLE 8. The effect of the length of one-year-old stem on the rate of translocation of ^{45}Ca to leaves of apple seedlings (counts per minute per 100 mg dry weight of leaf after 8 days).

Length of old stem above highest root, inches	Counts per minute	
	Non-scored	Scored
1.0 - 1.5	39 a	49 a
4 - 5	14 b	8 a
7 - 8	11 b	16 a

Means followed by the same letter are not significantly different (P 0.05 Tukey's test).

A comparison of the amount of ^{45}Ca accumulated in phloem and xylem in the plants with 7-8 inches of old stem shows that the phloem accumulated 5 times as much ^{45}Ca as the xylem (Table 9).

TABLE 9. Accumulation of ^{45}Ca by phloem and xylem of apple seedlings in solution culture (counts per minute per 100 mg dry tissue).

Tissue	One-year-old stem	Current season stem
Xylem	887	542
Phloem	4611	2761

The transpiration rates of apple plants with only young or old leaves were not significantly different (Table 10). The total amount of water transpired was related to leaf surface area (Fig. 12) in the expected manner.

TABLE 10. Transpiration rate by upper vs. lower leaves of apple.

Water use measurement	Plants with only: Upper leaves	Lower leaves
ml per cm^2 leaf area	2.275	2.808
% of rate before leaf removal	140	167
ml per g fresh weight of leaf	136.6	164.3
ml per g dry weight of leaf	466.5	579.4

*The differences are not significantly different.

The young leaves were typical of those which have absorbed large quantities of ^{45}Ca in previous experiments. That old leaves accumulate much less Ca than young leaves has been shown both by autoradiography (Fig. 13) and counts (Table 5).

Effect of the young growing shoot tip. Leaves below a rapidly-growing shoot tip accumulated significantly more ^{45}Ca than the same number of leaves on an adjacent shoot of the same plant from which the tip had been removed (Table 11). Therefore, it was concluded that the younger leaves do not compete with the older leaves below for the

Fig. 12. Correlation between leaf surface area per 'MM 106' apple layer and water used over a 3 day period. [*significant at the 5% level.]

