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Title: POSTHARVEST PHYSIOLOGY OF 'ANJOU' PEAR FRUIT: RELATIONS BETWEEN MINERAL NUTRITION AND CORK SPOT, RESPIRATION, AND ETHYLENE EVOLUTION

Abstract approved: Daryl G. Richardson

The objective of this investigation was to study the relations between mineral nutrition, and postharvest physiology of 'Anjou' pear (Pyrus communis L.) fruit, including the development of cork spot (a physiologic disorder), respiration, and ethylene evolution.

Results of fruit mineral analysis from nine orchards showed that: Fruit Ca was negatively correlated with both cork spot at harvest (r=-0.75) and after storage (r=-0.76) in both seasons. Cork spot at harvest and after storage was positively correlated (r=0.74 and 0.75) with N:Ca ratio. The correlations between cork spot and both Ca and N:Ca ratio were highly significant at harvest and during growing season as early as July. Critical minimum Ca concentration in 'Anjou' pear fruit for the development of corking disorder is about 7 mg/100 g of fresh weight. Cork spot is expected to be more than 30% if N:Ca ratio is higher than 10.

The correlations between cork spot severity and Ca were negative and highly significant (r=-0.86). Water soluble Ca was more correlated (r=-0.90) with severity of cork spot than was total Ca.

Orchard sprays with $Ca(NO_3)_2$ or $CaCl_2$ in June and July in six orchards reduced cork spot from 49.8 to 16.5% and increased fruit Ca 20% over control in 1975, but failed to do so in two out of three orchards in 1976.

When ^{45}Ca was injected in branch of 'Bartlett' pear on June 25 after removing terminal meristem, about 28% of total ^{45}Ca moved out of the application zone during three days compared with only 17.5% if the growing point was left intact. Within three days activity of ^{45}Ca in fruits on branches with growing point removed was three times higher than in fruits on branches with growing point left intact.

When ^{45}Ca was injected the second week of August into branches of 'Anjou' pear, 2% of total ^{45}Ca moved into the fruit, while 41% of total ^{45}Ca absorbed by the fruit from painting on fruit surface, moved out of the fruit within 40 days.

When ^{45}Ca was painted on leaf surfaces, 10% of total activity moved out of the leaf within 40 days.

Calcium concentration in the fruit was increased significantly by postharvest dips (up to 155%) or by vacuum infiltration (up to 54%) in $CaCl_2$ solutions. The increase in Ca concentration in the fruit caused a significant reduction in respiration rate and ethylene

evolution. There were highly significant negative correlations between fruit Ca concentration and respiration rate (r=-0.83) and ethylene evolution (r=-0.87), during ripening at 20°C. There was higher correlation between the rate of respiration and water soluble Ca (r=-0.91) than with total Ca at harvest. Fruit firmness was increased 1.1 kg by increasing Ca concentration in the fruit, but internal ethylene concentration of the fruit was also increased by dipping the fruit in 5% $CaCl_2$.

Calcium concentration in mitochondria isolated from 'Anjou' pear fruit affected with cork spot was significantly lower than Ca concentration in mitochondria isolated from normal fruits. State III respiration, respiratory control ratio, and ADP:O ratios were significantly higher in mitochondria isolated from normal fruits than in mitochondria isolated from cork spotted fruits. The results suggest that calcium deficiency reduced the functional integrity of the mitochondria isolated from ripe 'Anjou' pear fruit.

Postharvest Physiology of 'Anjou' Pear Fruit: Relations between Mineral Nutrition and Cork Spot, Respiration, and Ethylene Evolution

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This thesis is dedicated

with much love to my friend

Miss Susan J. Buntjer

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INTRODUCTION

Cork spot is a physiological disorder of 'Anjou' pear fruit which is characterized by sub-epidermal darkened lesions which become increasingly apparent in storage, largely localized in the distal two thirds of the fruit, and is often followed by abnormal ripening and accelerated senescence. A number of apparently related physiologic fruit disorders have been reported in the literature, such as blossom-end rot in tomatoes and pepper; water core, lenticel spot, internal breakdown, and bitter pit in apple; also black end found on 'Bartlett' and other pear cultivars. Corking disorders of 'Anjou' pears have been a problem to orchardists in the Pacific Northwest for many years. Cork spot of 'Anjou' pear is similar to various forms of bitter pit in apples. About 10 to 25% of the disordered pears may be detectable at harvest, the remainder appearing as storage duration increases. Although this disorder is predominant in the Anjou variety, rare occurrences of cork spot have been observed on 'Bartlett' and 'Bosc'. It has been established in the literature that nutritional imbalances in the fruit are major causes of many physiological fruit disorders. The association of low concentration (concn) of Ca in the

fruit with many apple disorders is well established, but work on 'Anjou' pear is limited. Therefore, the objective of this study was to identify those mineral elements most closely related to the incidence of cork spot. Special emphasis was given to Ca since localized deficiency in fruit is a primary cause of many physiological disorders.

A second objective of the investigation was to study the translocation of Ca to and from the fruit under field conditions by using radioactive ^{45}Ca. The third objective was to study the effects of fruit Ca and N concentrations on respiration and ethylene evolution to understand more about the abnormal ripening and accelerated senescence of the disordered fruits. This thesis was prepared as a series of chapters, each reporting a different subject area investigated in relation to the incidence of cork spot of 'Anjou' pear.

The titles of each chapter follow:

I. Relations between fruit mineral content and the incidence of cork spot.

II. Investigations about uptake and translocation of radioactive calcium after branch injection, and painting on the surfaces of the fruit and leaves of 'Anjou' pear trees under field conditions.

III. Effect of calcium and nitrogen on respiration and ethylene evolution of 'Anjou' pear fruit.

IV. Relation between Ca concn, respiration, respiratory control

ratio, and ADP:O ratio in mitochondria isolated from fruits of 'Anjou' pears affected with cork spot.

CHAPTER I

RELATIONS BETWEEN FRUIT MINERAL CONTENT AND THE INCIDENCE OF CORK SPOT

Literature Review

I. Introduction

Cork spot is a physiologic disorder that occurs mainly in apples and 'Anjou' pears, but can occur on 'Bosc' and 'Bartlett' pears. Cork spot is characterized by the development of localized desiccated tissue resembling cork, in the flesh of the fruit. This disorder is initiated during the growing season. The first symptom of the disorder is the appearance of a small blushed area on the skin above the affected brown spot (61). The brown spot can be anywhere between the skin and the core, but in most cases it is close to the surface of the fruit just beneath the skin (61, 158). A depression develops above the internal spots as the fruit enlarges, due to the reduced growth in the affected tissue. The brown spot is much harder than the surrounding tissue. The spots are usually more frequent at the calyx end of the fruit.

Bitter pit of apples is a disorder in which small, brown, dry areas develop in the flesh of the fruit generally during storage (68, 184). The skin over the affected tissue usually retains more green

color than the surrounding skin. Bitter pit may be initiated while the fruit is still on the tree and visual symptoms may show up before harvest, after storage, or sometimes not until a few days after removal from storage (61). The affected tissue of bitter pit is softer than the surrounding tissue (61, 68, 151). The affected tissue in both bitter pit and cork spot are bitter in taste. The disorders are usually classified as "tree pit" for cork spot and "storage pit" for bitter pit, but many authors group the two as the same disorder due to the difficulties in drawing any objective distinction between them (61, 68). The term cork spot is applied to pears and sometimes to apples, but bitter pit is currently applied only to apples. In this study we will use the term "cork spot" for the disorder in pears ('Anjou') only and "bitter pit" for the disorder in apples.

It was reported for the first time in 1936 by DeLong (45) that apple fruits affected by bitter pit contained less Ca than normal fruit. Some of the physiologic disorders in other plants associated with a localized Ca deficiency are: blossom-end rot of tomatoes (18, 57, 59, 72), blossom-end rot of pepper (78), black heart of celery (71), tipburn of lettuce and cabbage (2), cracking of prunes (39), blossom-end rot of watermelons (174), leaf tipburn of strawberry (113), anther failure in 'Redgauntlet' strawberry (77), internal browning of Brussels sprouts (151), and black-end of pears (44). Since DeLong's early work, it has been recognized that Ca deficiency is associated with

many physiological disorders of apple and pears (3-8, 13, 16, 17, 29-32, 36, 37, 41, 46, 48, 50, 52, 54-56, 60-62, 65, 68, 70, 75, 79, 82, 96-109, 110, 112, 114, 117, 119, 122, 123, 127, 128, 132, 138, 140-145, 147, 150-158, 161, 168, 170, 174, 177-180, 184-188).

II. History and Background

Bitter pit in apples was described for the first time, and called a spot disease in 1891, by Jones (83). The name bitter pit was used for the first time in 1895 by Cobb (41), because the abnormal tissue tasted bitter. The hot, dry summer of 1911 in Europe increased bitter pit up to 50% in some apple varieties (19). Nitrogen fertilizer was reported to promote bitter pit in 1913 for the first time (81). An association between bitter pit and the fungus Alternaria was reported in 1914 by Reed (137). All attempts in 1914 to isolate any pathogen from the spots failed (163). Excessive water supply late in the season was reported to increase bitter pit in 1920 (25), but heavy irrigation early in the season, followed by light irrigation the rest of the season reportedly reduced bitter pit (24).

Cork spot on pears was reported for the first time in 1921 by McAlpine (115), the author suggested that breeding for resistant cultivars is the most effective way to control cork spot. Early picking was reported to increase storage pit by Adams, in 1924 (1), and cold storage at 0°C retarded the appearance of it. The first mineral

analysis of apple fruit was in 1926; it showed that N and K were higher in pitted than normal fruits, but Ca was not assayed (26). In 1936, Ca was found to be lower than normal in fruits with bitter pit, on both dry (45) and fresh weight bases by DeLong (46). The research of Garman and Mathis, in 1956, was the first attempt to increase the Ca concn in the fruit by foliar sprays with Ca salts. The authors were able to reduce bitter pit in apples from 45% to 10% by using a $Ca(NO_3)_2$ spray program (70). Thereafter, reports of successful control were reported world wide (3, 7, 13, 14, 16, 17, 29, 35, 43, 54, 82, 117, 120, 140, 144, 166-168, 170). Also some reports of failure of control by Ca sprays were reported (6, 68, 149, 168).

III. Soil Management

Calcium is one of the most abundant elements in the soil. It accounts for more than 3% of the composition of the earth's crust (151). Calcium related physiologic disorders in fruits and storage tissues is mainly due to localized deficiency within the plant parts. Control of these physiologic disorders requires maintenance of adequate levels of Ca in the fruit. The uptake and movement of Ca into the fruit is a very complex process. It is affected by climatic and soil conditions as well as by many cultural practices.

Calcium uptake from the soil is affected by many factors.

including soil type, soil pH, soil moisture, salt concentration, and the type of N fertilizers (60, 149, 151). Calcium nutrition implies more than the uptake from the soil. All those factors which influence the uptake, translocation, distribution, and ultimate metabolic utilization of Ca may be considered as part of Ca nutrition. Thus, source and concn of nutrient supply, cultivation, irrigation, spray applications (hormones and growth regulators) and the timing and severity of pruning can influence Ca nutrition (8, 23, 149, 156).

Soil cultivation was reported to reduce bitter pit in apples (47). This was suggested to be due to the removal of the fine roots in the uppermost 6 to 7 cm of soil which resulted in a reduction of K uptake.

Straw mulching, which is used to reduce water stress, significantly reduced the Ca concn of the apple fruit, increased bitter pit, internal breakdown, and decay in the fruit. Fruit analyses showed that the fruits were significantly higher in K. The straw may have released large amounts of K or made the K in the soil surface more available to the roots. Excess K may have interfered with Ca uptake by the tree roots (W. J. Bramlage, communication by letter).

No relation has been shown between the levels of Ca in the soil and bitter pit in apples (30, 75, 140) and high soil Ca has not prevented bitter pit in apple (140).

Application of $Ca(NO_3)_2$ to the soil (119), or in the nutrient solution of tomato plant (9) increased Ca concn in the fruit and

reduced blossom-end rot. Increasing Ca concn in the nutrient solution of apple trees in sand culture, reduced bitter pit and increased Ca concn in the fruit (30, 110).

Soil applications of $Ca(NO_3)_2$ or $CaCl_2$ showed no effect on the incidence of bitter pit in apples (16, 100). Application of gypsum (calcium sulfate) to light soils reportedly has some advantage in reducing bitter pit in apples (167, 168). Failure to reduce bitter pit by increasing Ca uptake through application of gypsum to the soil is also reported (36, 43). Liming is reported to either have no effect on bitter pit (43, 53) or to increase bitter pit of apple (35, 116), or even reduced Ca in the fruit (36) This possibly can be explained on the basis that increasing the soil pH increased the uptake of K, N, and Mg, which in turn compete with the uptake, translocation, and possibly the utilization of Ca. From the preceding discussion we can conclude that disorders caused by Ca deficiency in the fruit can seldom, if ever be corrected by supplying additional Ca to the soil. The problem is mainly due to restricted translocation of Ca to the fruit

IV. Cultural Practice

A Water relations. It was observed earlier that bitter pit may be produced by a shortage of water or irregular water supply (115, 151). Abnormally high transpiration rates and too rapid growth

were thought to cause a shortage of water in fruit tissues and the development of "dry spot" (115). This is now considered unlikely. Moisture stress has an indirect effect on the development of cork spot and bitter pit by reducing the uptake and translocation of Ca. Low soil moisture is considered to be one of the important factors in the development of blossom-end rot in tomato (9). Experiments with tomato plants maintained near the wilting point after fruit set, showed that Ca concentration in the fruit was reduced from 0.36 to 0.23 mg/g of dry weight, and blossom-end rot was increased from 1.3 to 30.7% (9).

An irrigation experiment with apple trees showed an increased concn of leaf Ca and a decreased concn of fruit Ca with decreased soil moisture (74). During water stress the relative "pulling power" of fruits for water is less than that of the leaves; the fruits thus lose water to the leaves (61, 86). This could cause Ca to move with the water from the fruits to the leaves resulting in the development of cork spot close to the ends of the vascular bundles, the locus for cork spot in the fruit. Dry conditions are reported to induce more bitter pit and cork spot (61, 68, 74, 151, 168, 178, 188). If the level of Ca was already low in the fruit, the incidence of cork spot or bitter pit would be expected to be aggravated by moisture stress. Excessive irrigation could also promote the development of the physiologic disorders. Over-watering can induce water stress by

causing poor soil aeration, which reduces active uptake of divalent cations (151). Excessive late season irrigation results in larger fruits which are more susceptible to bitter pit Irregular water supply has been shown to increase bitter pit by causing growth fleshes and fluctuating nutrient uptake (61). Irrigation should be used regularly to maintain uniform soil moisture throughout the growing season, and thus avoid water stress and marked fluctuations in nutrient supply.

B. Pruning. Severe winter pruning increases the incidence of bitter pit, possibly by excessive stimulation of shoot growth (61, 151). Vigorous vegetative growth early in the spring was found to be highly correlated with bitter pit in apple (115). Also severe winter pruning resulted in larger fruits due to its thinning effect and greater leaf:fruit ratio. To reduce bitter pit, winter pruning should be light or moderate. Late summer pruning was reported to reduce bitter pit and the concn of N, K, P but increase the concn of Ca in the fruit (179). Removal of all the new terminal growth from apple trees in early August raised the Ca concn of the fruit and improved the storage life (W. J. Bramlage, personal communication). The increase in fruit Ca depends upon how late and how severe the summer pruning has been. The problem with summer pruning is that it causes multiple bud breaks the following spring and can cause bloom to occur in autumn. The practical aspect of

summer pruning is still not clear. Summer pruning reduces the leaf:fruit ratio and reduces the competition for available Ca late in the season. Adjusting the leaf:fruit ratio by defoliation was found to be very effective in reducing or preventing cork spot or bitter pit (61). Removing every second leaflet in tomato plants in the greenhouse reduced blossom-end rot (9).

C. Crop size. Light cropping results in larger fruits and high leaf:fruit ratio, whereas heavy cropping results in smaller fruit. It has often been reported that large fruits are more susceptible to bitter pit (61, 68, 98, 99, 145, 188), and cork spot (121) than small fruits. Heavy thinning results in a light crop with larger fruits and more bitter pit (61, 142, 188). Thinning of apples reduced Ca concn in the fruit from 6.2 to 5.7 mg/100 g of fresh weight and increased the incidence of bitter pit (142). Fruit thinning is necessary to reduce alternate bearing and avoid bitter pit in the light cropping years. Thinning should be moderate and delayed as late as possible to avoid excessively large size fruit where susceptibility to bitter pit is known.

D. Growth regulators. Several growth regulators have been shown to have an effect on Ca movement in apple (103, 119, 153, 160-162, 168), and pear (161) trees. Movement of ^{45}Ca to the mature leaves of apple seedlings was increased with sprays of kinetin (153). Sprays with TIBA reduced Ca concn in the fruits of 'Golden Delicious' apples and increased the development of bitter pit (160, 161, 162).

Sprays with shoot growth retardants (Alar and CCC) resulted in reduction of bitter pit, and increased Ca levels in the fruits (119, 168).

E. Rootstocks. Calcium uptake from the soil is affected by the plant species and the kind of rootstock (65, 76, 91, 147, 187). Cultivars differ in susceptibility to the disorders (76, 151), and rootstocks vary in their ability to extract Ca from the soil (22, 91, 135). The genetic resistance to blossom-end rot of tomatoes is based on the differences in Ca uptake efficiency, accumulation, and metabolism in the fruit. The highly resistant cultivars appear to be more efficient "absorbers" and "accumulators" of Ca. The highly susceptible cultivars "have a high Ca requirement but are inefficient at absorbing and translocating Ca" (76). Various apple cultivars are found to differ in their accumulation of Ca in leaves (147) and in fruits (65). Fruits from seedlings of cultivars which are susceptible to bitter pit accumulate less Ca than the resistant ones (65). Apple trees grafted on vigorous rootstocks are more susceptible to bitter pit than those grafted on dwarfing stocks (30, 135, 149, 179). Leaves from scions on 'Malling 9' and 'Malling '3' contained more Ca than from those of 'Malling 7' (149, 179). Trees on 'Malling 26' and 'Malling 7' developed more bitter pit than 'Malling 9' rootstock (179). Apple trees on 'Malling 4' have higher K in the fruit and leaves than those on 'Malling 7'. Leaf Ca was lower on 'Malling 4' than on 'Malling 7', but fruit Ca was equal (30). Several pear cultivars were found to

accumulate more Ca in the leaves, if they were grafted on 'Old Home' rootstock than any other rootstocks (91). Cork spot of 'Anjou' pear was the lowest on 'Old Home' compared to many other rootstocks (91). In planning new orchards, growers should carefully consider proper rootstock selection to avoid potential Ca deficiency disorders.

V. Antagonism between Calcium and Other Elements

The balance between Ca concn and the concn of other elements in the leaves (56, 148, 150, 153), and fruits (10, 11, 16, 32, 35, 60, 61, 106, 108, 109, 147-150, 153-156, 168, 173, 183, 185) is very important in trying to relate mineral composition to physiologic disorders in the fruits. The critical concn of Ca depends on the concn of other elements, especially N, K, Mg, and P in the fruit.

A. Nitrogen. High N in the fruit reportedly aggravates the development of Ca deficiency symptoms (12, 60, 106, 108, 109, 142-150, 153-155). Pitted tissues have lower Ca and higher N, protein, free amino acids, and other nitrogenous compounds (61, 67, 80, 106, 109). Excessive N fertilization may have direct effects on the uptake and translocation of Ca. High N can have indirect effects on Ca concn in the fruit either by increasing fruit weight thereby diluting Ca, or by increasing shoot growth which competes with the fruit for Ca moving in the transpiration stream (60, 61, 148-156).

Not only the amount of N but also the form of N should be

considered to avoid Ca deficiency in orchards. In experiments with apple trees in sand culture, forms of N were tested (106), and the results showed that ammonium sulphate reduced Ca, Mg, and K in the leaves and fruit compared with urea. Ammonium nitrate did this also but to a lesser extent, while urea had no effect on Ca, Mg, and K. Ammonium sulphate and ammonium nitrate increased bitter pit, while urea did not. Nitrate N increases cation uptake including Ca, from the soil (31, 148, 183). Nitrate also increased both water consumption of trees and Ca uptake late in the season (31). Nitrate may increase cation uptake by increasing soil pH. On the other hand, NH_4^+ reportedly competes with the uptake of cations, including Ca, by apple trees both in sand culture and in the field (31, 148, 183). Ammonium forms of fertilizers increased fruit and leaf N more than nitrate forms (156). Growth of apple trees receiving NH_4^+ was much reduced compared with trees receiving NO_3^- (156). The form of N not only affects concn of Ca in the fruit and shoot, but also influences the distribution of Ca within the plant. Nitrate N increased Ca accumulation in mature leaves, while NH_4^+ increased Ca accumulation in younger leaves (153, 155, 156).

Sprays of ammonium sulfate on mature leaves of apple trees also decreased Ca accumulation in these leaves (153). Ammonium ion in the root zone decreases the uptake of Ca and interferes with its translocation within the tree. In the presence of ammonium, Ca

is transported mainly to leaves instead of to fruits (60). Experiments with tomato and sweet corn plants showed that ammonium-N reduced shoot and root growth and reduced Ca, K, and Mg contents of the leaves and fruits compared with nitrate-N treatments (133, 176). When tomato plants were supplied with ammonium-N during fruiting, rapid development of blossom-end rot occurred, probably due to reduced Ca concn in the fruit. Ammonium-N decreased water uptake as compared with tomato plants the same size receiving nitrate-N, probably by increasing leaf and root resistance to water flux. The inhibition of water uptake may have resulted from a change in membrane structure. Deficiency of Ca induced by NH_4^+ could cause some loss of membrane integrity (133). The toxic effects of NH_4^+ on chloroplasts and mitochondria could be related to Ca deficiency induced by NH_4^+. This has been reported to accelerate respiratory breakdown of carbohydrates, uncouple photosynthetic phosphorylation and result in disruption of chloroplast membranes, according to Purith and Vines (as cited by Pill and Lambeth, 133). In tomato, the ammonium-N increased the synthesis of aspartic and glutamic acids and their amides in the roots, while nitrate-N shifted their site of synthesis to the leaves (80).

Application of $CaCl_2$ to fruits of tomato plant grown with NH_4^+ reduced the concn of amino acids to the levels of nitrate-N fruits. It has been suggested that ammonium toxicity is due to NH_4-induced

intracellular Ca deficiency (80). Application of $CaCl_2$ to fruits prevents or reduces blossom-end rot caused by NH_4^+ (176). The accumulation of free amino acids with ammonium-N nutrition indicates that they were not being fully utilized into protein. Protein synthesis may be blocked or severely reduced with high NH_4^+ (80). The reduced uptake of Ca and Mg in the presence of NH_4 occurs whether pH of the nutrient solution is controlled or not. The reduction in protein synthesis may be due to the reduction or inhibition of Ca and Mg uptake which are enzyme cofactors required for protein synthesis. It has been found that the NH_4^+ form of N can affect Ca nutrition in several portions of the nutritional cycle. The uptake of Ca can be reduced by NH_4^+ ion through a "competitive absorption." Ammonium ion in the root zone inhibits nitrate reductase activity, and may reduce the uptake of NO_3 (151). If as low as one seventh of the total N in the root zone of apple tree is in the NH_4^+ form nitrate reductase activity in the roots is reduced by one half, according to Frith (as cited by Shear et al., 156). Significant amounts of NH_4^+ could stay in the soil for two to five weeks after application, depending on soil temperature, pH, and amount of organic material (60). This may cause the tree to respond as an ammonium-fed tree with the uptake, translocation, distribution, and metabolism of Ca restricted (156). In general, N application should be limited during fruit growth, and the use of NH_4^+ forms of N should be avoided to

assure enough Ca in the fruit.

Shear (150) showed that the N:Ca ratio in the fruits and the leaves of apple were more highly correlated with bitter pit in apple than was Ca or N alone. High N can offset the beneficial effect of normal concn of Ca, so that a low N:Ca ratio and adequate Ca is required for good quality apple fruit (150, 153). The N:Ca ratio in the apple fruit flesh must be less than 10 to 14 to expect less than 10% bitter pit (150).

B. Boron. A disorder known as drought spot in apple has been identified as a B deficiency (61, 83), and sprays with B have reduced the disorder (61). The B deficiency disorder typically occurs within the core area extending outward and soon turns to a golden color and the skin becomes wrinkled. The effect of B on cork spot and bitter pit is unclear. Some reports show that B treatments are beneficial (58, 116) while many others have shown them useless (17, 20, 35, 61, 175, 185). In the cases where B sprays were reportedly beneficial in preventing bitter pit, the disorder may have been confused with drought spots, which is different than bitter pit. There are reports which showed B or borax sprays slightly promoted bitter pit (35). Soil applications of borax and boric acid indicate that the heavier the application the higher the percentage of flesh breakdown and water core. High positive correlations were found between the rate of B application and the amount of injury to the fruit (175).

The possible interrelationship between B and Ca was recognized early (70, 172). The lack of B in the nutrient solution reduced the uptake of Ca more than that of K or N (172). Increasing B in a nutrient solution or foliar sprays increases Ca accumulation in apple seedlings especially at low and critical concn of Ca (153, 156). Boron may be required for the transport of Ca into the fully developed tissues (61), although B as boric acid was ineffective in releasing Ca from exchange sites in pieces of apple stem (156). The increase in Ca accumulation in leaves sprayed with B could be due to the stimulation of metabolic activity of the parenchyma cells of the leaf (156). Accumulation of ^{45}Ca in the leaves of apple seedlings was increased by increasing B levels from 0.001 to 1.0 ppm in the nutrient solution (189), but there was no significant difference in ^{45}Ca activity in stems and roots. The lack of ^{45}Ca accumulation in the stems and roots suggests that B preferentially increased the translocation and accumulation of ^{45}Ca in the leaves. Soil application of B increased the effectiveness of Ca sprays by reducing the percentage of bitter pit (50). Sprays with B or B + Ca completely prevented apple fruit cracking (50). For the optimum utilization of Ca, the concn of B in the leaf could cause storage breakdwon (151), and increasing leaf B up to 60 ppm could result in early fruit maturation and preharvest drop (149). Under B deficiency conditions, the uptake and translocation of Ca may be restricted.

C. Magnesium. High levels of Mg in fruit is associated with increased percentage of bitter pit (7, 166, 183). Bitter pit can be induced by soil application of Mg (61, 94, 183), or by spraying with Mg salts (11, 61, 104, 106, 108, 151, 184). The concentration of Mg in the pit area in the fruit is higher than adjacent healthy tissues (7, 28, 64, 112). There is evidence of general migration of Ca, Mg, K, Na, B, P and N from healthy to affected tissue (7, 64). If the complete fruit is analyzed, there are contradictory results. Some reports show that fruits with bitter pit or cork spot are higher in Mg than normal fruits (7, 28, 112); others found no significant differences (30, 106, 185, 186).

It may be more meaningful to consider the Mg/Ca ratio in the fruit rather than the concn of Ca only (7, 146, 188). If the Mg/Ca ratio is less than 1.8 bitter pit is not expected, but if the ratio is above 2.0 bitter pit is anticipated (181).

D. Potassium. Sprays with K salts or excess K fertilization have been found to increase the incidence of bitter pit (29, 30, 61, 104, 140, 146, 183). The concn of K in the pit area was higher than healthy tissue (28, 151, 168, 183), and in the fruits with cork spot (112, 186). The K/Ca and (Ca+Mg)/K ratios in the leaf or in the fruit are more important in relation to bitter pit than the absolute K levels (4, 7, 9, 18, 61, 72, 146, 168, 185, 186). Cork spot and bitter pit were positively correlated with K/Ca and negatively correlated with

(Ca+Mg)/K ratios. Schumacher (146) suggested that (K+Mg)/Ca ratio was the most important single index to predict bitter pit, but various other factors may interact. It has been found that Ca deficiency greatly increases K uptake and the K/Ca ratio is increased in Ca-deficient trees and resulted in a high percentage of bitter pit (30, 61, 183). Sharples (143) suggested that extremely low K may cause a type of breakdown of apple in storage. Five years of records show that apples from plots receiving K had 50% less low temperature breakdown at 32°F, and 50% less senescent breakdown at 37°F than the control, while bitter pit was increased 50% above the control (178, 180).

Competitive effects of Mg and K on the uptake and translocation of Ca are very important in planning fertilizer programs. Bangerth (11) suggested that Mg and K are the main ions that antagonize the function of Ca in bitter pit development, perhaps because their concentration is high enough to compete with Ca in the fruit but not in the leaf. In the fruits there is an extremely high (Mg+K)/Ca ratio (on the order of 20 to 50) while in the leaves this ratio is low (about 1 to 2). Bangerth (11) also suggested that Mg and K may have occupied some nonspecific Ca attachment site and antagonized the function of Ca in the membrane. The most serious effect on Ca is caused by Mg; this suggests that the sites for these antagonistic effects may be at the membrane interfaces, since results of root studies indicated

that large portions of Ca in the membrane are exchanged by Mg, K, or H^+; this exchange increases the permeability and fragility of the membranes (9, 11). It has been shown that treating apple fruit with Mg can increase the permeability and the respiration rate of the tissue (11). Treating apple slices with Mg can also replace Ca and increase sugar uptake and permeability (12). The critical concn of Ca in apple fruit is affected by the concns of Mg and K. Calcium deficiency is relative, and conditioned by the concn of Mg, K, and N. The relative Ca deficiency may precondition the replacement of Ca in the membranes by Mg, K, and H^+ (9).

E. Phosphorus. Phosphorus has been reported to affect keeping quality of apples (143). It was found that if P levels are less than 10 mg/100 g fresh weight the fruits will show "low-phosphorus breakdown" even if calcium concn is above the critical concn. This can be corrected by four sprays of 1% KH_2PO_4 between mid-June and mid-July (143). Highly significant negative correlations between bitter pit in apple and P content were reported by Martin (99), and the variation of P between seasons was attributed to cropping level. Data on the effects of fruit P concn on bitter pit are conflicting (61). Increasing levels of P are not always associated with a reduction of bitter pit (151). There is also report that bitter pit was associated with high P content (61). Although in this case high P was found to be associated with bitter pit, the real cause was low Ca (61).

Phosphorus content was high in cases associated with low K, however, the observed results in reduction of bitter pit was likely due to low K not high P. The high P concentration in pitted tissue is not surprising because of the general migration of all mineral elements into the pitted tissue (7, 61, 69). The fact that a mineral element is in a high concn in the lesions of bitter pit can be an artifact due to death and dehydration of the tissue and may not be of any specific importance.

F. Other elements. Many other elements have been applied as sprays at several times throughout the season to control bitter pit, but they were ineffective, except for Na and Zn sprays which apparently increased bitter pit (61). However, some reports indicate that Zn spray (151) or soil application (94) may reduce bitter pit.

VI. Relations between Calcium and Organic Acids

Organic acids could cause the fixation of Ca along the pathway from root to the fruit and deposition of Ca in the fruit into insoluble forms. Susceptibility of plants to Ca deficiency was not related to Ca absorption as demonstrated by Brumage and Hiatt (27). The upper portions of the susceptible plant contained less Ca than of non-susceptible plants, indicating some interference with the translocation of Ca to that portion of the plant (27). Oxalic acid content of the upper half of susceptible tobacco varieties stalks was higher than the

nonsusceptible varieties. Crystals of Ca oxalate were found within the cells of tobacco leaves (27) and along the vascular bundles of the pedicel of apple fruit (164). The precipitation of Ca as insoluble Ca-oxalate would make it unavailable for translocation to the fruit or to developing tissues of the plant. It was found that the Ca content of the stalk portion of the plant was about 15 to 20 times greater than Ca content of the leaves of the plant. Because of the high oxalic acid content in susceptible varieties there is less available Ca and high levels of oxalic acid may have interfered with the translocation and utilization of absorbed Ca (27). Seasonal samples from the xylem sap of apple demonstrated that about half of the Ca in these samples was complexed with citrate and malate (22). Doesburg (51) found that the alcohol insoluble fraction of Ca in the ripening fruit increased at the time that pectin was becoming more soluble. Some of the Ca that was released from the breakdown of the cell walls was precipitated by organic acids such as oxalic acid. The solubility of pectin and the changes in pH during ripening apparently controls the precipitation of Ca (51). Successive reduction of Ca solubility as well as citric acid accumulation in pit lesions may cause bitter pit in apple (52). The predominant organic acid in bitter pit was citric, while malic acid is the major constituent in the normal tissues (61). The high acidity in the pitted tissue may cause the cations to move into the affected tissues to neutralize the excess acidity (61). On the other hand, inorganic

ions may move to the site of metabolic activity during the first stages of the disorder, which is accompanied by high respiration, increased protein synthesis, pectin synthesis, and increased ethylene production (61). Injections of K oxalate in the tree promoted bitter pit, while Ca acetate reduced it (13, 14).

Excessive N fertilizer increases asparagine which can form stable Ca-complexes and promote bitter pit (11). Pitted tissues contain high concns of amino acids (aspartic and glutamic), more protein, and more organic acids than healthy tissues (8, 11, 67, 146, 168). Bruised apple fruit, without actual breakage of the skin, showed the same analytical results as the pitted tissues, except the organic acids and amides content failed to increase in mechanically-induced lesions (8). Organic acids in the fruit can have antagonistic effects on Ca by providing H^+ (11). Organic acids can also remove Ca from its binding sites such as in the membrane by acting as chelators for Ca (11). Citric acid-Ca complexes have a high stability constant, and an increase in citric acid may increase the development of bitter pit (11). Removal of Ca from the membrane by the action of organic acids can increase the permeability, and with increased respiration, ethylene evolution, and senescence.

VII. Spray with Calcium Salts

Pre-harvest sprays with calcium nitrate to control bitter pit in

'Baldwin' apples was first reported by Garman and Mathis in 1956 (70). Martin et al. (100) were able to reduce bitter pit, in the cultivar 'Cleopatra', from 35% to 7% by spraying with Ca nitrate. This success in control of bitter pit was followed by reports of partial control by sprays with calcium salts from all over the world. The control of bitter pit was never complete and the time and number of sprayings with calcium salts was important to obtain commercially useful results. The use of various additives or surfactants has a great effect on Ca uptake and increases the degree of response to treatment. Martin et al. (98, 103, 105, 107) studied the effect of various additives to Ca salts on the incidence of both bitter pit and breakdown of apples and concluded that dimethylsulphoxide (DMSO) was the most effective chemical added to Ca nitrate. Ca nitrate (0.05 M, 1970 ppm Ca) plus 500 ppm DMSO sprayed two times during the growing season were as effective as four or six sprays of Ca nitrate (0.05 M) alone. The additive diphenylamine (DPA) 3000 ppm reduced scald and fungal rotting but increased breakdown if it is sprayed immediately before harvest.

A group of Australian workers (90, 96-109) have extensively studied orchard sprays containing calcium salts and their conclusions are given below. Spraying with Ca nitrate or Ca chloride are equally effective in reducing bitter pit in apple, but Ca chloride was recommended when the nitrogen concn is already high. Low

concentrations of Ca nitrate (5 lb/100 gal) were recommended for earlier sprays and high concentrations of Ca chloride (10 lb/100 gal) were recommended for later spraying. The applied Ca penetrated the fruit and increased the Ca content of the fruit. ^{45}Ca applied to the surface of the fruit penetrated into the fruit readily and moved in the vascular tissue of the fruit. Repeated applications of Ca sprays over many years showed that there were no residual effects on bitter pit in the season after treatment. Their results showed that a valuable reduction in bitter pit can be accomplished with Ca sprays. The workers could not find any satisfactory method for full elimination of the disorder, even with five applications. The degree of control depends on the number of application and there is little possibility that a single well-timed application will be sufficient. The workers suggested that the more applications the better control of bitter pit in apple. Soil dressings with Ca nitrate (1 kg/tree) had no effect on fruit Ca concn and increased the percentage of bitter pit by increasing available nitrogen from the soil without increasing Ca. Magnesium sprays increased the percentage of bitter pit. Boron had no effect on bitter pit and spray with borax at full bloom tended to reduce the effect of calcium salts.

Before spraying commercial orchards, preliminary tests should be carried out on a small number of trees to find the best concentration and the timing. Spray programs from one part of

of the world cannot necessarily be applied to other parts without modifications due to differences in environmental conditions and cultural practices.

In England the East Malling Research Station has prepared an annual advisory note with the current recommendations for growers (68, 143-145). They recommended that foliar sprays with Ca nitrate or Ca chloride are valuable in controlling bitter pit. Calcium chloride may be more effective than Ca nitrate but the chloride can cause leaf scorch under certain conditions. The rate of Ca nitrate application should be about 0.8% calcium nitrate (10 lb/100 gal). Commercial Ca nitrate contains about 80% of pure calcium nitrate and 15.5% nitrogen. The suggested rate of Ca chloride application is about 0.57% Ca chloride (8 lb/100 gal). Commercial Ca chloride contains 71% pure Ca chloride. A spray program of four to seven calcium sprays is recommended for best results. The spray should be at a high volume (200 gal/acre), with a suitable wetting agent in order to obtain good coverage of leaves and fruits. All the fruits on the tree should be covered by the calcium spray in order to get the best results. High volume sprays should be repeated at 21-day intervals starting at the beginning of June until harvest. If the orchards are badly affected with bitter pit, the spray should be repeated at 10-day intervals. Spraying with Ca salts, especially $CaCl_2$, at high concentrations may cause a slight leaf scorch, especially early in the

season, so the concentration should be reduced to 5 lb/100 gal in the June spray and repeated after 10 days. Spraying during hot weather (21°C and above) may damage the leaves so the spraying should be reduced to 5 lb/100 gal. Damage to the fruits also could happen from a high concentration of calcium nitrate spray (143-145). All these recommendations are applied for apples in East Malling Research Station in England.