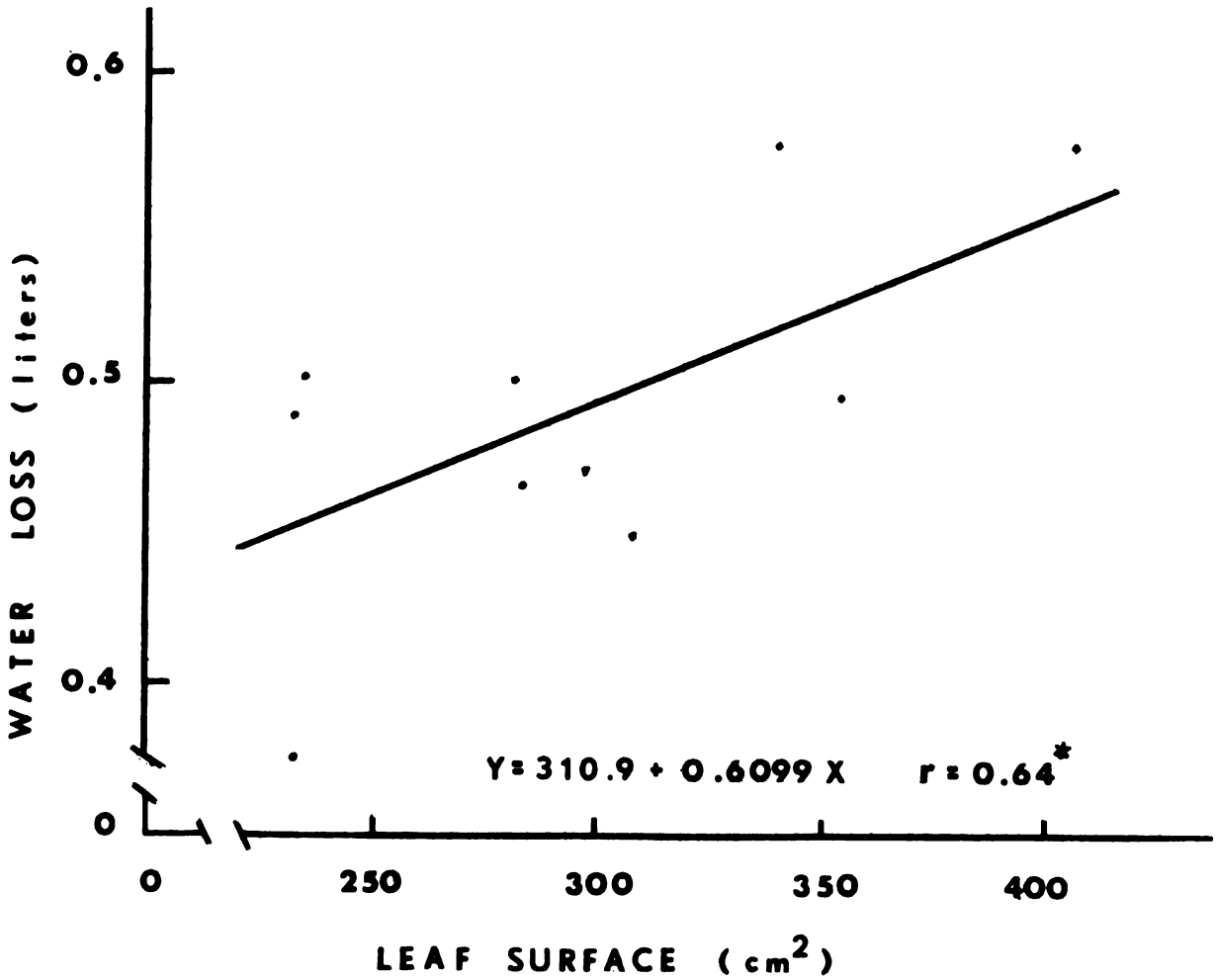
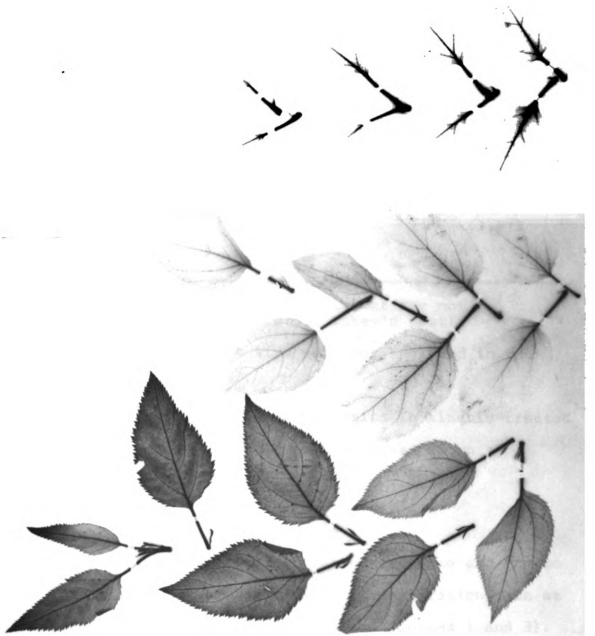

0.6
0.5
0.4
0
WATER LOSS (liters)
Y= 310.9 + 0.6099 X
r = 0.64*
0
250
300
350
400
LEAF SURFACE (cm2)

Fig. 13. Accumulation of ^{45}Ca by leaves of varying age on a single apple seedling. Left, young leaves; right, old leaves.



available supply of Ca. Leaves below a growing tip probably receive more ^{45}Ca simply because of the greater supply moving up in the phloem.

TABLE 11. The effect of the presence of a rapidly-growing shoot tip on ^{45}Ca absorption of fully-expanded leaves below (counts per minute per total dry weight of leaves). The results are for non-pruned and pruned shoots on the same plant.

Treatment		
Tip removed	Tip remaining	Shoot tip[1]
26,641	51,711	63,979
a	b	

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

[1]Counts from shoot tips were not included in the statistical analysis.

No tendency for ^{45}Ca accumulation in kinetin-treated spots on old leaves of apple seedlings was shown by autoradiography.

DISCUSSION

More ^{45}Ca accumulated in leaves of apple and tomato and tomato fruit at higher rates of transpiration than at lower rates (Tables, 1, 2, 3 and 4 and Figures 1 and 3). This is in agreement with the findings of Wiersum (36, 37) for tomato and peanut fruit, but is contrary to the results of Koontz and Foote (15) for leaves of Phaseolus vulgaris. The possibility exists that the latter workers did not

actually establish differential rates of transpiration, since different rates of air flow and shading were used to obtain the different rates. One must assume, although they gave no data on humidity, that the air had been (completely) dried by passage through a $CaSO_4$ column prior to entering the leaf chamber, otherwise regardless of the transpiration rate, water trapped from air leaving the leaf chamber would be proportional to flow rate.

Since the rate of ^{45}Ca accumulation in leaves of apple seedlings was greater in plants with short (1.0-1.5 inch), old stems, as opposed to long (4-5 or 7-8 inch) old stems (Table 8), it is believed that the rate of translocation as well as root absorption affected the amount of ^{45}Ca accumulated in leaves. If ^{45}Ca moved freely in the transpiration stream, one would not expect to find much difference in rate of ^{45}Ca accumulation between seedlings with 1.0-1.5 inches of old stem as compared with seedlings with 4-5 inches of old stem. However, if Ca moves upward through the xylem only by exchange, as proposed by Bell and Biddulph (2) and supported by Jacoby (14) and Faust (9), stem length would be an important limiting factor. If Ca moves primarily through the phloem, the length of stem would be a limiting factor since the cells adjacent to the phloem accumulate Ca crystals (8).

Since girdling the one-year-old stem of apple seedlings and layers severely restricted accumulation of ^{45}Ca in leaves (Tables 5 and 6 and Fig. 6), it is concluded that ^{45}Ca is translocated primarily in the phloem of the one-year-old apple stems. These results do not agree with those of Mason and Maskell (21, 21) with cotton and Koontz and Foote with Phaseolus vulgaris (15). A girdling experiment can indicate acropetal transport of Ca in phloem only if the girdle severely restricts accumulation of Ca above the girdle. Phloem might be a normal pathway of translocation of Ca in some plants in which girdling does not severely restrict translocation of Ca. Girdling such plants would not stop translocation but only force lateral transport into the xylem stream. One would expect that transport of Ca in both phloem and xylem would normally occur in such plants.

Since the experiment with apple layers with a fresh cut in the nutrient solution (Fig. 8) showed that ^{45}Ca transport in the xylem is rapid, it is concluded that lateral transport from phloem into xylem of one-year-old stems was limited. Otherwise ^{45}Ca would have by-passed the phloem girdle and entered the xylem in much greater quantities.

Increasing absorption of ^{45}Ca by roots with increasing water use by leaves probably accounts for some of the increased ^{45}Ca accumulation in leaves shown in

Fig. 3. Since leaves below a polyethylene bag accumulated more ^{45}Ca than leaves in the bag (Table 1, Fig. 1), it is concluded that restricting transpiration rates restricts the rate of translocation as well as absorption.

The fact that the rate of accumulation of ^{45}Ca increases with increasing transpiration rate appears to contradict the phloem transport hypothesis. Transpiration rate is generally thought not to influence the rate of acropetal translocation in the phloem. Increasing rates of transpiration may increase the rate of translocation of ^{45}Ca by increasing the rate of movement from phloem to xylem, particularly in the current-season's growth. The failure of the 0.5 cm girdle on current season shoots in the first kinetin experiment to severely restrict ^{45}Ca accumulation in leaves above suggests that ^{45}Ca moves more readily from phloem to xylem in the current season's growth. When the phloem is fully turgid and the transpiration rate is relatively low, as under conditions of low VPD, very little lateral transport may take place. As the transpiration rate increases, the relative concentration of Ca in phloem may increase and consequently Ca may move into the transpiration stream more rapidly. Lateral transport through living ray cells and possibly through the cambium, with subsequent leakage into tracheids would logically be expected to be a function of concentration. This process might be more rapid toward the shoot tip. The restricted movement of ^{45}Ca into old leaves is consistent with this

hypothesis. Once in the xylem, the movement of Ca into old leaves is unrestricted (Fig. 8).