It has been reported that Ca nitrate is compatible at a high volume with most wettable powders, including captan, dinocap and liquid formulation of dinocap (68). Satisfactory disease and bitter pit control was obtained by mixing 10 lb/acre of Ca nitrate with either captan or dinocap. These pesticides are normally applied every 10-14 days, which is the same as Ca-spray recommendations. Captan is used to reduce fungus infections as a spray late in the season and can be mixed with Ca nitrate to control bitter pit. More field-scale studies are needed to ascertain the compatibility between calcium salts and pesticides.

Woodbridge (184-188) studied the effect of various calcium nutrients including Ca EDTA in apples. The concentration was 1 lb/100 gallons of water sprayed at weekly intervals during May and June. Calcium ethylene diamine tetraacetate (Ca-EDTA) treatment gave the best results in reducing the percentage of bitter pit in apples. However, our own studies (unpublished) have shown Ca-chelates to be

ineffective. A higher concentration of Ca nitrate is very effective. There was no effect of the earlier spray. Spray trials to test the compatibility of Ca salts with insecticides were also performed (185). Calcium nitrate (5 lb/100 gal) or Ca chloride (3 lb/100 gal) were mixed with the following pesticides: Diazinon, thiodan, zolone, omite, Guthion and Imidan separately. The materials were sprayed in three cover sprays on one group of trees and six cover sprays on the second group. All treatements were effective in controlling insects and bitter pit. The reduction of bitter pit in apple was about 50 to 100% depending on the number of sprays with Ca chloride or Ca nitrate. The authors (188) recommended the following spray program: spray with Ca nitrate (5 lb/100 gal) three times; the first spray about mid-June, the second spray in mid-July and the third spray in mid-August. It is important to cover the tree and the entire fruit with the sprayed material. Coverage is more important than the number of sprays and the concentration.

From the previous review we can see that the association of low concn of Ca in the fruit and the incidence of bitter pit in apple is well established but the work on cork spot of 'Anjou' pear is limited. Therefore, the objective of the study in this chapter was to identify those mineral elements most closely related to the incidence of cork spot in 'Anjou' pear. Special emphasis was given to Ca since its localized deficiency in other fruits is the primary cause

of many physiological disorders. Sprays with Ca salts is a practical method to raise the Ca concn in the fruit, therefore a large scale experiment of sprays with Ca salts in the commercial orchards was planned to test their effectiveness in controlling the incidence of cork spot in 'Anjou' pear.

Materials and Methods

'Anjou' pear fruits were sampled from two experiment station sites and selected commercial orchards. Trees at the Southern Oregon Experiment Station in Medford (Medford Station) and in the commercial orchards were 60 year old 'Anjou' pear on French (Pyrus communis L.) seedling rootstocks. Trees at the Hanley station were 12 year old 'Anjou' pear on either "Old Home" (OH) or "Old Home" x "Farmingdale" (OH x F) rootstocks.

I. Sampling for Mineral Analyses

A. Hanley Station. Twenty 'Anjou' pear trees were selected for unifor size and vigor, and two fruits from each tree were sampled biweekly from June through September 22. Ten fruits from each of five trees were combined and treated as one sample at all sampling dates except the final harvest date (September 4, 1976 and September 22, 1975). At the final harvest date (September 4, 1976), one box (90 to 100 fruit) was harvested from each tree and used for cork

spot evaluation at harvest, while in 1975 one box was harvested from two trees because the crop was light. Samples of five typical, normal fruit were taken from each box for mineral analyses, and if the trees showed cork spot, another sample of five fruits with cork spot was taken for mineral analyses.

B. Medford Station. Six 'Anjou' pear trees were selected based on vigor, crop and history of cork spot incidence. Samples of five typical fruits from each tree were collected biweekly from June 10 through September 22. At the final harvest date (September 22, 1975 and September 4, 1976), two boxes (100 to 110 fruit per box) were harvested from each tree, and used for cork spot evaluation. Samples of five normal fruits and five fruits with cork spot were taken from each box for mineral analyses.

C. Commercial orchards. Orchards with histories of cork spot (Clancy, Beebe, Corey) and another orchard with history of normal fruit (Naumes), were selected at different locations in Medford area in 1975. Two additional orchards (Eden Valley and Pinnacle-104), were sampled in 1976. Five to ten uniform trees were labeled in each orchard, and samples of five typical fruits were analyzed from each tree biweekly from June 24 through September 4 in 1976. In 1975, the fruit samples were collected from the blocks by walking between the rows and picking one fruit from the outside of the tree at random, except fruits which were not typical for size

or were misshapen were omitted and only typical trees for size and vigor were used. Samples of 25 fruits were analyzed biweekly from each block starting June 10 through September 22, 1975. At the final harvest date (September 4) in 1976, two boxes (100 to 130 fruit per box) were harvested from each tree and were evaluated for superficial cork spot. Samples of five normal fruits were taken from each box for mineral analyses. If the tree had fruits with cork spot, additional samples of five fruit with cork spot were taken for mineral analyses. At the final harvest date (September 22) in 1975, five boxes were harvested from each block by picking no more than five fruits from the outside of each tree at random, only typical appearing trees were used. Trees with pear decline were sampled separately. Fruit in all boxes was used for cork spot evaluation, and each of five normal and cork spotted fruit were taken from each box for mineral analyses.

II. Sprays with Calcium Salts in 1975

Spray treatments were applied in commercial orchards with histories of cork spot. Large scale sprays were done by the growers with the supervision of the farm adviser (Mr. R. Rackham).

A. Beebe orchard. Ten trees on one side of the orchard were left for controls and the rest of the orchard was sprayed with $Ca(NO_3)_2$ (5 lb/100 gal water), 400 gal per acre, on May 15, June 15,

and July 15. Five boxes of fruits were collected from the sprayed block and another five boxes from the control trees for use in cork spot evaluation. The fruits were collected using the sampling procedure described above for the 1975 season, and each box was treated as one sample. Five normal fruits and five fruits with cork spot were taken from each box for mineral analyses.

B. Cory (Ed Earnest orchard). Eight rows were sprayed with $Ca(NO_3)_2$ (5 lb/100 gal water), 250 gal per acre, and eight rows were sprayed with $CaCl_2$, (5 lb/100 gal water), 250 gal per acre, and eight rows were left as control. Spray treatments wer applied on May 30 and June 26, 1975. Samples for mineral analyses were collected from the treatment and the control on July 8, August 5, 19, and September 4 and 22, 1975. At the final harvest date September 22, 1975, samples of five boxes were collected from the treatments and the control for cork spot evaluation and storage, using the sampling procedure described above for the 1975 season, and each box was treated as one sample. Samples of five normal fruits and five fruits with cork spot were taken from each box for mineral analyses. Trees with pear decline were sampled separately.

C. Clancy (Rogue River orchard). Sixteen rows were sprayed with $Ca(NO_3)_2$, (5 lb/100 gal), 250 gal per acre, on June 20 and July 20, 1975, and eight rows were left as control. Samples for mineral analyses were collected on August 19 and September 22, 1975.

Some of the trees in this orchard were also showing pear decline, so additional samples were taken as described earlier. At the final harvest date (September 22, 1975), samples of five boxes of fruit were collected from the treatments and controls for cork spot evaluation and storage. Each box was treated as one replicate, and samples of five normal fruits, and five fruits with cork spot were taken from each box for mineral analyses.

D. Pinnacle orchard. Ten rows of trees were sprayed with $Ca(NO_3)_2$ (5 lb/100 gal water), 250 gal per acre, on May 30, and June 26, 1975, and ten rows were left as control. Samples for mineral analyses were collected July 8, August 13, and September 4 and 22, 1975. Samples of three boxes were harvested at the final harvest date (September 22, 1975), and treated as described above for Beebe orchard.

III. Spray with Calcium Salts in 1976

Calcium sprays were applied in two locations (Clancy and Eden Valley orchards) having a history of cork spot. Twenty trees were labeled randomly in each orchard. Ten trees were sprayed with $Ca(NO_3)_2$ (5 lb/100 gal water), and the other ten trees were sprayed with $CaCl_2$ (5 lb/100 gal water). Spray application was by high pressure hand gun sprayer, and surfactant (X-77) was added at the rate of 4 oz per 100 gal. All the tree was covered with the spray until the

liquid started to drip from the leaves. The sprays were applied on August 13 and 26, 1976. Labeled trees for the seasonal sampling were used as controls. Fruit samples for mineral analyses were collected from the labeled trees immediately before the sprays were applied, as described for other samples. Trees in both orchards were not uniform in size, vigor, and crop, and some trees had pear decline. Some of the trees showed about 15 percent cork spot even prior to the first spray. At the final harvest date (September 4, 1976) one box from each tree was harvested and used for cork spot evaluation and storage. Samples of five normal fruits and five fruits with cork spots were taken from each box for mineral analyses.

A. Beebe orchard. The orchard was sprayed in 1976 by the grower, the same way as described for 1975. Three trees were left as controls. Ten boxes were harvested from five trees labeled in a different site in the orchard, and another ten boxes were harvested from the control trees for cork spot evaluation and storage. Samples of five normal fruits and five fruits with cork spots were taken from each box for mineral analyses.

IV. Cork Spot Evaluation

All samples were evaluated for superficial cork spot at harvest and the percentage of cork spot was referred to as harvest cork or cork spot at harvest. All the fruits with cork spots were removed and

the normal fruits were stored in commercial cartons, with perforated plastic film liners, at 0°C. Samples were placed in storage on the day of harvest. In 1975, the fruit samples were evaluated periodically on December 15, February 20, and April 5, 1975, for the development of superficial cork spot in storage. At each evaluation date all the fruits with cork spot and rot were removed from the box. After the last evaluation date (April 5), the incidence of cork spot was calculated as a total percentage of cork spot over the six months period of storage, and referred to as total cork spot, storage cork or cork spot after storage. The fruits were not peeled in 1975. In 1976, the fruit samples were stored until May 5th, at which time the samples were evaluated. The fruits were tested for the superficial cork spot, and all the normal fruits were peeled to check for development of internal cork spot. The superficial and internal cork spot is designated as total cork spot after eight months of storage, or storage cork.

V. Cork Spot Rating

At harvest (September 22, 1975) samples of 93 boxes of fruits (95 to 120 fruit per box) were evaluated for superficial cork spot and grouped according to the percentage of cork spot into five groups as follows: group 1, 0.0 to 5% cork spot; group 2, 0.5 to 10%; group 3, 11 to 20%; group 4, 21 to 40% and group 5, 41 to 100% cork spot. The samples were grouped this way to study the differences in mineral

content of the fruit between the five groups. The incidence of cork spot at harvest in 1976 was unusually low and the study was limited to the 1975 season.

The incidence of cork spot after eight months in storage (May 5, 1976) was also grouped in five groups (130 boxes were used) for the same reason. All the fruits were peeled and the internal cork spot was added to the superficial. The five groups are: group 1, 0.0 to 20%; group 2, 21 to 40%; group 3, 41 to 60%; group 4, 61 to 80%; and group 5, 81 to 100% cork spot. The means of fruit mineral content were tested across the five groups using Duncan's multiple range test (Tables 9 and 10).

VI. Severity of Cork Spot

Samples of 20 boxes of fruits (95 to 120 fruit per box) were categorized as follows: normal, light, medium, and severe cork spot based on the percentage of the fruit surface which was covered with the spots. The same treatment was repeated on 43 boxes of fruits after five months in storage and another 53 boxes of fruits after eight months in storage. Means of the fruit mineral content of each group were compared with the normal fruits using LSD test (Tables 7 and 8).

VII. Preparation of Samples for Mineral Analyses

All harvested samples were sealed in plastic bags (129, 178),

and placed in cold storage in Medford until it was possible to transfer them to the cold storage in Corvallis. Samples for seasonal studies were analyzed within one week from the sampling date. Samples from the last harvest date were analyzed within a month from harvest. All fruits were washed with distilled water and allowed to dry in the air on paper towels (126, 132) just before cutting sections for analyses. Pairs of opposite quarters were cut longitudinally (125, 126, 131, 132) from five to ten fruit samples and bulked. Seeds and stems were removed to prevent errors in analytical results (126, 131, 182). Samples of 500 g were blended with 500 ml deionized water in a commercial blender for 2 min. About 400 ml of the mixture was further homogenized (Virtis 45, 000) for one min to make a fine suspension (124, 125, 129, 130, 132), which is referred to as the fruit water suspension.

VIII. Dry Ashing

The hygroscopic nature of dried pear fruit makes it hard to grind and get accurate dry weight of the ground fruit powder (124, 178). For this reason a suspension of fruit tissue was used for dry ashing.

Ten ml of the fruit water suspension was transferred by syringe into a crucible and dried at 70°C (124, 129, 130), and then ashed at 500°C for four hrs. The ash was dissolved in five ml of

1.8 N HCl (the solution also contained 0.1% Co and 0.5% Li) and the minerals (Ca, Mg, P, K, and B) were determined by "emission" spectrometry (Jarrell-Ash model 750).

The extremely low concn of Ca in the fruit compared with that in the leaves make the dry ashing technique not suitable for the determination of fruit Ca. Dry ashing frequently leads to losses of several mineral elements, especially Ca (124). Low temperature (500°C) dry ashing causes the losses of elements due to residual carbon, and ashing at high temperature (700 to 900°C) causes the losses of elements due to volatilization (88, 89). For these reasons Ca was extracted with concentrated HCl (130, 134), as described below.

IX. Extraction of Calcium with Hydrochloric Acid

Calcium was extracted by the method suggested by Perring (130), with the following modification: Ten ml of fruit water suspension was syringed into a 50 ml Erlenmyer flask and 10 ml concn HCl (37.8% A.R.) added. The suspension was boiled for 20 min on a hot plate with a small funnel placed in the neck of the flask to prevent drying. The digested contents were filtered through Whatman No. 31 filter paper. The sides of the flask were rinsed three times and the filter paper was washed twice with deionized water. The filtrate was diluted to 100 ml with deionized water and is referred to as the "acid extract," which was used to determine total Ca.

X. Extraction of Calcium with Ethanol

Forty ml of the fruit water suspension was mixed with 40 ml of 95% ethanol and the mixture further homogenized for 15 seconds. Twenty ml of the fruit ethanol suspension was filtered through Whatman No. 2 filter paper. The filter paper was washed three times with deionized water. The filtrate was diluted to 100 ml with deionized water and used for the determination of "ethanol extractable Ca" or the "ethanol soluble Ca" (M. A. Perring, personal communication).

XI. Extraction of Calcium with Water

Twenty ml of the fruit water suspension was filtered through Whatman No. 2 filter paper. The filtrate was diluted to 100 ml with deionized water and used for the determination of "Water extractable Ca" or the "Water soluble Ca" (M. A. Perring, personal communication). Fruit Ca was not extracted with either ethanol or water in 1976 seasonal sampling.

XII. Measurement of Calcium

Strontium chloride was added as an internal standard to a final concn of 3% to all the extracts. Calcium was determined by atomic absorption spectrophotometry (Perkin Elmer, model 303). A

calibration graph was prepared by reading a series of Ca carbonate standards in the range of 0.0 to 25 ppm, before and after each group of samples.

XIII. Measurement of Total Nitrogen

Five ml of the fruit water suspension was transferred to a Kjeldahl tube and dried at 70°C. Digestions and automated N determinations were made with Auto-Analyzer modules (Technicon Instruments Co. Ltd.) (129).

XIV. Statistical Procedure

Simple linear correlations between the incidence of cork spot and the mineral content of the fruit were calculated on the CDC 3300 computer. The incidence of cork spot is the percentage of fruits showing either superficial cork spot at harvest or after six months in storage in 1975 or total of superficial and internal cork spot after eight months in storage in 1976. The percent of cork spot is based on one box fruit sample, and the values of fruit Ca are the means of duplicated sample analyses. Multiple correlation coefficients were obtained with "stepwise" and "Backstep" programs, which are add best and drop worst, respectively. The multiple correlations and the simple linear correlations were used to select the variables which were most closely correlated with the incidence of cork spot.

In the multiple correlations, no more than five independent variables were involved.

Calcium spray treatments, the effect of locations, severity of cork spot, and cork spot rating were analyzed using one factor analyes of variance program.

Results

I. Correlations between Cork Spot and Mineral Elements

Results of one on one linear correlation coefficients (r-values) of mineral elements in relation to cork spot of 'Anjou' pear fruit are shown in Tables 1 and 2. These tables are the results of seasonal fruit mineral analysis of all orchards sampled in 1975 and 1976. The incidence of cork spot was negatively correlated with Ca concn in the fruit during growth and at harvest. This correlation is highly significant in both years. [Unless otherwise specified, "Ca" or "Total Ca" refer to acid extractable calcium.] Tables 1 and 2 show that the correlation between Ca and the incidence of cork spot increases and became more significant, as the sampling date approaches the final harvest date. The incidence of cork spot at harvest in 1976 was considerably lower. This was anticipated as the disorder often follows an alternate year pattern. The correlation between Ca and the incidence of harvest cork spot is -0.42 on

Table 1. Linear correlations between the incidence of harvest cork (% CS at harvest) and the mineral content of the fruit during development and at harvest (r values).