The results of the girdling experiment are in agreement with the findings of Martin (19) who reported that ^{45}Ca appeared to move exclusively in the phloem of apple trees.

Most of the Ca in an apple is absorbed early in the season, according to Kohl (16). This may be due to lateral transport from the phloem into the young xylem of the cluster base and stem. Later, as the xylem matures, lateral transport may be restricted. The phloem, as described by Evert (8), is also more active early in the season than later. Translocation may be hindered by deposition of crystals in the phloem as suggested by Lauchlii (17).

Although the evidence clearly indicates that translocation of ^{45}Ca in apple stems increases with increasing transpiration rate, whether this is primarily due to an increased rate of movement in the xylem or to an increased rate of transfer from phloem to xylem remains to be resolved. The evidence clearly indicates that in the one-year-old apple stem, ^{45}Ca moves primarily in the phloem rather than in the xylem.

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APPENDIX

TABLE A-1. Mineral content of Jonathan leaves (July samples).

Element	Survey 1968 Mean	Survey 1968 Standard deviations	Survey 1969 Mean	Survey 1969 Standard deviations
N (%)	1.89	0.22	1.99	0.16
P (%)	0.26	0.08	0.31	0.11
K (%)	1.17	0.24	1.29	0.22
Ca (%)	1.46	0.19	1.90	0.20
Mg (%)	0.40	0.03	0.24	0.04
Mn ppm	56.5	43.5	91	75
B ppm	33.0	4.6	42	5
Fe ppm	147	24	138	25
Cu ppm	12.5	4.9	18.6	5.2
Zn ppm	15.0	3.8	26.5	10.3
Al ppm	442	153	340	128

	Graham Station 1968		Graham Station 1969	
N (%)	1.76	0.20	1.86	0.19
P (%)	0.184	0.022	0.222	0.030
K (%)	1.23	0.13	1.30	0.17
Ca (%)	1.69	0.22	1.30	0.18
Mg (%)	0.34	0.05	0.41	0.07
Mn (ppm)	67.9	12.4	56.7	8.1
B (ppm)	32.7	1.3	35.8	3.2
Fe (ppm)	263	23	248	42
Cu (ppm)	11.9	3.2	13.3	1.8
Zn (ppm)	24.6	2.8	10.1	2.1
Al (ppm)	562	83	154	23

TABLE A-2. Mineral content of leaves (July samples) and incidence of water core and internal breakdown in fruit after storage, 1969 survey.

Orchard	Tree	Internal Breakdown[a]	Water Core[b]	N (%)	K (%)	Mn (ppm)	B (ppm)
1	1	10	6	2.04	1.32	62	47.2
	2	1	0	2.02	1.32	70	49.0
	3	7	5	1.90	1.32	95	52.7
	4	3	0	1.90	1.56	45	41.7
2	1	0	0	1.92	1.42	73	39.2
	2	0	0	1.78	1.42	51	35.4
	3	0	0	1.82	1.28	103	47.8
	4	1	0	1.90	1.46	68	41.0
3	1	47	38	1.94	0.80	366	34.1
	2	89	87	1.86	0.86	268	32.9
	3	57	50	2.02	0.96	328	35.4
	4	60	38	1.80	1.12	270	32.9
4	1	32	9	1.78	1.80	84	47.8
	2	1	0	1.92	1.80	81	44.1
	3	1	1	2.00	1.66	79	41.7
	4	2	1	2.00	1.24	56	34.8
5	1.	4	1	1.96	1.20	73	41.0
	2	0	0	2.26	1.32	79	41.7
	3	20	12	1.80	1.32	59	42.3
	4	0	0	2.04	1.32	51	40.4
6	1	3	1	2.14	1.38	135	42.9
	2	0	0	2.30	1.12	79	43.5
	3	2	0	2.42	1.04	56	39.2
	4	0	0	2.18	0.96	73	37.3
7	1	24	8	2.12	1.20	106	42.9
	2	43	1	1.96	1.32	43	35.4
	3	22	11	1.94	1.12	43	35.4
	4	1	0	2.28	1.12	48	40.4