Sampling date	d.f.	Ca-acid extract (total)	N	K	P	Mg	B	N/Ca	K/Ca	Mg/Ca	(K+Mg)/Ca	Total Ca/fruit	Ca-ethanol extractable	Ca-water extractable	Water extractable	Total Ca	Water extractable/Water-non extractable Ca
Survey 1975																	
7/8	21	-0.48*	0.24 NS	-0.51*	0.09 NS	0.02 NS	0.41*	0.53**	-0.01 NS	0.46*	0.05 NS	-0.28 NS	-0.22 NS	-0.40 NS	0.39 NS		0.40 NS
7/22	19	-0.21 NS	-0.11 NS	-0.09 NS	0.36 NS	0.24 NS	0.30 NS	0.07 NS	0.12 NS	0.30 NS	0.15 NS	-0.15 NS	-0.26 NS	-0.44*	0.01 NS		0.05 NS
8/5	23	-0.59**	0.04 NS	-0.53**	0.19 NS	-0.29 NS	0.30 NS	0.62**	0.14 NS	0.21 NS	0.15 NS	-0.40*	-0.37 NS	-0.61**	0.33 NS		0.27 NS
8/19	29	-0.65**	-0.08 NS	-0.52**	0.41*	0.14 NS	0.18 NS	0.39*	0.02 NS	0.35 NS	0.05 NS	-0.06 NS	-0.15 NS	-0.35 NS	0.32 NS		0.33 NS
9/4	34	-0.75**	0.11 NS	-0.40*	0.32 NS	0.05 NS	0.12 NS	0.45**	0.33*	0.45**	0.34*	-0.54**	-0.47**	-0.28 NS	0.63**		0.58**
9/22	92	-0.66**	0.40**	-0.31**	0.20*	-0.02 NS	0.08 NS	0.74**	0.49**	0.45**	0.50**	-0.54**	-0.55**	-0.50**	0.41**		0.42**
Survey 1976																	
6/24	30	0.42*	-0.20 NS	-0.47**	0.16 NS	-0.11 NS	0.60**	0.33 NS	-0.57**	-0.28 NS	-0.58**	-0.29 NS	--	--	--	--	--
7/14	46	0.04 NS	-0.37**	-0.50**	0.09 NS	-0.04 NS	0.65**	-0.23 NS	-0.43**	-0.08 NS	-0.43**	-0.21 NS	--	--	--	--	--
7/29	46	-0.05 NS	-0.25 NS	-0.40**	0.16 NS	-0.05 NS	0.54**	-0.14 NS	-0.31**	-0.03 NS	-0.31*	-0.19 NS	--	--	--	--	--
8/13	50	-0.32*	-0.06 NS	-0.43**	0.07 NS	0.08 NS	0.26 NS	0.20 NS	-0.34*	0.27 NS	-0.33*	-0.31*	--	--	--	--	--
8/26	50	-0.31*	-0.10 NS	-0.31*	0.15 NS	-0.04 NS	0.31*	0.15 NS	-0.08 NS	0.20 NS	-0.08 NS	-0.41**	--	--	--	--	--
9/4	128	-0.42**	0.19*	-0.06 NS	-0.01 NS	-0.04 NS	0.00 NS	0.55**	0.39**	0.30**	-0.40**	-0.37**	--	--	--	--	--

NOTE: Calcium was not extracted with water or ethanol in 1976.

*Significant at the 5% level; **Significant at the 1% level; NS Not Significant

Table 2. Linear correlation between incidence of storage cork (% of cork spot after storage) and the mineral content of the fruit during development and at harvest (r values).

Sampling date	d. f.	Ca-acid extract (total)	N	B	K	P	Mg	N/Ca	K/Ca	Mg/Ca	(K + Mg)/Ca	Total Ca/fruit	Ca-ethanol extract	Ca-water extract	Water extractable / Total Ca	Water extractable / Water nonextractable Ca
Survey 1975																
7/8	21	-0.63**	0.20 NS	0.69**	-0.62**	0.07 NS	-0.16 NS	0.67**	0.04 NS	0.43*	0.10 NS	-0.37 NS	-0.27 NS	-0.58**	0.47*	0.49*
7/22	19	-0.25 NS	-0.04 NS	0.41 NS	-0.30 NS	0.33 NS	0.31 NS	0.16 NS	0.03 NS	0.37 NS	0.07 NS	-0.01 NS	-0.38 NS	-0.47*	0.03 NS	0.06 NS
8/S	23	-0.59**	0.17 NS	0.28 NS	-0.72**	0.16 NS	-0.24 NS	0.72**	-0.01 NS	0.31 NS	0.00 NS	-0.54**	-0.50**	-0.70**	0.29 NS	0.23 NS
8/19	29	-0.7S**	0.01 NS	0.2S NS	-0.SS**	0.S0**	0.16 NS	0.S4**	0.08 NS	0.44*	0.12 NS	-0.30 NS	-0.49**	-0.62**	0.12 NS	0.12 NS
9/4	34	-0.71**	0.13 NS	0.16 NS	-0.44**	0.41*	0.07 NS	0.4S**	0.24 NS	0.44**	0.26 NS	-0.S7**	-0.40*	-0.21 NS	0.62**	0.56**
9/22	92	-0.76**	0.3S**	0.14 NS	-0.43**	0.35**	0.02 NS	0.68**	0.42**	0.S0**	0.44**	-0.64**	-0.54**	-0.43**	0.S7**	0.S1**
Survey 1976																
6/24	30	0.11 NS	0.11 NS	0.4S**	-0 37*	0.16 NS	-0.46**	0.10 NS	-0.34	-0.51**	-0.36**	-0.4S**	--	--	--	--
7/14	46	-0.29*	0.03 NS	0.22 NS	-0.44**	-0.07 NS	-0.24 NS	0.19 NS	-0.18 NS	-0.09 NS	-0.19 NS	-0.41**	--	--	--	--
7/29	46	-0.50**	0.21 NS	0.37**	-0.28 NS	0.29*	0.11 NS	0.38**	0.23 NS	0.36*	-0.24 NS	-0.52**	--	--	--	--
8/13	50	-0.S9**	0.36**	0.08 NS	-0.54**	0.14 NS	0.23 NS	0.S8**	-0.21 NS	0.49**	-0 20 NS	-0.56**	--	--	--	--
8/26	S0	-0.7S**	0.26*	0.32*	-0.44**	0.01 NS	-0.21 NS	0.71**	0.22 NS	0.21 NS	0.22 NS	-0.76**	--	--	--	--
9/4	128	-0.76**	0.S1**	-0.07 NS	-0.27**	0.12 NS	0.00 NS	0.7S**	0.28**	0.42**	0.29**	-0.67**	--	--	--	--

NOTE: Calcium was not extracted with ethanol or water in 1976.

*Significant at the S% level; **Significant at the 1% level; NS Not Significant

September 4, 1976 compared with -0.75 on September 4, 1975. The incidence of cork spot after storage is positively correlated with N in both years, but that correlation is not significant in 1975, except on September 22 (the final harvest date), while in 1976 it is significant on the last three sampling dates (Table 2).

Cork spot incidence after storage is positively correlated with N:Ca ratio in both years, and that correlation increased as the sampling date approached the final harvest date, reaching 0.68 and 0.75 in 1975 and 1976, respectively (Table 2). The correlation between N:Ca ratio and the incidence of cork spot at harvest is not as high as that with storage cork especially in 1976 in which the percentage of cork spot at harvest is very low. The correlation between harvest cork and theN:Ca ratio increased toward the final harvest date and reached 0.74 on September 22, 1975 and 0.55 on September 4, 1976 (Table 1).

Total Ca in the fruit (mg/fruit) and the incidence of cork spot after storage is negatively correlated in both years and on all sampling dates and the significance of that correlation increased as the sampling date approached the final harvest date, and reached -0.64 on September 22, 1975, and -0.76 on August 26, 1976 (Table 2). The correlation between the incidence of cork spot and total Ca in the fruit is higher after storage than at harvest.

Water extractable Ca (water soluble) is significantly correlated

with the incidence of cork spot after storage and is always negative. This correlation is higher than the correlation with the acid extractable Ca, on the sampling dates July 22, 1975 and Augus5 5, 1975. (Table 2). Storage cork spot is significantly correlated with the ratio of water extractable:total Ca at the last two sampling dates (September 4 and 22, 1975), and the same is true for the ratio of water extractable:water nonextractable Ca (total Ca-water soluble Ca) (Table 2).

The incidence of cork spot at harvest is always negatively correlated with fruit K on all sampling dates and in both years, and that correlation decreases as the sampling date approaches the final harvest date in both years and becomes nonsignificant at the last sampling date in 1976 (Table 1). The correlation between K and the storage cork spot is always negative and highly significant on all sampling dates in both years except on July 22, 1975, and July 29, 1976 which are not significant (Table 2).

Boron has no significant correlation with the incidence of cork spot after storage on all sampling dates in 1975, except on July 8, 1975, and this correlation decreased as the sampling date approached the final harvest date. In 1976 the significance of B is not consistent throughout the season, and the highest correlation is on the first sampling date, May 24, 1976 (Table 2). The same is true for the correlation between B and the incidence of cork spot at harvest in 1975. In 1976 the correlation between B and cork spot at harvest is

always positive and highly significant at the first three sampling dates (Table 1). The incidence of both cork spot at harvest and after storage have no significant correlation with Mg on all sampling dates and in both years except on May 24, 1976 (Table 2).

Phosphorus is positively correlated with the incidence of cork spot after storage on the last three sampling dates, August 19, September 4 and 22, 1975 (Table 2), while it had no significance in 1976. The same is true for the incidence of harvest cork except on August 19 and September 22, 1975 (Table 1).

The results in Tables 1 and 2 may not have established the cause of cork spot, but they have showed a strong relationship between the incidence of cork spot and fruit mineral content. At this point we can not say that Ca deficiency in the fruit is the main cause of cork spot but we can safely conclude that Ca deficiency in the fruit is strongly associated with the incidence of cork spot of 'Anjou' pear fruit at harvest and after storage.

II. Multiple Correlations between Cork Spot and Mineral Elements

Results of multiple linear correlations of mineral elements in relation to cork spot of 'Anjou' pear fruit are expressed in coefficient of determination (r^2-values), as shown in Tables 3 and 4. These tables are the results of seasonal fruit mineral analyses of all

Table 3. Multiple linear correlations (coefficient of determination) between the incidence of harvest cork spot (% cs at harvest) and the mineral content of the fruit during development and at harvest (r^2 values).

Sampling date Survey 1975	Dependent variable	Independent variables (1)	r^2 Value
7/8	CS-Harvest	-K + N/ Ca	0. 34 NS
df = 21	" "	-Mg + Mg/ Ca	0. 35 NS
	" "	+P - K + N/ Ca	0. 38 NS
	" "	- Mg + B + Mg/Ca	0. 37 NS
	" "	+ P - K + N/ Ca + Mg/ Ca	0. 38 NS
	" "	+ Ca + Mg + B + Mg/ Ca	0. 40 NS
	" "	+ P - K + N/ Ca + Mg/ Ca + Ca/ fruit	0. 39 NS
7/22	" "	-Ca (water soluble)- N/ Ca	0. 35 NS
df = 19	" "	- Ca (water soluble)- Ave f. w.	0. 26 NS
	" "	+ Mg - Ca (water soluble) - N/ Ca	0. 54 NS
	" "	-N - Ca (water soluble) - Ave f. w.	0. 32 NS
		- N - Ca (water soluble) - Ave f. w. + Ca/ fruit	0. 46 NS
	" "	+ Mg - Ca (water soluble) - Ca (ethanol soluble) - N/ Ca	0. 59*
	" "	-N - Ca (water soluble) - Ave f. w. + Ca/ fruit + soluble/ nonsol. Ca	0. 64 NS
	" "	+ Mg + K - Ca (water soluble) - Ca (ethanol soluble) - N/ Ca	0. 64 NS
8/5	" "	-Ca (water soluble) + N/ Ca	0. 49*
df = 23	" "	-Ca (water soluble) + B	0. 53*
	" "	-Ca (water soluble) + B + N/Ca	0. 56*
	" "	- Ca (water soluble) + B + water soluble/total Ca	0. 56*
	" "	- Ca (water soluble) + B + water soluble/total Ca - soluble/nonsoluble Ca	0. 65*
	" "	- Ca (water soluble) + B + water soluble/total Ca - soluble/ nonsoluble Ca - (K + Mg)/ Ca	0. 65 NS

Table 3. (Continued)

Sampling date Survey 1975	Dependent variable	Independent variables (1)	r^2 Value
8/19	CS- Harvest	- Ca + Ave f. w	0.52**
df = 29	" "	- Ca/fruit + Ave f. w.	0.55**
	" "	- Ca - K + Ave f. w.	0.60**
	" "	- Ca/fruit + Ave f. w. - (K + Mg)/Ca	0.62**
	" "	- Ca - K + Ave f. w. - Ca/fruit	0.67**
	" "	- Ca/fruit + Ave f. w. - (K + Mg)/Ca + Ca (water soluble)	0.63**
	" "	- Ca - K + P + Ave f. w. - Ca/fruit	0.71**
	" "	- Ca/fruit + Ave f. w. - (K + Mg)/ Ca + Ca (water soluble) - water soluble/total Ca	0.69**
9/4	" "	- Ca + P	0.60**
df = 34	" "	- N + N/ Ca	0.59**
	" "	- Ca - Ca (ethanol soluble) + P	0.62**
	" "	- N + P + N/Ca	0.62**
	" "	-Ca - Ca (ethanol soluble) + P + water soluble/total Ca	0.65**
	" "	-N + P + N/ Ca - Ca (ethanol soluble)	0.65**
	" "	-Ca - Ca (ethanol soluble) + P + water soluble/total Ca - Ave f. w.	0.66**
9/22	" "	+ N /Ca - N	0.79**
df = 92	" "	+ Ca + N/Ca - N	0.81**
	" "	+ N/Ca - N - water soluble/total Ca	0.80**
	" "	+ Ca + N/Ca + P - N	0.82**
	" "	- N + N/ Ca + water soluble/nonsoluble Ca - soluble/total Ca	0.81**
	" "	+ Ca + N/Ca + P - N - Ca (ethanol soluble)	0.83**

Table 3. (Continued)

Sampling date Survey 1976	Dependent variable	Independent variables (1)	r^2 Value
6/24			
df = 30	CS-Harvest	- K + B	0.43*
	" "	+ Ca - K	0.40 NS
	" "	- K + B + Ca	0.49 NS
	" "	+ Ca - K + (K + Mg)/ Ca	0.53*
	" "	- K + B + Ca - Mg	0.51*
	" "	- K - Mg + Ca + (K + Mg//Ca	0.56*
	" "	- K + B + Ca - Mg + P	0.54*
	" "	- K + Mg + Ca + (K + Mg)/ Ca + P	0.59*
7/14	" "	- K + B	0.47**
df = 46	" "	+ B - Mg/ Ca	0.45*
	" "	- K + B - Mg/ Ca	0.53**
	" "	+ B - Mg/ Ca - K/ Ca	0.47**
	" "	- K + B - Mg/ Ca - N	0.56**
	" "	- Ca + B - Mg/ Ca - K/ Ca	0.52**
	" "	- Ca - K + B - Mg/ Ca - N	0.56**
	" "	- Ca + B - M/ Ca - K/ Ca - N	0.56**
7/29	" "	- P + B	0.36*
df = 46	" "	- K + B	0.34 NS
	" "	- P + B - N	0.39 NS
	" "	- K + B - Mg/ Ca	0.37 NS
	" "	- Ca - P + B - N	0.41 NS
	" "	- K + B - Mg/ Ca + K/ Ca	0.37 NS
	" "	- Ca - P - K - N + B	0.42 NS
	" "	- K + B + Mg - Mg/ Ca + K/ Ca	0.42 NS

Table 3. (Continued)

Sampling date Survey 1976	Dependent variable	Independent variables (1)	r^2 Value
8/13	CS-Harvest	- N - K	0.21 NS
df= 50	" "	- Ca - (K + Mg)/ Ca	0.23 NS
	" "	- N - K + B	0.24 NS
	" "	- Ca + K - (K + Mg)/ Ca	0.33 NS
	" "	- N - K + B + N/ Ca	0.30 NS
	" "	- Ca + K - N - (K + Mg)/ Ca	0.36 NS
	" "	- N - K + B + N/ Ca - (K + Mg)/ Ca	0.39 NS
	" "	- Ca + K - N - (K + Mg)/ Ca + N/ Ca	0.46 NS
8/26	" "	- N - Ca/ fruit	0.26 NS
df = 50	" "	- Mg + Mg/ Ca	0.20 NS
	" "	- N + B - Ca/ fruit	0.30 NS
	" "	- Mg + Mg/ Ca - Ave f. w.	0.26 NS
	" "	- N + B - Ca/ fruit - (K + Mg)/ Ca	0.31 NS
	" "	- Mg + Mg/ Ca - Ave f. w. - N/ Ca	0.39 NS
	" "	- Ca - N + B - Ca/ fruit - (K + Mg)/ Ca	0.32 NS
	" "	- Mg + Mg/ Ca - Ave f. w. - N/ Ca + Ca/ fruit	0.41 NS
9/4	" "	- N + N/ Ca	0.44**
df= 128	" "	- K + (K + Mg)/ Ca	0.38**
	" "	- N + Ca + N/ Ca	0.53**
	" "	- K + (K + Mg)/ Ca - Ave f. w.	0.40**
	" "	- N + Ca + N/ Ca - Ave f. w.	0.57**
	" "	- K + (K + Mg)/ Ca - Ave f. w. + Ca/ fruit	0.62**
	" "	- K - Ca + (K + Mg)/ Ca - Ave f. w. + Ca/ fruit	0.64**

(1): (-) or (+) indicate sign of partial correlation.

NS Not significant; *Significant at the 5% level; **Significant at the 1% level.

NOTE: Ca was not extracted with ethanol or water in 1976.

Table 4. Multiple linear correlations (coefficient of determination) between the incidence of storage cork spot (% CS after storage) and the mineral content of the fruit during development and at harvest (r^2-value).

Sampling date Survey 1975	Dependent variable	Independent variables (1)	r^2 Value
7/8	CS-Storage	- K + N/Ca	0.53*
df=21	" "	- N + N/Ca	0.52*
	" "	- N - K + N/Ca	0.60*
	" "	- N + N/Ca - (K + Mg)/Ca	0.65**
	" "	+ Ca - N - K + N/Ca	0.71**
	" "	+ Ca - N - K + N/Ca - (K + Mg)/Ca	0.71**
	" "	+ Ca - N + N/Ca + Mg/Ca - (K + Mg)/Ca	0.71**
	" "	+ Ca - N - K + N/Ca + soluble/nonsoluble Ca	0.73**
7/22	" "	+ B - Ca (water soluble)	0.40 NS
df= 19	" "	- Ca (water soluble) - Ave f. w.	0.36 NS
	" "	+ B - Ca (water soluble) - N/Ca	0.48 NS
	" "	- Ca (water soluble) - Ave f. w. + Ca/fruit	0.37 NS
	" "	+ Mg + B - Ca (water soluble) - N/Ca	0.58 NS
	" "	- Ca (water soluble) - Ave f. w. + Ca/fruit + soluble/nonsoluble Ca	0.54 NS
8/5	" "	+ N/Ca - water soluble/nonsoluble Ca	0.74**
df = 23	" "	- N + N/Ca - water soluble/nonsoluble Ca	0.80**
	" "	- N + B + N/Ca - water soluble/nonsoluble Ca	0.86**
	" "	- N + B + N/Ca - water soluble/nonsoluble Ca - (K + Mg)/Ca	0.89**
8/19	" "	- Ca + P	0.63**
df = 29	" "	- N + N/Ca	0.64**
	" "	-Ca + P - B	0.68**
	" "	- N + N/Ca - water soluble/total Ca	0.69**
	" "	- Ca + P - B - water soluble/nonsoluble Ca	0.71**
	" "	- N + N/Ca - water soluble/total Ca - (K + Mg)/Ca	0.71**
	" "	- Ca + P - B - water soluble/nonsoluble Ca + Ca (water soluble)	0.72**

Table 4. (Continued)

Sampling date Survey 1975	Dependent variable	Independent variables (1)	r^2 Value
9/4	CS-Storage	- Ca + P	0.59**
df = 34	" "	- Ca (ethanol soluble) + water soluble/total Ca	0.49**
	" "	- Ca + P + water soluble/total Ca	0.60**
	" "	- Ca (ethanol soluble) + P + water soluble/total Ca	0.60**
	" "	- Ca + P + Ca (ethanol soluble) + water soluble/total Ca	0.63**
	" "	- Ca (ethanol soluble) + P + N/Ca + water soluble/total Ca	0.63**
	" "	- Ca + P + Ca (ethanol soluble) + water soluble/total Ca - water soluble/ nonsoluble Ca	0.64**
	" "	- Ca (ethanol soluble) + P - N + N/Ca + water soluble/total Ca	0.65**
9/22	" "	- Ca + N/Ca	0.62**
df = 92	" "	- N + N/Ca	0.70**
	" "	- Ca - N + N/Ca	0.72**
	" "	- N + P + N/Ca	0.76**
	" "	- Ca N + P + N/Ca	0.77**
	" "	- N + P - Ca (ethanol soluble) + N/Ca	0.77**
	" "	- Ca - N + P - Ca (ethanol soluble) + N/Ca	0.77**
	" "	- Ca (ethanol soluble) - N + P + N/Ca + water soluble/total Ca	0.78**
Survey 1976			
6/24	" "	- Ca/fruit - Mg/Ca	0.38*
df = 30	" "	- K - Mg/Ca	0.37*
	" "	- Ca/fruit - Mg/Ca + P	0.46*
	" "	- K - Mg/Ca + P	0.55*
	" "	- Ca/fruit - Mg/Ca + P - K	0.59**
	" "	- Ca/fruit - Mg/Ca + P - K - Ca	0.67**

Table 4. (Continued)

Sampling date Survey 1976	Dependent variable	Independent variables (1)	r^2 Value
7/14	CS-Storage	- Ca + B	0.30 NS
df = 46	" "	- Ca/fruit - K/Ca	0.26 NS
	" "	- Ca + B - Ave. f. w.	0.33 NS
	" "	- Ca/fruit - K/Ca - Mg	0.34 NS
	" "	- Ca + B - Ave. f. w. (K + Mg)/ Ca	0.35 NS
	" "	- Ca/fruit - K/ Ca - Mg + P	0.37 NS
	" "	- Ca + B + P- Ave. f. w. - (K + Mg)/ Ca	0.37 NS
	" "	- Ca - Ca/fruit - K/Ca - Mg + P	0.42 NS
7/29	" "	+ P - Ca/fruit	0.36*
df = 46	" "	- K + (K + Mg)/ Ca	0.2S NS
	" "	+ P - Ca/ fruit - Ca	0.41*
	" "	- K + (K + Mg)/ Ca + P	0.39*
	" "	+ P - Ca/ fruit - Ca + B	0.40 NS
	" "	- K + (K + Mg)/ Ca + P - Mg/ Ca	0.40 NS
	" "	+ P - Ca/fruit - Ca + B + Ave f. w.	0.45 NS
	" "	- K + (K + Mg)/ Ca + P - Mg/ Ca + Mg	0.43 NS
8/13	" "	- Ca - K/Ca	0.41**
df = 50	" "	- Ca - Ave f. w.	0. 37*
	" "	- Ca - K/Ca + N/Ca	0.44*
	" "	- Ca - Ave f. w. + Ca/fruit	0.45**
	" "	- Ca + K - K/Ca + N/Ca	0.46*
	" "	- Ca - Ave f. w. + Ca/fruit + P	0.48**
	" "	- Ca + K - K/Ca + N/Ca + P	0.47 NS
	" "	- Ca - Ave f. w. + Ca/fruit + P - K/Ca	0. S0*

Table 4. (Continued)

Sampling date Survey 1976	Dependent variable	Independent variables (1)	r^2 Value
8/26	CS-Storage	- Ca - Ca/fruit	0.65**
df = 50	" "	- N + N/Ca	0.69**
	" "	- Ca - Ca/fruit + B	0.69**
		-N + B + N/Ca	0.73**
	" "	- Ca - Mg + B - Ca/fruit	0.71**
		-N + B + N/Ca - Ca/fruit	0.75**
	" "	- Ca - Mg + B - Ca/fruit + N/Ca	0.72**
		-N + B + N/Ca - Ca/fruit - Mg/Ca	0.77**
9/4	" "	- Ca + N	0.66**
df= 128		- Ca + N/Ca	0.65**
	" "	- Ca + N - K	0.67**
		-Ca + N/Ca - (K + Mg)/Ca	0.67**
	" "	- Ca + N - K + N/Ca	0.67**
		- Ca + N/Ca - (K + Mg)/Ca + K/Ca	0.67**
	" "	- Ca + N - K + N/Ca - (K + Mg)/Ca	0.68**
		- Ca + N/Ca - (K + Mg)/Ca + P + K/Ca	0.68**

*Significant at the 5% level

**Significant at the 1% level

NS - Not significant

(1): (-) or (+) indicate sign of partial correlation.

NOTE: Ca was not extracted with ethanol or water in 1976.

orchards sampled in 1975 and 1976. The coefficient of determination (r^2) increases as the sampling date approaches the final harvest date. Multiple linear correlations between mineral elements and the incidence of cork spot at harvest and after storage show that Ca and the N:Ca ratio are the most closely related variables to the incidence of cork spot in both years (Tables 3 and 4). In 1976, K and K:Ca ratio was associated with the incidence of cork spot at harvest and after storage, but in 1975 this association was not as close. The possible involvement of B in the incidence of cork spot is mainly in the early sampling dates and it is associated with cork spot at harvest more than the cork spot after storage. Phosphorus was positively associated with harvest and storage cork spot on the last three sampling dates in 1975, and on nearly all sampling dates in 1976 (Tables 3 and 4). Water soluble Ca (water extractable) was more negatively associated with the incidence of harvest cork spot, and the incidence of cork spot after storage, than the total Ca (acid extractable) in the earlier sampling dates, whereas the ethanol soluble (ethanol extractable Ca) was more important than the water soluble Ca later in the season (Tables 3 and 4).

III. Severity of Cork Spot

A. Correlations between the severity of cork spot and the mineral elements.

Results of one on one linear correlation

coefficient (r-values) of mineral elements in relation to the severity of cork spot at three sampling dates are shown in Table 5. Severity of cork spot is significantly correlated with Ca at harvest, after five months in storage, and after eight months in storage, and the r-values are -0.86, -0.86, and 0.80, respectively. The next important variable is the N:Ca ratio which had r-values of 0.84, 0.80 and 0.77 in respect to each harvest and storage sampling date. Severity of cork spot had no significant correlations with B, N, P, K, and Mg. Water soluble Ca (water extractable) had the highest correlation with the severity of cork spot at harvest (r-value = -0.90), but the correlation decreased after storage. The same is true for the ethanol soluble Ca (ethanol extractable) with the r-values of -0.76, -0.53 and -0.59 in the three sampling dates. Total Ca per fruit (mg per fruit) is closely correlated with cork spot severity and the r-values are -0.88, -0.74, and -0.75 for the three sampling dates.

B. Multiple correlations between the severity of cork spot and mineral elements. Results of multiple linear correlations of mineral elements in relation to severity of cork spot are expressed in coefficient of determination (r^2-values), as shown in Table 6. The two most important variables which are closely associated with cork spot severity on the three sampling dates are the water soluble Ca and the ratio of water soluble Ca:total Ca; the r^2-value for the three sampling dates are 0.86, 0.81, and 0.72, respectively (Table 6).

Table 5. Linear correlations between the severity of cork spot and the mineral content of the fruit at harvest and during storage (r-values).

Sampling date	d.f.	Total Ca	Mg	N	B	K	P	N/Ca	K/Ca	Mg/Ca	(K+Mg)/Ca	Total Ca/fruit	Ca-ethanol extractable	Ca-water extractable	Water extractable / Total Ca	Water extractable / Water nonextractable
		**	NS	NS	NS	NS	NS	**	**	**	**	**	**	**	**	**
9/10/76	19	-0.86	-0.22	+0.30	+0.26	-0.37	+0.34	+0.84	+0.80	+0.70	+0.80	-0.88	-0.76	-0.90	+0.59	+0.55
		**	NS	NS	NS	NS	NS	**	**	**	**	**	**	**	**	**
2/2/77	42	-0.86	-0.18	+0.07	-0.26	-0.05	+0.08	+0.80	+0.69	+0.62	+0.69	-0.74	-0.53	-0.46	+0.68	+0.64
		**	NS	NS	NS	NS	NS	**	**	**	**	**	**	**	**	**
5/3/77	52	-0.80	-0.19	+0.15	-0.11	-0.14	+0.16	+0.77	+0.66	+0.60	+0.66	-0.75	-0.59	-0.61	+0.57	+0.67

*Significant at the 5% level; ** Significant at the 1% level; NS Not significant

Table 6. Multiple correlations (coefficient of determination) between the severity of cork spot and the mineral content of the fruit at harvest and during storage (r^2 values).

Sampling date	Dependent variable	Independent variables (1)	r^2 Value
9/10/1976	CS-Severity	-Ca (water soluble) + water soluble/total Ca	0.86**
df = 42	" "	-Ca (water soluble) + water soluble/total Ca + B	0.90**
	" "	-Ca (water soluble) + water soluble/total Ca-N/Ca	0.89**
	" "	-Ca (water soluble) + water soluble/total Ca+B-N	0.96**
	" "	-Ca (water soluble) + water soluble/total Ca-N/Ca + Mg/Ca	0.93**
2/2/1977	" "	-Ca + water soluble/water nonsoluble Ca	0.77**
df = 42	" "	-Ca (water soluble) + water soluble/total Ca	0.81**
	" "	-Ca-Ca (ethanol soluble) + water soluble/nonsoluble Ca	0.81**
	" "	-Ca (water soluble) + water soluble/total Ca-B	0.83**
	" "	-Ca-B-Ca (ethanol soluble) + water soluble/nonsoluble Ca	0.83**
	" "	-Ca- water soluble) - Ca (ethanol soluble) - B + soluble/total Ca	0.86**
	" "	-Ca-Ca(ethanol soluble) + water soluble/nonsoluble Ca - B + Mg/Ca	0.88**
5/3/1977	" "	-Ca - Ca (ethanol soluble)	0.68**
df=52	" "	-Ca (water soluble) + water soluble/total Ca	0.72**
	" "	-Ca - Ca (ethanol soluble) + Mg/Ca	0.72**
	" "	-Ca (water soluble) - Ca (ethanol soluble) + water soluble/total Ca	0.75**
	" "	-Ca - Ca (ethanol soluble) + Mg/Ca + K/Ca	0.74**
	" "	-Ca (water soluble) - Ca (ethanol soluble) + Mg/Ca + water soluble/total Ca	0.75**

(1): (-) or (+) indicate sign of partial correlation.

*Significant at the 5% level; **Significant at the 1% level; NS is Not significant.

Total Ca (acid extractable) became more important after five months in storage, while the ethanol soluble (ethanol extractable) fraction became more important after eight months in storage. Boron and the N:Ca ratio are relatively important at harvest.

C. Severity of cork spot and fruit mineral content. If the mineral content of severely cork spotted fruit is compared with the normal fruit at harvest, we find that the Ca (acid extractable) content of the normal fruit is almost two fold higher than that of fruits with severe cork spot. The same holds true for water soluble Ca and ethanol soluble Ca. These differences also hold for the sampling after five months in storage (Tables 7 and 8). The concn range of Ca from normal to severely cork spotted 'Anjou' fruits is as follows: 8.1, 5.7, 4.8 and 3.8 mg/100 g of fresh weight (Table 8).

Nitrogen concn was also significantly different comparing severely cork spotted fruit with normals at harvest (Table 7), but not after five months in storage (Table 8). However calculating from Table 8 the N:Ca ratios after five months in storage yields the following values: 7.3, 11.3, 12.8, and 16.0 for normal, light, medium, and severe cork spot, respectively. At harvest the K content in normal fruit is significantly higher than those showing severe cork spot, 101.8 and 86.8 mg/100 g of fresh weight, respectively. The differences were not significant after storage (Tables 7 and 8). Phosphorus is higher in the fruits with severe cork spot than normal

Table 7. Severity of superficial cork spot at harvest (September 10, 1976) and the mineral content of 'Anjou' pear fruit. Each value is the mean of ten samples.

Severity of cork spot df=27 and 2	Mineral content of the fruit (mg/100 g of f.w.)							ppm
	Ca-acid extract	Ca-ethanol extract	Ca-water extract	N	K	P	Mg	B
Normal	6.3	4.8	3.2	52.8	101.8	12.4	5.2	14.9
Light	4.3	3.9	2.5	66	86.4	13.2	4.3	14.8
Severe	3.5	2.9	1.9	58.8	86.8	14.0	4.6	17.6
$LSD_{0.05}$	0.42	0.24	0.08	3.40	18.54	2.29	0.64	0.74
$LSD_{0.01}$	0.57	0.33	0.11	4.65	25.35	3.13	0.87	1.01

NOTE: The normal fruits were used as control.

Table 8. Severity of superficial cork spot after five months in storage and mineral content of 'Anjou' pear fruit. Each value is the mean of ten samples.

Severity of cork spot df= 36 and 3	Mineral content of the fruit (mg/100 g of f. w.)							ppm
	Ca-acid extract	Ca-ethanol extract	Ca-water extract	N	K	P	Mg	B
Normal	8.1	4.6	3.0	58.7	89.8	14.12	5.2	21.1
Light	5.7	4.2	2.4	64.5	84.0	13.12	4.4	19.1
Medium	4.8	3.5	2.1	61.4	85.6	14.44	3.9	19.2
Severe	3.8	3.3	2.2	60.5	87.8	14.5	5.0	15.6
$LSD_{0.05}$	0.45	0.09	0.22	4.56	24.64	2.88	0.17	3.31
$LSD_{0.01}$	0.60	0.11	0.29	6.34	32.89	3.85	0.23	4.42

NOTE: The normal fruits were used for statistical comparison.

fruits, which are respectively 70 and 62 mgP/100 g of fresh weight at harvest. The differences in P between the normal and severe groupings were not significant after storage. Magnesium is higher in the normal fruits than the fruits with cork spot at both sampling dates. Boron is lower in normal fruits at harvest but higher in normal fruit compared with severely cork spotted fruit after storage (Tables 7 and 8).

IV. Cork Spot Rating at Harvest

The fruit mineral content of the five groups was compared using Duncan's multiple range test as shown in Table 9. The mean total Ca concn in each of the five rating groups was: 7.3, 5.9, 5.0, 4.9, and 4.3 mg/100 g of fresh weight, respectively. Those differences are significant at the 5% level and the same is true for water soluble and ethanol soluble Ca. There were no significant differences in N concn between the five groups, but the N:Ca ratios for the five groups were 6.6, 9.6, 11.0, 10.3, and 14.6, respectively (calculated from Table 9). Potassium content was highest in the second group (118.8 mg K/100 g of fresh wt.), and lowest in the fifth group (72.8 mg K/100 g of fresh wt.). Phosphorus is the lowest in the first group (11.3 mg P/100 g of fresh wt.), and the highest in the fifth group (19.4 mg P/100 g of fresh wt.). The differences in Mg concn were not significant. Boron concn of the last four groups was slightly

Table 9. Rating of cork spot incidence and mineral content of 'Anjou' pear fruit from Medford orchards surveyed September 22, 1975. Each value is the mean of 15 samples.

% CS at harvest df=70 and 4	Mineral content of the fruit (mg/100 g of f.w.)							ppm
	Ca-acid extract	Ca-ethanol extract	Ca-water extract	N	K	P	Mg	B
0.0-0.5%	7.3[a]	4.6[a]	3.2[a]	48.0[a]	102.6[b]	11.49[b]	5.8[b]	16.1[b]
0.5-10%	5.9[b]	4.2[b]	2.7[b]	56.4[a]	118.8[a]	12.8[c]	6.0[b]	27.0[a]
11-20%	5.0[c]	4.0[b]	2.7[b]	55.1[a]	85.6[d]	17.0[b]	5.7[b]	24.0[a]
21-40%	4.9[c]	3.7[c]	2.9[c]	50.2[a]	94.4[c]	16.2[b]	7.1[a]	18.5[b]
41-100%	4.3[d]	3.4[d]	2.2[d]	62.9[a]	72.8[e]	19.38[a]	6.2[b]	27.8[a]

NOTE: Means followed by different letters are significantly different at the 5% level (Duncan's multiple range test)

higher than the concn of the first group (Table 9).

V. Cork Spot Rating after Storage

The fruit mineral content of each group compared by Duncan's multiple range test. The total Ca concn range in the five groups was: 7.5, 6.4, 6.2, 5.8, and 5.1 mg Ca/100 g of fresh weight (Table 10). Nitrogen concn was lowest in the first group and the highest in the fifth group (81 to 100% cork spot). The range of N:Ca ratios is as follows: 7.4, 8.8, 9.6, 10.5, and 13.2, respectively (calculated from Table 10). Potassium concn ranges from 110 to 89 mg K/100 g of fresh weight, respectively. There were no significant differences in P concn between the five groups. Magnesium concn was 6.6 mg/100 g of fresh weight in the first group (0.0 to 20% cork spot), and 5.1 mg/100 g of fresh weight in the last group (81 to 100% cork spot). There were no significant differences in the B concn between the five groups (Table 10).

VI. Sprays with Calcium Salts in 1975

Three early sprays on May 15, June 15, and July 15 in the Beebe orchard with 5 lb $Ca(NO_3)_2$ per 100 gal at 400 gal per acre reduced the incidence of harvest cork spot from 24.7% to 3.0% and reduced storage cork spot from 51.1% to 8.5%. Fruit Ca was increased from 5.3 to 6.6 mg/100 g of fresh weight (Table 11). Water

Table 10. The relation between cork spot incidence rating and the mineral content of 'Anjou' pear fruit from orchards in the Medford area, harvested September 4, 1976. Each value is the mean of 15 samples.

% CS after storage df = 70 and 4	Mineral content of the fruit (mg/100 g of f. w.)					ppm
	Ca-acid extract	N	K	P	Mg	B
0.0-20%	7.5a	55.5b	110.0a	16.0a	6.6a	20.3a
21-40%	6.4b	56.4b	105.4a	14.8a	6.2b	18.1a
41-60%	6.2c	59.2b	98.8a	16.6a	6.2b	20.4a
61-80%	5.8d	61.0a	83.2b	18.4a	6.6a	23.4a
81-100%	5.1e	67.0a	89.0b	15.2a	5.1c	20.4a

NOTE: Means followed by different letters are significantly different at the 1% level (Duncan's test).

soluble Ca increased from 2.7 to 3.4 and ethanol soluble Ca increased from 3.1 to 5.3 mg/100 g of fresh weight. The increase in Ca (all solubility fractions) and the reduction in both storage cork and harvest cork is significant at the one percent level. Calcium sprays had no significant effects on the fruit N or K concentrations (Table 11).