TABLE A-2. (Cont.)

Orchard	Tree	Internal Breakdown[a]	Water Core[b]	N (%)	K (%)	Mn (ppm)	B (ppm)
8	1	14	3	1.86	1.32	37	46.6
	2	4	1	2.08	1.00	45	34.1
	3	0	0	1.96	1.08	62	41.7
	4	0	0	1.80	1.24	40	38.5
9	1	8	5	1.80	1.20	89	35.4
	2	18	9	1.90	1.24	73	36.7
	3	13	9	1.98	1.20	87	44.1
	4	10	8	1.72	1.12	124	44.8
10	1	2	0	2.04	1.08	98	57.5
	2	10	0	2.00	0.92	45	46.6
	3	14	2	1.98	1.04	68	50.3
	4	4	0	2.08	0.96	56	42.9
11	1	0	0	1.98	1.46	40	51.5
	2	2	0	2.12	1.46	54	44.8
	3	3	0	1.98	1.46	62	52.1
	4	0	0	2.20	1.56	62	48.4
12	1	1	0	2.20	1.32	81	47.8
	2	0	0	1.96	1.24	65	42.3
	3	6	0	2.00	1.38	51	37.9
	4	23	3	2.06	1.50	56	37.9

[a]The sums of the number of fruit from the first harvest with internal breakdown in samples examined 12/15/69 and 2/19/70.

[b]The sums of the number of fruit from the first harvest with water core in samples examined 12/15/69 and 2/19/70.

TABLE A-3. Mineral content of cortical tissue of fruit (September samples), 1969 survey.

Orchard	Tree	N (%)	K (%)	Ca (%)	Mn (ppm)	B (ppm)
1	1	0.24	0.68	0.0275	0.408	20.0
	2	0.20	0.56	0.0338	0.408	22.0
	3	0.16	0.62	0.0338	0.438	23.6
	4	0.24	0.82	0.0300	0.438	28.5
2	1	0.20	0.82	0.0275	0.266	17.6
	2	0.16	0.66	0.0362	0.485	20.0
	3	0.12	0.66	0.0250	0.266	20.0
	4	0.06	0.62	0.0300	0.408	23.6
3	1	0.18	0.44	0.0200	0.485	16.8
	2	0.18	0.50	0.0338	0.671	22.7
	3	0.20	0.52	0.0200	0.485	16.3
	4	0.18	0.52	0.0200	0.438	15.2
4	1	0.18	0.76	0.0440	0.798	22.7
	2	0.20	0.76	0.0288	0.438	18.3
	3	0.22	0.68	0.0300	0.438	14.3
	4	0.20	0.62	0.0437	0.673	16.5
5	1	0.22	0.52	0.0338	0.408	22.7
	2	0.30	0.76	0.0463	1.311	31.8
	3	0.16	0.66	0.0288	0.313	35.6
	4	0.20	0.68	0.0238	0.360	38.9
6	1	0.24	0.72	0.0288	0.438	18.1
	2	0.34	0.66	0.0325	0.438	20.9
	3	0.18	0.66	0.0262	0.313	22.7
	4	0.28	0.60	0.0362	0.485	25.7
7	1	0.20	0.62	0.0338	0.625	15.0
	2	0.14	0.66	0.0175	0.219	16.5
	3	0.14	0.66	0.0200	0.171	13.2
	4	0.20	0.68	0.0288	0.438	22.0

TABLE A-3. (cont.)