Two sprays, the first in June and the second in July were applied in six commercial orchards in the Medford area in 1975 (5 lb/100 gal, of $CaCl_2$ of $Ca(NO_3)_2$, 250 gal per acre), resulted in an average reduction of harvest cork spot from 26.9% to 8.7% and storage cork spot was reduced from 49.8% to 16.5% (Table 12). The sprays increased fruit Ca (acid extract) from 5.05 to 6.03 mg/100 g of fresh weight, the ethanol soluble Ca from 3.62 to 4.56 mg/100 g of fresh weight, and the water soluble Ca from 2.87 to 3.03 mg/100 g of fresh weight. The reduction in cork spot and the increase in total Ca and ethanol soluble Ca is significant at the one percent level, and the increase in the water soluble Ca fraction is significant at the five percent level using LSD test (Table 12). The sprays had no significant effect on fruit N or K concentrations.

VII. Sprays with Calcium Salts in 1976

Sprays with 5 lb/100 gal of $Ca(NO_3)$ or $CaCl_2$ on August 13 and August 26, in the Eden Valley and Clancy orchards did not reduce the

Table 11. Mineral content and cork spot incidence of 'Anjou' pear fruit from Beebe orchard sprayed with $Ca(NO_3)_2$, 5 lb/100 gal, 400 gal per acre on May 15, June 15, and July 15, 1975. Each value is the mean of five samples.

Treatment df = 9	Mineral content of the fruit (mg/100 g of f. w.)					% Cork spot	
	Ca-acid extract	Ca-ethanol extract	Ca-water extract	N	K	at harvest	after storage
$Ca(NO_3)_2$ spray	6.6	5.3	3.4	59	100.6	3.0	8.5
Control	5.3	3.1	2.7	58	101.0	24.7	51.8
$LSD_{0.05}$	0.81	0.78	0.09	16.67	28.47	4.29	3.87
$LSD_{0.01}$	1.27	1.21	0.14	25.42	43.41	6.60	6.03

Table 12. Aggregate effects of $Ca(NO_3)_2$ or $CaCl_2$ sprays (5 lb/100 gal, 250 gal per acre, on June and July) in six orchards on control of cork spot incidence and calcium content of 'Anjou' pear fruit. Each value is the mean of 23 samples.

Treatment df = 44	Mineral content of the fruit (mg/100 g of f. w.)					% Cork spot	
	Ca-acid extract	Ca-ethanol extract	Ca-water extract	N	K	at harvest	after storage
Ca-spray	6.03	4.56	3.03	51.8	97.8	8.7	16.5
Control	5.05	3.62	2.87	51.8	92.8	26.9	49.8
$LSD_{0.05}$	0.40	0.32	0.15	5.95	14.36	4.55	4.49
$LSD_{0.01}$	0.58	0.46	0.42	8.73	20.69	6.56	6.46

incidence of cork spot significantly and did not increase fruit Ca (Table 13). In the Eden Valley orchard B is significantly higher in the control than in the fruits from sprayed trees, while there were no significant differences in N, K, and Mg concn between the treatment and the control. In the Clancy orchard N and Mg are significantly higher in the fruits from control trees than sprayed. While there is no significant difference in K and B concn between the treatment and the control (Table 13).

The Beebe orchard was sprayed three times in 1976, the second week of May, the second week of June and the third spray in the second week of July, with $Ca(NO_3)_2$ 5 lb/100 gal water, 400 gal per acre. The spray increased Ca concn of the fruit significantly and reduced the incidence of cork spot at harvest from 6.6% to 1.5% and reduced the storage cork spot significantly from 61.9% to 23.6% (Table 13). Nitrogen is higher in the control than the treatment, while B is lower in the control. No significant differences were observed in the K and Mg concn in the fruit comparing the treatments and the control (Table 13).

VIII. Comparison of Orchards in 1975

Samples from orchards with varying percentage of cork spot were compared with the Naumes orchard which had a consistent history of freedom from cork spot at harvest and only minimal cork

Table 13. Cork spot incidence and mineral content of 'Anjou' pear fruit from commercial orchards sprayed with 5 lb/100 gal water, of Ca $(NO_3)_2$ or $CaCl_2$. Harvested September 4, 1976. Each value is the mean of ten samples.

Orchard	Treatment	Mineral content of the fruit (mg/100 g of f.w.)				ppm	% cork spot	
	df=72 and 7	Ca-acid extract	N	K	Mg	B	at harvest	after storage
Eden Valley	$CaCl_2$ spray	6.0	64.5	74.8	7.4	17.3	2.2	53.6
	$Ca(NO_3)_2$ spray	6.1	62.4	77.2	7.1	19.5	4.4	50.0
	Control	6.3	61.2	85.4	6.9	22.8	0.1	39.3
Clancy (Rogue River)	$CaCl_2$ spray	6.3	65.4	82.8	6.8	21.4	11	43.6
	$Ca(NO_3)_2$ spray	5.7	65.4	77.2	6.9	23.4	15	69.3
	Control	6.1	69.9	83.8	7.7	23.3	1.2	48.4
Beebe	$Ca(NO_3)_2$ spray	6.8	65.0	116.6	6.1	17.3	1.5	23.6
	Control	5.8	71.5	115.4	5.9	13.8	6.6	61.9
	$LSD_{0.05}$	0.33	3.48	12.19	0.57	2.25	7.42	8.13
	$LSD_{0.01}$	0.42	4.40	15.37	0.72	2.90	9.36	10.26

spot after storage. Results are shown in Table 14 for 1975, and in Table 15 for 1976.

A. Clancy orchard (Rogue River). All of the Ca fractions (acid, ethanol, and water soluble) are significantly lower than the Ca content of the fruits from the Naumes orchard. The incidence of harvest cork and storage cork is also significantly higher than in the Naumes orchard. There were no significant differences in fruit N, K and Mg concn. Boron and P are significantly higher in 'Anjou' fruit from the Clancy orchard than from Naumes (Table 14).

B. Beebe orchard. Harvest cork and storage cork were significantly higher and all fractions of Ca significantly lower than in the Naumes orchard. There were no significant differences in N, Mg, and B, between the two orchards. Potassium and P are significantly higher than in the Naumes orchard. Nitrogen, P and B were significantly higher than Naumes orchard. Potassium was significantly lower in the declined trees, while Mg was not significantly different.

D. Cory (normal trees). The incidence of cork spot at harvest and after storage is significantly higher than the Naumes orchard, but significantly lower than the incidence of cork spot in the declined trees. Acid extractable and water extractable Ca are significantly lower than Naumes, but all the Ca fractions are significantly higher than the Naumes orchard. Potassium is significantly higher in the

Table 14. Mineral content of 'Anjou' pear fruit and percentage of cork spot from various Medford orchards evaluated September 22, 1975. Each value is the mean of ten samples.

Orchard df=63 and 6	Mineral content of the fruit (mg/100 g of f.w.)							ppm	% cork spot	
	Ca-acid extract	Ca-ethanol extract	Ca-water extract	N	K	P	Mg	B	at harvest	after storage
Clancy	4.7	3.8	2.6	50.1	93.2	17.54	7.0	18.7	34.2	52.9
Beebe	5.4	3.9	2.5	55	103.0	15.2	7.5	15.7	12.5	29.5
Naumes	6.6	4.4	3.0	51.1	94.0	11.8	6.0	15.2	0.0	0.0
Cory (trees with pear decline)	4.3	3.5	2.5	70.2	69.6	19.34	6.0	27.5	54.3	67.8
Cory (normal trees)	5.6	4.4	2.8	59.0	123.8	11.34	5.8	18.4	11.2	26.4
Medford station	6.3	4.1	2.9	65	128.0	12.0	5.0	15.6	7.3	14.2
Hanley station	6.8	4.0	2.8	49.2	149.4	12.0	5.9	17.2	6.4	6.4
$LSD_{.05}$	0.42	0.33	0.19	7.64	14.94	3.2	1.69	3.01	7.29	6.98
$LSD_{.01}$	0.54	0.42	0.24	9.75	19.0	4.1	2.16	3.84	9.30	8.91

NOTE: Naumes orchard was used as control because of consistent history of normal fruit; the mean of each orchard was compared with the mean of Naumes orchard.

Table 15. Mineral content and cork spot incidence of 'Anjou' pear fruit from various Medford orchards evaluated September 4, 1976. Each value is the mean of 15 samples.

Orchard df=126 and 8	Mineral content of the fruit (mg/100 g of f.w.)					ppm	% cork spot	
	Ca-acid extract	N	K	Mg	P	B	at harvest	after storage
Eden Valley	6.1	64.0	79.0	6.8	17.48	21.4	13.7	53.8
Clancy	6.0	67.0	79.2	6.8	17.54	21.5	18.9	59.4
Beebe	5.9	70.4	117.2	5.9	14.24	15.2	8.9	53.4
Naumes	6.6	59.4	125.4	4.4	8.40	9.5	0.0	22.0
Cory (trees with pear decline)	5.4	68.5	72.8	5.1	17.56	29.9	43.7	79.9
Cory (normal trees)	6.4	52.7	118.2	4.4	12.78	12.6	3.3	39.0
Medford station	6.5	53.8	125.6	5.1	14.16	21.9	8.5	24.6
Hanley station	6.4	48.0	125.4	5.3	14.28	22.6	0.7	24.6
Pinnacle (104)	7.3	60.6	111.4	8.7	20.4	19.8	0.13	28.0
$LSD_{0.05}$	0.31	2.65	8.16	0.51	2.65	1.90	8.58	8.15
$LSD_{0.01}$	0.39	3.33	10.19	0.64	3.33	2.39	10.79	10.24

NOTE: Naumes orchard was used as control because of consistent history of normal fruits and with the least CS after storage; the mean of each orchard is compared with the mean of Naumes orchard.

fruits from the normal trees than from the declined trees, but N and P are significantly lower than the declined trees.

E. Medford station. Both harvest cork and storage cork were significantly higher than Naumes. There were no significant differences in any of the Ca fractions from Naumes, but N and K were significantly higher in Medford station than Naumes orchard.

F. Hanley station. There were no significant differences in either harvest cork or storage cork compared to the Naumes orchard. Acid extractable Ca and N, P, Mg and B were not significantly different from Naumes orchard, but water soluble Ca and ethanol soluble Ca are significantly lower than Naumes orchard, while K is significantly higher than Naumes orchard (Table 14).

IX. Orchard Comparison in 1976

The same orchards were compared with the Naumes orchard in 1976, and two more orchards were added (Eden Valley and Pinnacle 104). Calcium was not extracted with ethanol or water in 1976. The fruits were stored eight months in 1976 as compared to six months in 1975. The cork spot evaluation was based on superficial cork spot only in 1975, whereas in 1976 all the fruits were peeled to include the internal cork spot in the total percentage of cork spot after storage.

A. Eden Valley orchard. Cork spot at harvest and after storage was significantly higher and Ca was significantly lower than that of

the Naumes orchard (Table 15). Nitrogen, Mg, P and B were significantly higher than Naumes, but K was significantly lower than Naumes.

B. Clancy orchard. The incidence of storage cork and harvest cork were significantly higher and Ca was significantly lower than Naumes. Boron, N, Mg and P were significantly higher than Naumes, whereas K is significantly lower (Table 15).

C. Beebe orchard. Calcium and K were significantly lower while storage cork and harvest cork were significantly higher than Naumes orchard. Nitrogen, Mg, P, and B were significantly higher than Naumes.

D. Cory orchard (trees with pear decline). Calcium and K were significantly lower and cork spot at harvest and during storage was significantly higher than the Naumes orchard. Boron, N, P and Mg were significantly higher than Naumes.

E. Cory orchard (normal trees). Harvest cork spot, Ca, and Mg are not significantly different than the Naumes orchard, but storage cork spot, B, and P were significantly higher than Naumes. Potassium and N were significantly lower than Naumes. Calcium and K were significantly higher and both storage cork and harvest cork were significantly lower than the declined trees.

F. Medford station. Storage cork and harvest cork, Ca and K were not significantly different from the Naumes orchard. Nitrogen

was lower and B, Mg, and P were higher than Naumes (Table 15).

G. Hanley station. No significant differences were observed in Ca and K and both storage cork and harvest cork compared to Naumes. Boron, P, and Mg were significantly higher, while N was significantly lower than Naumes.

H. Pinnacle (104) orchard. Calcium was significantly higher but both storage cork and harvest cork were not significantly different than the Naumes orchard. Boron, P, and Mg were significantly higher, while K was lower and N was not different.

X. Orchards Soil Analyses

The Naumes orchard showed the lowest in Ca, Mg, and soil pH, which also had no harvest cork and the lowest incidence of storage cork. The Clancy orchard had the lowest K and the highest cork spot at harvest and after storage, but Beebe orchard soil had the highest K and Ca, and yet had a very high incidence of cork spot after storage (53.4%) (Table 16). The results in Table 16 show no clear relations between cork spot incidence and soil mineral content or soil pH.

Discussion

I. Linear Correlation Coefficient (r-values) between Cork Spot and Mineral Elements

Simple linear regressions of storage cork spot in relation to

Table 16. Soil analyses in relation to the incidence of harvest cork and storage cork from different orchards in Medford area, surveyed on September 4, 1976. A comparable sample was collected from the top six inches from different sites in each orchard.

Orchard	pH	Extractable Cations				% Cork spot	
		P (ppm)	K (ppm)	Ca (meq/100 g)	Mg (meq/ 100 g)	at harvest	after storage
Beebe	6.6	80	658	19.0	4.4	8.9**	53.4**
Eden Valley	6.3	47	256	17.5	5.1	13.7**	53.8**
Clancy	5.5	16	152	8.4	2.7	18.9**	59.4**
Naumes	4.9	75	398	6.9	1.7	0.0	22.0

**Significant from the Naumes standard at the 1% level using LSD test.

NOTE: Naumes orchard was used as a control, because it had a consistent history of no cork spot at harvest.

Ca concn (acid extract) of the fruit in the last four sampling dates in both years and harvest cork in 1975 was strongly negative and highly significant (Tables 1 and 2). These results mean that Ca deficiency in the fruit is a major factor in the incidence of cork spot both at harvest and after storage in 'Anjou' pears grown under Medford conditions. The data in our study show a high negative correlation between the incidence of cork spot and Ca, which has not been reported before in 'Anjou' pear. This correlation could be used to determine the relative importance of Ca in the development of cork spot and also as a predictive index for the disorder. The correlation between Ca and the incidence of harvest cork spot in 1975 was low but highly significant despite the fact that cork spot at harvest was extremely low and most of the trees did not show any superficial cork spot (Table 2).

The seasonal analyses for Ca suggest that the relation between Ca concentration during fruit growth and the incidence of cork spot at harvest and after storage could be used as predictor of probable cork spot. From Table 2 the correlation between the storage cork and Ca (acid extract) in the fruit is significant as early as July 8 in 1975, and July 14 in 1976. Tables 17 and 18 show the expected cork spot incidence at harvest and after storage, calculated from the relation between Ca during the growing season and the cork spot incidence. To expect less than 5% cork spot at harvest, the Ca concn in the fruit must be above 7 mg/100 g of fresh weight in

Table 17. Expected cork spot incidence at harvest in relation to seasonal fruit calcium concentration. Values in the table are percentage of fruit with cork spot.*

Sampling date	Ca concn in the fruit (mg/100 g of f.w.)									
	1	2	3	4	5	6	7	8	9	10
9/22/75	66.0	55.0	45.0	35.0	25.0	15.0	5.0	0.0	0.0	0.0
9/4/75	37.7	32.5	27.3	22.1	16.9	11.7	6.5	1.3	0.0	0.0
8/19/75	39.4	35.9	32.	28.9	25.4	21.9	18.4	14.9	11.4	7.9
8/5/75	23.5	22.3	21.1	19.9	18.7	17.5	16.3	15.1	13.9	12.7
7/8/75	26.3	25.4	24.5	23.6	22.7	21.8	20.9	20.0	19.1	18.2

*the predicted cork spot was not calculated for earlier dates due to the low significance.

Table 18. Expected cork spot incidence after six months (1975) and eight months (1976) of storage in relation to seasonal fruit calcium concentration. Values in the table are percentage of fruit with cork spot.

Sampling date	Ca concn in the fruit (mg/100 g of f. w.)									
	1	2	3	4	5	6	7	8	9	10
9/22/75	95.0	81.0	67.0	53.0	40.0	26.0	12.0	2.0	0	0
9/4/75	77.2	66.6	56.0	45.4	34.8	24.2	13.6	3.0	0	0
8/19/75	84.3	76.6	68.9	61.2	53.5	45.8	38.1	30.4	22.7	15.0
8/5/75	45.2	42.7	40.2	37.7	35.2	32.7	30.2	27.7	25.2	22.7
7/8/75	61.9	59.6	57.3	55.0	52.7	50.4	48.1	45.8	43.5	41.2
9/4/76	100	100	100	84.7	65.7	46.7	27.7	8.7	0	0
8/26/76	100	100	99.9	85.9	71.9	57.9	43.9	29.9	15.9	1.9
8/13/76	100	100	91.7	82.3	72.9	63.5	54.1	44.7	35.3	25.9
7/29/76	81.9	77.7	73.5	69.3	65.1	60.9	56.7	52.5	48.3	44.1

September. Calcium concn in the fruit must be above 10 mg/100 g of fresh weight in the first week of July to anticipate less than 18% cork spot at harvest (Table 17). To expect less than 13% superficial cork spot after six months in storage, the Ca concn in the fruit in September (at harvest) must be above 7 mg Ca/100 g of fresh weight. If Ca concn in the fruit at harvest is below 3 mg/100 g of fresh weight the expected superficial cork spot after six months in storage is above 80%. In the first week of July, the Ca concn in the fruit must be above 10 mg/100 g of fresh weight to expect less than 40% superficial cork spot after six months in storage (Table 18, 1975 data). In 1976, all the normal fruits were peeled and tested for internal cork spot and the internal was added to the superficial cork to get total cork spot. Also the fruits were stored eight months in 1976 to permit the development of more incipient cork spot. In this study, the 1975 data can be used as an index to predict the superficial cork spot at harvest and after six months in storage (Tables 17 and 18), and the 1976 data can be used as an index to predict the total cork spot (superficial and internal) after eight months in storage (Table 18). If Ca concn in the fruit at harvest is less than 4 mg/100 g of fresh weight the expected cork spot after eight months in storage is 100%. To expect less than 8% cork spot after eight months in storage, the Ca conc in the fruit at harvest (September) must be above 8 mg/100 g of fresh weight. In the last week of July, the Ca

Table 19. Expected cork spot incidence after eight months of storage based on seasonal total fruit calcium (mg per fruit). Values in the table are percentage of fruits with cork spot.*

Sampling date	Total Ca in the fruit (mg per fruit)									
	2	4	6	8	10	12	14	16	18	20
9/4/76	100	94.2	81.2	68.2	55.2	45.2	29.2	16.2	3.2	0.0
8/26/76	100	98.7	82.7	66.7	50.7	34.7	18.7	2.7	0.0	0.0
8/13/76	86.4	76.0	65.6	55.2	44.8	34.4	24.0	13.6	3.2	0.0
7/29/76	71.6	63.6	55.6	47.6	39.6	31.6	23.6	15.6	7.6	0.4

*the predicted cork spot was not calculated for earlier dates due to the low significance.

concn in the fruit must be above 10 mg/100 g of fresh weight, to expect less than 44% of total cork spot after eight months in storage (Table 18, 1976 data).

Total Ca in the fruit also could be used as an index to predict the incidence of these disorders. Two variables are included in the total Ca in the fruit values, the fresh weight and the Ca concn in mg/100 g of fresh weight. If the fruit size or fruit weight is an important variable in the development of cork spot, then the total Ca per fruit should be a better index than the Ca concn alone. In this study there is not a good correlation between the fresh weight and the incidence of cork spot, first because the orchard trees in the study are older than 60 years except for the Hanley station which were 12. Older pear trees usually bear small to medium sized fruits with medium to heavy crop loads. Young trees are more vigorous and bear light loads which results in rapid fruit growth and possibly a growth dilution of the mineral elements, especially Ca. Fruits of this kind are more susceptible to cork spot. Second, trees with pear decline were included in the study and such trees bear small fruits with more cork spot. If the total Ca in the fruit (mg/fruit) is lower than 6 mg in late August and early September, the total cork spot after eight months in storage is expected to be higher than 95%. In the last week of July, the total Ca in the fruit must be above 18 mg to expect less than 7% total cork spot after eight months in storage.

Our results showed the N:Ca ratio in 'Anjou' pear fruit to be more highly correlated with cork spot than was Ca or N alone (Tables 1 and 2). The results of Shear (155) with apple agree with that. High N can offset the beneficial effect of normally adequate concn of Ca, and the N:Ca ratio can reflect the degree of balance between Ca and N. The N:Ca ratio is a good index to predict the incidence of cork spot after storage as shown in Table 20.

The correlation between K and the incidence of both harvest cork and storage cork in both years was always negative (Tables 1 and 2). Our results show that low K in the fruit may be related to the incidence of cork spot (r-values from -0.72 to -0.27, Talbe 1). These results are in contrast to the results from work with bitter pit in apple (29, 30, 61, 104, 140, 146, 183), which suggests that excess K fertilization or sprays with K salts increased the incidence of bitter pit in apple. The results of Sharples (143), whow that extremely low K may cause a type of breakdown of apples in storage. Whether the lower K in fruits is a direct relationship or merely reflective of other physiological events (such as altered root physiology) can only be speculated at this time.

The positive correlations between the incidence of both storage cork and harvest cork and B is significant only in the very early sampling dates in both years. Our results disagree with some of the reports on apple bitter pit, which suggest that B sprays were

Table 20. Expected cork spot incidence after six months (1975) and eight months (1976) of storage in relation to seasonal fruit N:Ca ratio. Values in the table are percentage of fruit with cork spot in 'Anjou' pear.

Sampling date	Fruit N:Ca ratio									
	20	18	16	14	12	10	8	6	4	2
9/22/75	62.7	55.9	49.1	42.3	35.5	28.7	21.9	15.1	8.3	1.5
8/19/75	82.2	71.8	61.4	51.0	40.6	30.2	19.8	9.4	0.0	0.0
8/5/75	49.8	41.6	33.4	25.2	17.0	8.8	0.6	0.0	0.0	0.0
7/8/75	55.1	46.5	37.9	29.3	20.7	12.1	3.5	0.0	0.0	0.0
9/4/76	100	88.6	76.6	64.6	52.6	40.6	28.6	16.6	4.6	0.0
8/26/76	100	100	100	92.1	74.1	56.1	38.1	20.1	2.1	0.0
8/13/76	100	99.8	87.0	74.2	61.4	48.6	35.8	23.0	10.2	0.0
7/29/76	86.3	78.3	70.3	62.3	54.3	46.3	38.3	30.3	21.9	14.1

beneficial in reducing bitter pit (58, 116), but in a sense agrees with some other reports showing B sprays were ineffective (16, 35, 61, 175, 185). Boron does not appear to play a role in 'Anjou' cork spot.

The results with Mg were inconsistent. There were negative correlations in some dates and positive correlations in others and were not significant in both years except for one sampling date (May 24, 1976) in which r = -0.46. Evidence for (7, 166, 183) and against (30, 106, 185, 186) involvement of Mg in bitter pit of apple can be found.

Phosphorus is positively correlated with the incidence of cork spot in both years (Tables 1 and 2), but only significant in the last three sampling dates in 1975 (Table 1). High concn of P in 'Anjou' pear fruit could be related directly or indirectly to the development of cork spot. These findings do not agree with the results of Martin (99), which showed a highly significant negative correlation between bitter pit in apple and P, but some other reports have shown a positive correlation between P and bitter pit in apple (61, 151). The low significance of P in the second year is likely due to the variation in the crop as suggested by Martin (99).

II. Multiple Correlations between Cork Spot and Mineral Elements (Coefficient of Determination = r^2)

The combinations of the independent variables in relation to the incidence of cork spot at harvest and after storage for the last five sampling dates and for both years are shown in Tables 3 and 4. About 58% of the variation in cork spot after storage can be accounted for by the linear regression of fruit Ca in both years (r^2=0.578) (from Table 2). Fifty-six percent of the variation in harvest cork spot can be accounted for by the linear regression on fruit Ca in 1975 (r^2= 0.563). We can safely conclude that 56% to 58% of the observed cork spot at harvest and after storage was associated with the concn of Ca in the fruit. Sixty-two percent of the variation in the superficial cork spot after six months storage can be accounted for by the Ca and N:Ca ratio (r^2=0.62), at harvest. Sixty-five percent of the variation in the total cork spot (superficial and internal) after eight months storage can be accounted for by the Ca and N:Ca ratio (r^2=0.65), at harvest. About 50 percent of the variation in the superficial cork spot at harvest can be accounted for by the water soluble Ca and N:Ca ratio of the fruit in the first week of August. These results show that the combination of both N:Ca ratio and Ca, can be used effectively as indices to predict the incidence of cork spot at harvest and after storage (Table 4). To expect less than 10% superficial cork spot after six months in storage, the Ca concn in the fruit must be above

7 mg/100 g of fresh weight and the N:Ca ratio must be below six. If the Ca concn in the fruit is below 5 mg/100 g of fresh weight and the N:Ca ratio is above ten, the expected superficial cork spotafter six months in storage is expected to be above 50% (Table 21 and Table 4). Expected total cork spot (superficial and internal) is above 90% after eight months in storage, if Ca concn in the fruit is below 4 mg/100 g of fresh weight and the N:Ca ratio is above 12 at harvest. If the Ca concn in the fruit is above 8 mg/100 g of fresh weight and the N:Ca ratio is below eight at harvest, no superficial or internal cork spot is expected after eight months in storage (Table 22). Water soluble Ca and the N:Ca ratio early in the growing season can be used as indices to predict the superficial cork spot at harvest (Tables 3 and 23). To expect less than 10% superficial cork spot at harvest, the water soluble Ca in the fruit on the first week of August must be above 3 mg/100 g of fresh weight and the N:Ca ratio must be below six. If the water soluble Ca is below 2 mg/100 g of fresh weight and the N:Ca ratio is above ten, in the first week of August the expected superficial cork spot at harvest would be above 30% (Table 23).

III. Severity of Cork Spot

The severity of cork spot at three sampling dates was highly significant and negatively correlated with the following variables: Ca, total Ca per fruit, Ca-ethanol soluble, and Ca-water soluble (Table 5).

Table 21. Expected superficial cork spot incidence after six months of storage in relation to calcium and N:Ca ratio at harvest (September 22, 1975). Values in the table are percentage of fruit with corkspot.*

Ca	N:Ca ratio in the fruit									
mg/100 g f.w.	20	18	16	14	12	10	8	6	4	2
1	91.5	88.5	85.5	82.5	79.5	76.5	73.5	70.5	67.5	64.5
2	81.5	78.5	75.5	72.5	69.5	66.5	63.5	60.5	57.5	54.5
3	71.5	68.5	65.5	62.5	59.5	56.5	53.5	50.5	47.5	44.5
4	61.5	58.5	55.5	52.5	49.5	46.5	43.5	40.5	37.5	34.5
5	51.5	48.5	45.5	42.5	39.5	36.5	33.5	30.5	27.5	24.5
6	41.5	38.5	35.5	32.5	29.5	26.5	23.5	20.5	17.5	14.5
7	31.5	28.5	25.5	22.5	19.5	16.5	13.5	10.5	7.5	4.5
8	21.5	18.5	15.5	12.5	9.5	6.5	3.5	0.5	0.0	0.0
9	11.5	8.5	5.5	2.5	0.0	0.0	0.0	0.0	0.0	0.0
10	1.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

*Cs-storage = 71.5 - 10 (Ca) + 1.5 (N/Ca), from Table 4.

Table 22. Expected total cork spot incidence after eight months of storage in relation to calcium and N:Ca ratio at harvest (September 4, 1976). Values in the table are percentage of fruit with cork spot.*

Ca	N:Ca ratio in the fruit									
mg/100 g f.w.	20	18	16	14	12	10	8	6	4	2
1	100	100	100	100	100	100	93.9	87.3	80.7	74.1
2	100	100	100	97.0	95.8	89.2	82.6	76.0	69.4	62.8
3	100	100	97.7	91.1	84.5	77.9	71.3	64.7	58.1	51.5
4	99.6	93.0	86.4	79.8	73.2	66.6	60.0	53.4	46.8	40.2
5	88.3	81.7	75.1	68.5	61.9	55.3	48.7	42.1	35.5	28.9
6	77.0	70.4	63.8	57.2	50.6	44.0	37.4	30.8	24.2	17.6
7	65.7	59.1	52.5	45.9	39.3	32.9	26.1	19.5	12.9	6.3
8	52.8	47.8	41.2	34.6	28.0	21.4	14.8	8.2	1.6	0.0
9	43.1	36.5	29.9	23.3	16.7	10.1	3.5	0.0	0.0	0.0
10	31.8	25.2	18.6	12.0	5.4	1.2	0.0	0.0	0.0	0.0

*CS-storage = 78.8 - 11.3 (Ca) + 3.3 (N/Ca), from Table 4.

Table 23. Expected superficial cork spot incidence at harvest in relation to water soluble calcium and N:Ca ratio of August 5, 1975 (two months before harvest). Values in the table are percentage of fruit with cork spot.*

Ca (water soluble) mg/100 g of f.w.	N:Ca ratio in the fruit									
	20	18	16	14	12	10	8	6	4	2
1	37.9	35.7	33.5	31.3	29.1	26.9	24.7	22.5	20.3	18.1
2	32.6	30.4	28.2	26.0	23.8	21.6	19.4	17.2	15.0	12.8
3	27.3	25.1	22.9	20.7	18.5	16.3	14.1	11.9	9.7	7.5
4	22.0	19.8	17.6	15.4	13.2	11.0	8.8	6.6	4.4	2.2
5	16.7	14.5	12.3	10.1	7.9	5.7	3.5	1.3	0.0	0.0
6	11.4	9.2	7.0	4.8	2.6	0.4	0.0	0.0	0.0	0.0
7	6.1	3.9	1.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0
8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

*CS-Harv = 21.2 - 5.3 (Ca-water soluble) + 1.1 (N/Ca), from Table 3.

This suggests that the severity of cork spot is related to the severity of Ca deficiency in the fruit. Up to the present there has been no study of this kind reported in the literature. Eighty-one percent of the variability in the severity of cork spot at harvest can be accounted for by water soluble Ca (r^2=0.81). The deficiency of water soluble Ca in the fruit could be caused by movement of the water soluble Ca fraction out of the fruit especially late in the season. Under moisture stress conditions, water can move out of the fruit in the transpiring leaves and it may take along the water soluble Ca. Unpublished results from the seasonal Ca study (Appendix A, Figure 3) showed that the ratio of water soluble:total Ca decreased near harvest time which may suggest that some of the water soluble Ca fraction moved out of the fruit. Unpublished results from ^{45}Ca studies showed that about 40% of the ^{45}Ca taken by the fruit, from surface painting applications, moved out of the fruit to the adjacent leaves and stems (see Chapter II, Table 1).

The association between the severity of cork spot and total Ca is much lower (r^2=0.74), than with the water soluble Ca fraction at harvest, but after eight months in storage the correlation with total Ca is much higher than with the water soluble Ca fraction. The severity of cork spot that developed during storage is more related to the total Ca, probably because of the interchanges between the Ca

fractions during storage (M. A. Perring, special communication by letter).

The multiple correlations show that 86% of the variation in cork spot severity at harvest can be accounted for by the water soluble Ca and the ratio of water soluble Ca:total Ca (r^2=0.86), and 89% of the variation in the severity of cork spot can be attributed to the N:Ca ratio, water soluble Ca, and the ratio of water soluble Ca:total Ca (r^2=0.89) (Table 6). Eighty-one percent of the variation in the severity of cork spot after five months in storage may be due to the water soluble Ca and the ratio of water soluble Ca:total Ca (r^2=0.81) and 72% of the variation in the severity of cork spot after eight months in storage may be due to the same two variables (r^2=0.72).

From the results in Tables 7, 8, 9, and 10, we can recommend the following range of major element concns in the fruit to avoid the risk of the incidence of cork spot during storage (Table 24). These critical concns are applied for the 'Anjou' pears from the Medford area; however, growers in other areas could use the range of concns as a guide but the critical concns would have to be established experimentally in each situation. The critical concn of each element depends on the concns of other elements as has been suggested by others (10, 31, 60, 106, 108, 156, 173). If the Ca concn in the fruit is critical but N concn is above the optimum some cork spot could be expected to develop during storage. When Ca is at the critical concn

(7.3 mg/100 g f.w.), other elements especially N, P, K, and Mg should be in minimum concns in the fruit to provide a balance with the concn of Ca, to reduce the risk of cork spot development during storage. But if Ca is in the critical concn and the other elements are high, the risk of cork spot development is very high. If the concns of N, P, K, and Mg are higher than the optimum, the critical Ca concn in the fruit should be increased from 7.3 to 8 mg/100 g of fresh weight or even higher. In our study high K concn does not appear to be a problem and the optimum is about 100 mg/100 g of fresh weight. In some orchards with heavy K fertilization, fruit K has been increased above 160 mg/100 g of fresh weight, and could assist the development of bitter pit (143), probably by antagonizing the uptake of Ca, or antagonizing the function of Ca at the membrane or by competing with Ca at active sites on membranes (11). The same may be true with high levels of Mg (11). Nitrogen concn in the fruit should be kept at the minimum to reduce the risk of cork spot development in storage. High levels of N in the fruit have been reported to aggravate the development of Ca deficiency symptoms in apples (11, 108, 109, 147, 150). Excessive N fertilization, especially ammonium forms may have direct effects on the uptake and translocation of Ca (60, 61, 151-157).

Phosphorus is positively correlated with the incidence of cork spot, so its concn should be kept at the minimum. In orchards with

Table 24. Relationship of other influential mineral elements to critical calcium concn in the fruit of 'Anjou' pear at harvest and risk of cork spot during storage.

Risk of cork spot	For Ca mg/100 g of fr. wt.	In relation to other elements (mg/100 g of fresh wt.) N	P	K	Mg
Very low	≥ 8.0	≤60.0	≤16.0	≤110.0	≤ 6.0
Critical	7.3	>53.8	>13.5	>101.1	> 5.7
High	≤ 6.0	≥48.0	≥11.0	≥ 90.0	≥ 5.0

NOTE: The values in this table are taken from Tables 7, 8, 9, and 10.

problems of cork spot, a carefully planned fertilization program should be followed to maintain a balance between Ca and the other major elements.

Fruit samples from the orchards should be analyzed for main elements at harvest or earlier and only the fruits that have the optimum range of concns should be used for long storage (more than six months) and the fruits from orchards with critical Ca conc should be stored for short time only (about 2-4 months). Fruits that have below the critical Ca concn should not be stored at all, but instead could be used for processing.

IV. Spray with Ca Salts

Sprays with $Ca(NO_3)_2$ helped in controlling the cork spot in 1975 in all commercial orchards significantly but did not eliminate the disorder completely. We are not aware of any reports that claimed 100% control of bitter pit in apple by spray with Ca salts, but most reports have shown a significant reduction in bitter pit (70, 90, 96-109, 143, 144, 145, 150-154, 184, 185, 186-188). The sprays also increased all fractions of Ca (acid soluble, ethanol soluble, and water soluble) significantly (Tables 11 and 12). The spray with $Ca(NO_3)_2$ in 1975 did not increase the nitrogen content of the fruit significantly, and had no significant effect on K content of the fruit (Tables 11 and 12).

Sprays with Ca salts in 1976 had no significant effect on the cork spot incidence or on the Ca content of the fruit, except in the Beebe orchard (Table 13). Spray with $Ca(NO_3)_2$ in the Beebe orchard in 1976 increased Ca content of the fruit significantly from 5.8 to 6.8 mg/100 g of fresh weight, and reduced the cork spot after eight months in storage from 61.9% to 23.6%. Sprays with $CaCl_2$ or $Ca(NO_3)_2$ had only a slight effect in the Eden Valley and Clancy orchards mainly because the sprays were too late in the season (August 13 and 26). At the first spray (August 13) there was about 15% cork spot already developed on some of the trees that were sprayed. Beebe orchard was sprayed on the second week of May, second week of June, and second week of July. Another reason why the spray was not significant in the other two orchards is that these orchards had many trees with pear decline, which were included in the spray treatment, but not in the control. Table 13 shows that the treated trees have higher percentage of cork spot and lower Ca in the fruit, and that is mainly due to some trees with pear decline. The Beebe orchard does not have any trees with pear decline. The trees with pear decline were included in the study in 1975 but they were sampled separately, which should be done in all orchards which have some pear decline trees in order to obtain more accurate results of the Ca-spray treatments. Some reports in the literature claimed no significant effects of Ca-sprays on the control of bitter pit in apple (6, 68, 149, 168), and this

may be due to improper sampling, non-uniform trees, inadequate spray coverage and poor timing for the spray treatments.

Our conclusions are that sprays with Ca-salts are beneficial in controlling the incidence of cork spot in 'Anjou' pear, if the correct timings are used.

V. Effect of Location

Orchards with a consistent history of cork spot had the lowest Ca concn in the fruit in both years. Calculation of the N:Ca ratio from Tables 14 and 15 shows that all orchards with a consistent history of high incidence of cork spot have the highest N:Ca ratio in both years.