Orchard	Tree	N (%)	K (%)	Ca (%)	Mn (ppm)	B (ppm)
8	1	0.16	0.62	0.0213	0.171	29.0
	2	0.16	0.62	0.0300	0.265	22.2
	3	0.16	0.60	0.0338	0.485	30.9
	4	0.14	0.62	0.0362	0.438	32.8
9	1	0.16	0.52	0.0200	0.313	20.0
	2	0.18	0.56	0.0238	0.408	25.7
	3	0.20	0.56	0.0326	0.625	24.9
	4	0.14	0.52	0.0213	0.265	24.1
10	1	0.32	0.60	0.0262	0.360	28.5
	2	0.22	0.62	0.0262	0.408	45.2
	3	0.24	0.50	0.0200	0.313	29.6
	4	0.34	0.62	0.0588	1.236	44.3
11	1	0.24	0.72	0.0188	0.219	32.5
	2	0.28	0.72	0.0188	0.360	30.6
	3	0.20	0.72	0.0188	0.171	45.2
	4	0.24	0.72	0.0175	0.140	25.2
12	1	0.22	0.66	0.0248	0.265	16.8
	2	0.20	0.66	0.0462	0.844	22.2
	3	0.24	0.62	0.0650	1.125	26.8
	4	0.80	0.72	0.0175	0.313	23.2

TABLE A-4. Mineral content of leaves (June samples) and incidence of water core and internal breakdown in fruit after storage, 1968 survey.

Orchard	Tree	Breakdown[a]	Breakdown[b]	Water Core[c]	N (%)	K (%)	Mn (ppm)	B (ppm)
1	1	9	20	3	1.88	1.46	51	29.6
	2	1	9	3	2.06	1.32	63	35.4
	3	3	6	15	2.02	1.42	51	36.0
	4	24	43	5	1.92	2.18	40	35.4
2	1	1	3	1	2.08	1.50	84	37.3
	2	0	8	0	1.90	1.70	48	31.6
	3	7	12	4	1.92	1.56	67	32.5
	4	9	17	0	2.12	1.66	65	32.2
3	1	1	1	3	1.86	1.04	234	33.5
	2	0	0	0	1.80	0.66	166	29.6
	3	3	5	11	1.90	1.24	182	29.8
	4	0	1	12	1.88	1.16	202	32.9
4	1	0	2	7	1.38	1.70	26	32.9
	2	0	1	2	1.36	1.66	40	37.3
	3	4	4	0	1.68	1.86	37	30.9
	4	0	8	10	1.46	1.38	37	31.6
5	1	1	1	2	1.68	1.24	40	32.2
	2	1	5	17	1.64	1.42	54	32.9
	3	0	0	19	1.54	1.32	48	39.2
	4	6	7	6	1.64	1.46	48	34.1
6	1	1	1	0	2.08	1.32	59	21.8
	2	4	4	1	2.04	1.16	62	25.1
	3	1	1	0	2.12	1.38	40	23.8
	4	2	2	0	1.88	1.16	43	22.4

TABLE A-4. (Cont.)

Orchard	Tree	Breakdown[a]	Breakdown[b]	Water Core[c]	N (%)	K (%)	(ppm)	(ppm)
7	1	1	4	13	1.64	1.46	28	22.4
	2	0	2	5	1.70	1.50	31	25.1
	3	2	6	0	1.68	1.56	26	23.1
	4	8	13	6	1.98	1.32	23	23.8
8	1	0	0	1	1.95	1.08	34	23.8
	2	0	0	3	1.78	1.08	31	23.1
	3	12	33	9	1.72	1.12	26	23.8
	4	1	5	18	1.94	0.96	20	21.1
9	1	21	50	14	1.96	0.96	79	21.1
	2	11	20	0	2.00	1.08	62	21.1
	3	8	14	3	2.14	1.04	89	23.8
	4	10	23	3	1.94	0.92	76	21.8
10	1	0	15	2	2.26	0.96	45	23.8
	2	0	18	7	2.38	0.86	87	29.0
	3	0	16	6	2.28	0.88	45	24.4
	4	5	32	14	2.18	0.88	51	23.8
11	1	0	0	0	2.13	1.56	34	23.4
	2	10	17	1	2.29	1.70	45	29.0
	3	0	0	0	1.91	1.46	54	30.3
	4	0	0	0	1.99	1.38	45	27.0
12	1	0	0	1	2.40	1.38	45	23.1
	2	0	1	0	2.30	1.32	45	23.8
	3	0	0	0	1.96	1.56	43	21.8
	4	0	0	0	2.08	1.56	48	23.8

[a]Number of fruits from the 1968 survey out of Ca storage with internal breakdown on 5/7/69.

[b]Sum of number of fruits with internal breakdown examined 2/25/69, 3/18/69 and 5/7/69.

[c]Number of fruits with water core in sample examined 12/28/68.

TABLE A-5. Mineral content of fruit (including peel and core), 1968 survey.

Orchard	Tree	N (% Sept.)	K (% Sept.)	Ca (% June)	Mn (ppm June)	B (ppm Sept.)
1	1	0.34	0.76	0.32	9	23.8
	2	0.26	0.70	0.35	14	18.4
	3	0.32	0.68	0.35	11	17.0
	4	0.28	0.76	0.32	11	21.1
2	1	0.36	0.72	0.37	17	22.4
	2	0.28	0.76	0.29	14	16.4
	3	0.24	0.64	0.32	20	16.4
	4	0.26	0.80	0.32	17	18.4
3	1	0.26	0.46	0.27	17	16.4
	2	0.22	0.50	0.29	23	17.7
	3	0.26	0.56	0.32	20	16.4
	4	0.24	0.56	0.32	28	17.7
4	1	0.19	0.76	0.42	11	26.4
	2	0.16	0.78	0.37	11	20.4
	3	0.19	0.76	0.37	11	16.4
	4	0.17	0.76	0.37	9	21.1
5	1	0.19	0.68	0.42	17	22.4
	2	0.24	0.80	0.40	17	25.1
	3	0.24	0.81	0.47	11	27.7
	4	0.26	0.74	0.42	17	15.7
6	1	0.32	0.76	0.35	20	19.1
	2	0.28	0.66	0.37	23	22.4
	3	0.27	0.59	0.32	11	21.1
	4	0.24	0.51	0.35	14	19.8
7	1	0.24	0.70	0.27	9	17.0
	2	0.27	0.66	0.29	6	17.0
	3	0.34	0.70	0.32	9	17.0
	4	0.32	0.76	0.24	3	26.4

TABLE A-5. (Cont.)

Orchard	Tree	N (% Sept.)	K (% Sept.)	Ca (% June)	Mn (ppm June)	B (ppm Sept.)
8	1	0.22	0.56	0.27	0	23.8
	2	0.21	0.61	0.29	6	21.1
	3	0.25	0.54	0.29	6	26.4
	4	0.20	0.63	0.32	6	19.8
9	1	0.18	0.46	0.22	6	19.1
	2	0.49	0.53	0.27	11	24.4
	3	0.20	0.50	0.27	11	25.1
	4	0.19	0.53	0.24	11	23.1
10	1	0.30	0.51	0.32	9	26.4
	2	0.39	0.51	0.32	9	31.6
	3	0.34	0.58	0.27	6	30.3
	4	0.31	0.56	0.32	9	31.6
11	1	0.31	0.91	0.37	9	27.7
	2	0.25	0.80	0.29	14	27.7
	3	0.26	0.80	0.35	17	32.9
	4	0.22	0.78	0.35	9	25.1
12	1	0.29	0.78	0.32	9	17.0
	2	0.25	0.68	0.27	11	19.8
	3	0.25	0.68	0.27	9	22.4
	4	0.27	0.81	0.27	0	25.7