Potassium in general is low in all orchards in both years compared with what has been reported in apple fruit (130-160 mg/100 g of fresh weight) from the East Malling Research Station (142) which probably is what caused the negative correlations in our results, between the incidence of cork spot and K in both years. Reports have shown that apple fruits with bitter pit (28, 151, 168, 183), and pear fruits with cork spot have higher than normal K levels (112, 186). Trees with pear decline have very low K in the fruit in both years, which is 69.6 to 72.8 mg/100 g of fresh weight, compared with 118.2 to 123.8 mg/-00 g of fresh weight in the normal trees. The differences in P concn are not consistent and there was a high variation

between seasons. This may be due to the differences in the crop load between seasons as suggested by Martin (99).

The differences in fruit mineral content between locations may be due to differences in soil conditions, cultural practices, fertilization programs, crop load, and tree vigor.

VI. Soil Analysis

Table 16 showed no relationship between the soil mineral concns or soil pH and the incidence of cork spot. Our data agrees with reports on bitter pit in apple (16, 30, 35, 36, 43, 53, 75, 100, 116, 140, 167, 168), which conclude that there is no relation between soil Ca and fruit Ca or bitter pit, and application of Ca-fertilizers to the soil have no effect on fruit Ca or bitter pit.

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CHAPTER II

INVESTIGATIONS OF RADIOACTIVE CALCIUM UPTAKE AND TRANSLOCATION AFTER BRANCH INJECTION, AND PAINTING ON THE SURFACE OF FRUITS AND LEAVES OF 'ANJOU' PEAR TREES UNDER ORCHARD CONDITIONS

Literature Review

I. Introduction

Interest in Ca translocation has increased after the discovery that many physiological disorders in fruits and vegetables are associated with localized Ca deficiency. It is important to understand the mechanism of Ca translocation and distribution to effect control of these physiologic disorders.

Calcium translocation has become a controversial topic. Most of the literature (4, 5, 11, 35, 40) has suggested that Ca is immobile in the phloem, while others (8, 29, 30) have suggested that Ca is mobile in both phloem and xylem. The immobility of Ca in the phloem is based on the observation that Ca deficiency appears first in the young leaves and growing points, and the poor movement of ^{45}Ca when applied to leaves. Calcium in the older tissues is part of the structure of the cell, mainly the cell wall and the plasmalemma. This fraction of Ca apparently cannot be retranslocated as long as the tissue remains intact. Probably there are at least two

fractions of Ca in the tissues, therse are the structural Ca which is relatively immobile, and metabolic Ca which may be more mobile. Metabolic Ca is required for maintaining the membranes in a functional state and maintaining cellular compartmentation, as well as for enzyme cofactors, and for the selective uptake and accumulation of other ions such as K (10, 11).

II. Phloem and Xylem Translocation

Immobility of Ca in the phloem presumably is caused by its failure to enter the sieve tubes (11). Although Ca has been referred to as "immobile" in the phloem careful studies with ^{45}Ca showed that Ca did move into the phloem of oats (30), and was redistributed through the phloem of subterranean clover (23, 24, 25). Application of ^{45}Ca to the leaf surface of apple tree showed that ^{45}Ca moved in the phloem (21, 29, 49), and darkness-pretreatment of the leaf reduced ^{45}Ca translocation in the phloem significantly (29). Results from girdling experiments showed that Ca translocation was in the phloem (39), but results from transpiration experiments showed that root absorved Ca was translocated in the xylem (4, 5, 6, 53). Analysis of phloem exudate from Yucca flaccida showed that Ca was present in the phloem in a concentration of 0.08 mg/g of dry weight (8). This may indicate that Ca either translocated in the phloem or fixed there. Studies with ^{47}Ca showed that ^{47}Ca is

is present in the bark above the girdling zone of dogwood trees (40). This suggested that Ca could be exchanged between xylem and phloem. Wiersum (53) immersed one leaf blade in solution containing ^{45}Ca and showed that the radioactivity was detected in a nearby leaf which was exposed to a dry atmosphere. This suggested that ^{45}Ca entered the phloem, then the xylem and followed the transpiration stream to the rapidly transpiring leaf. Calcium can be released from the phloem to the xylem and redistributed along gradients of water stress (6). This transfer could occur by a lateral diffusion. Transfer of Ca from xylem to the phloem and vice-versa, was also demonstrated by others (2, 8, 30).

Shear and Faust (12, 31) showed that when ^{45}Ca was added to the nutrient solution of apple seedling radioactivity appeared in the developing leaves, bypassing the older mature leaves. They performed many experiments of girdling and xylem removal and concluded that Ca can move in both xylem and phloem but phloem translocation is very slow compared with xylem translocation (12).

The mechanism of Ca translocation in the xylem is described as via an exchange mechanism by many authors (2, 6, 13, 32, 40, 41). The xylem cylinder of the stem may operate as an exchange column for the upward translocation of Ca in the stem of bean plant (2, 6). Lignin was suggested to be one of the possible exchange sites

for Ca translocation (32), and Ca can be released by all divalent cations from the xylem of excised stem pieces of apple seedlings (13, 31, 32). Supplying $CaCl_2$, $SrCl_2$ or $MgCl_2$ to the roots of bean plant in the nutrient solution caused ^{45}Ca to be freed for ascent in the transpiration solution, but KCl was ineffective (2). The non-exchangeable ^{45}Ca in the stem of bean plants was found to be mostly in calcium oxalate crystals (6). Calcium might ascend in the xylem by mass flow if all exchange sites are occupied by ions which cannot be replaced by Ca (40). The rate of Ca uptake and the rate of transpiration both can increase the rate of Ca exchange in the xylem, which allows it to move by mass flow.

Calcium in the xylem was found to be weakly bound on lignin and can be easily replaced by any divalent cation in vitro. In vivo, however, this replacement is sujbect to the laws of mass action (33). Translocation of Ca in the xylem is dependent upon Ca concn and can be influenced by transpiration. If Ca is available to the xylem its translocation is proportional to water uptake and transpiration (12).

Chelation and the presence of other cations increased the speed of Ca movement through excised stem pieces (13, 32), and in Brazil nut tree (34). Application of EDTA to the leaves or injection of divalent cations in subterranean clover plant increased the movement of previously deposited ^{45}Ca, but was not affected by water or diphenylamine (23, 24, 25). Movement of ^{45}Ca in the stem after injection

occurred in the phloem and in the xylem (25).

Application of B to the leaf (31) or to the nutrient solution of low B (56) increased translocation of Ca to the plant top. The form of N has been shown to influence the translocation and distribution of Ca within the plant (12, 26, 31). If NO_3^- is used as a source of N, Ca accumulated in the mature leaves; but if NH_4^+ is used as a source of N, Ca accumulated in the young leaves (31). Calcium content of plants which were growing on NH_4^+ as a N source was lower than the Ca content of the plants which were growing on NO_3^- as a N source. Ammonium ions may interfere with both the uptake and translocation of Ca (31, 33). If the nutrient solution was supplied with H^+ ion by lowering the pH of the solution to pH = 2 before the addition of Ca, no Ca was transported through the xylem (31), the authors did not show the effect of low pH on Ca translocation in the phloem.

III. Effect of Hormones and Growth Regulators

Calcium translocation also can be affected by hormones (12, 31, 33, 50), and growth regulators (36-38, 50). Faust and Shear (12, 38) suggested that root absorbed Ca could be translocated via the phloem and is under hormonal control. Calcium translocation can be shifted from the phloem to the xylem by removing the terminal buds of the plant or by killing the roots in hot water. In both cases

the source of hormones is removed and Ca can be diffused from the phloem to the xylem and move rapidly via the transpiration stream (31). Killing the roots with hot water also may increase the permeability which may increase Ca uptake by passive diffusion. Movement of Ca in a cut branch is predominantly via the xylem and it is influenced by transpiration (53). Increasing the metabolic activity of the older leaves with kinetin and benzyladenine increased ^{45}Ca movement to the old leaves (32). IAA increased ^{45}Ca translocation to bean shoots, but reduced ^{45}Ca translocation to the pea shoot (50), and GA_3 decreased ^{45}Ca translocation in both species. Application of TIBA (2, 3, 5-triiodobenzoic acid) decreases Ca transport into apple fruit (3, 36-38), and to bean and pea shoot (50), but has no effect on Ca translocation to apple shoots if ^{45}Ca is introduced into the cut end of shoots previously sprayed with TIBA (3). TIBA is known to inhibit Ca translocation in the phloem and reduction of ^{45}Ca movement to the fruit is evidence that the fruit receives some of its Ca through the phloem (12).

IV. Effect of Temperature and Metabolic Inhibitors

Calcium uptake and translocation are affected by temperature and metabolic inhibitors (9). Low temperature (2 to 4°C) only slightly reduced ^{45}Ca uptake by the root but strongly inhibited the translocation of ^{45}Ca to the shoots. Metabolic inhibitors such as

cyanide caused only a slight reduction in ^{45}Ca uptake by the roots, but strongly inhibited the translocation of ^{45}Ca to the shoots (9). High temperature (30°C) caused severe Ca deficiency symptoms in Nicotinia tabacum susceptible varieties, but no deficiency symptoms were developed if the same plants were grown at 21°C or 23°C. Increased temperature resulted in increased Ca accumulation in the stems (8). Application of ^{45}Ca to the tobacco leaf showed that ^{45}Ca absorption was not affected by low temperature, high temperature, darkness or metabolic inhibitors. This suggests that the absorption of Ca by the leaf is nonmetabolic. But all of the above factors affect ^{45}Ca translocation significantly (29). In apple, high temperature (24°C) late in the season reduced Ca translocation to the fruit, but increased Ca translocation to the fruit early in the season (42). This may relate to differences in fruit transpiration rates as it develops, the rate slowing as the fruit enlarges and with cuticular wax development.

V. Season or Time of Application

Translocation and distribution of Ca can be affected by the season or time of application. Application of ^{45}Ca to the roots of poplar seedlings in pot culture during April and May, resulted in uniform distribution of ^{45}Ca in all parts of the plant. When ^{45}Ca was applied on August 30 and September 27, the translocation of ^{45}Ca was

reduced. Application of ^{45}Ca on October 4 and November 3, resulted in even less ^{45}Ca accumulation in both roots and shoots (16). Application of ^{45}Ca to the roots of fruit-bearing apple trees in sand culture under outdoor conditions at different times during the growing season was tested (22, 51), to study the translocation of ^{45}Ca to the fruit. The summary of the results is as follows: When ^{45}Ca was applied immediately after dormancy, ^{45}Ca was found in the infloresences several days before full bloom. This shows that the flowers and young fruits acted as a strong sink. When ^{45}Ca was applied during the second half of August, the translocation of ^{45}Ca to all the upper parts of the tree was reduced significantly, and only a negligible amount was moved to the fruit. However, in the meristematic tissues such as the leaf buds, the accumulation of ^{45}Ca was relatively high. Mid-season (11 to 14 weeks after full bloom) application of ^{45}Ca resulted in more accumulation in the young rather than the old leaves. The concn of ^{45}Ca was much higher in the bark than in the wood.

Calcium nutrition of the fruit during the early stage (6 to 8 weeks after full bloom) of development appears to be very important to the fruit perhaps because of the high metabolic activity and cell division. Most of the Ca in the mature apple fruit accumulates during the early stage of development (54, 55).

VI. Secondary Translocation of Calcium

The formation of insoluble Ca compounds such as Ca-phosphate (27), or Ca-oxalate (6) could prevent translocation of Ca from older to younger plant parts. When ^{45}Ca was applied to the roots of bean plants in the nutrient solution and the plants were then moved to a nutrient solution with low Ca, the ^{45}Ca was secondarily translocated into the upper young shoot (46). Inoculation of ^{45}Ca in the xylem of dogwood tree stem, resulted in 73% of ^{45}Ca movement to the foliage, one month after inoculation. In the second season, after inoculation, 81% of the ^{45}Ca remaining in the trees moved to the leaves, and 84% of the ^{45}Ca remaining in the trees in the third season moved to the leaves (41). Extraction of ^{45}Ca from the xylem showed that 12% of the remaining ^{45}Ca after the first season is water soluble, and 28% after the second season, and 46% is water soluble after the third season (41). The author suggested that ^{45}Ca is immobilized on exchange sites in the xylem, where it can be replaced by other cations and thus re-enter the solution phase.

Wieneke and Fuhr (15, 52) studied the secondary translocation of Ca that had been deposited in the tree during the previous season and its contribution to fruit Ca in the following season, in apple trees growing in sand culture. The summary of their results is as follows: supplying the trees with ^{45}Ca after dormancy until the end of bloom showed that ^{45}Ca moved to all new growth (fruits, leaves, seasonal

shoots) during the following two years. Supplying ^{45}Ca during June (2-16) or August (16-30), showed that ^{45}Ca was secondarily translocated to the fruit in the second season more than the ^{45}Ca that moved into the first season fruits by direct translocation. The portion of secondary Ca deposited in the fruit during the second season was about 20 to 25% of the total Ca in the fruits. Most of the ^{45}Ca reserves which were deposited in the trees during the first season remobilized and secondarily translocated at the beginning of the second season. The authors suggested that ^{45}Ca may be remobilized by an exchange process with cations moving in the xylem.

Martin (21) studied the movement of ^{45}Ca in apple trees and fruits and his results can be summarized as follows: Radioactive Ca injected into the base of the branch in late spring, moved into the developing leaves rather than mature leaves. When ^{45}Ca was applied to the leaves after fruit harvest, it moved back into the spurs and appeared in the next season's leaves and fruits. Radioactive Ca injected into the fruit or applied to the fruit surface moved in the vascular tissue of the fruit and out of the fruit to adjacent leaves.

VII. Role of Transpiration

Root-absorbed Ca is mostly translocated in the transpiration stream. The distribution of ^{45}Ca follows the same pattern as that

of the dye, light green, which is carried along in the transpiration stream (53). Calcium movement in the xylem depends on water movement, and it accumulates in the most rapidly transpiring organs (4-6, 53). The total volume of the transpiration water reaching the organ was found to be the most important factor in determining the amount of Ca accumulation (5, 39). Fruits in general transpire water at a very low rate so that only a small proportion of water reaches them via the transpiration stream. The same is true for swelling buds which are not in the mainstream of transpiration (11). This phenomenon likely explains the low Ca concn in the fruits compared with the leaves (the ratio is of the order 1:20 to 1:80). Calcium supply to the fruit is correlated with the amount of water entering the fruit through the xylem (53). Since the rate of transpiration of the leaves is so much higher than the fruits, Ca generally bypassed the fruits and accumulated in the leaves. Enclosing the fruits in a plastic bag further reduces the transpiration rate and causes Ca deficiency disorders (53). An irrigation experiment with apple showed an increased concn of leaf Ca and a decreased concn of fruit Ca with decreased soil moisture (14, 17). Moisture stress may even cause the movement of water out of the fruit to the leaves due to a higher rate of transpiration from the leaves. Defoliation increased the Ca content of the fruits and reduced bitter pit in apples (53). It was found that apple fruits growing on the outside of the tree

have better keeping qualities in regard to bitter pit than those of the more shaded fruits; this could be explained by the higher fruit transpiration rate and more Ca accumulation in exposed fruits (53). Other authors (14, 54, 55) have suggested that exposed fruits can be under more water stress which may lead to a preferential movement of available Ca to the adjacent leaves, or movement of mobile Ca with the water out of the fruits to the leaves, due to higher relative transpiration rate.

VIII. Calcium Uptake by the Fruit

There is strong evidence that most of the Ca moves into fruits during early stage of development. Therefore the amount of Ca in the fruit early in the season is important in relating physiological disorders to Ca content (14, 54, 55). The movement of mobile Ca could be partitioned between the leaves and the fruits; since developing fruits are major sinks, it is likely that they receive part of their water via the phloem, which may translocate at least some of the fruit Ca (11). Examination of cross sections of the pea fruit stalk with an electron probe analyzer shows that Ca is present in the sieve tubes (11). This result is consistent with the concept that part of fruit Ca can be received via the phloem. Wilkinson (54) suggested that the movement of Ca into apple fruit can be considered in two stages. In stage one Ca uptake increases continuously until the end of July.

Stage two is from the end of July until harvest, in which Ca uptake may rise or fall. If Ca uptake rises in stage two it is at a lesser rate than stage one. If the amount of Ca falls in stage two it means that Ca has moved back from the fruit to the tree. Stage one is the period during which the cell walls are being formed and most of the Ca moves into the fruit during the first few weeks after fruit set. Stage two is the period of cell enlargement, during which the movement of Ca could be in either direction. If 1 mg of total Ca per fruit moved out of the fruit, it could make the difference between good and bad storage quality (54). Factors affecting Ca uptake in stage one are not known but the amount of Ca available to the fruit early in the season might determine the final level of Ca in the fruit (32, 54). The pattern of Ca movement between tree and fruit in stage two varies from continuing uptake, to return of Ca to the tree. Some Ca (1 to 1.5 mg) in stage two is in free mobility (i.e., water soluble) between the tree and fruit, which is sufficient to affect storage behavior. Among the factors which may affect Ca movement into or out of apple fruit in stage two is the competition between shoot growth and fruit growth, and the weather conditions (54). Abnormally dry weather may cause the movement of water out of the fruits to the leaves, and this may cause water soluble forms of Ca to move with the water out of the fruit. Diurnal studies of water potential of pear fruit showed that water loss from the fruit occurred mostly through the

xylem (19). This suggested that Ca may move out of the fruit through the xylem along with the water. Calcium uptake by the fruit was increased in stage two during the wet seasons, while in the dry seasons Ca moved out of the fruits (54). The amount of Ca per apple was higher in fruit from irrigated trees than in nonirrigated controls (54). Fast growing fruits may receive most of their water via the phloem and little water via the xylem (53). This may result in a very low Ca content and very high N and K In stage two the Ca content of the fruit undergoes a relative dilution more than N and K (53). Any treatment that increases the amount of additional water drawn toward the fruit by means of the xylem should result in increased Ca supply to the fruit.

Painting or spot application of ^{45}Ca on the skin of apple fruit at different stages of development showed that the penetration of Ca was most effective in the early stages of young fruit and decreased with further development of the fruit (21, 45). The speed of ^{45}Ca penetration from a drop on the apple fruit skin was found to be much higher under conditions of 50 to 80% relative humidity than at 100% relative humidity of the air (43). Dry conditions caused the evaporation of the drop too soon before penetration occurred and reduced the absorption of Ca. Painting of ^{45}Ca on the stem end of apple fruit showed that ^{45}Ca was absorbed by the fruit and translocated to the calyx end (47). Translocation of ^{45}Ca from the stem

end of the fruit to the calyx end was reduced by simultaneous lateral deposition and fixation. Fixation and precipitation of Ca as insoluble fractions occurred in the petiole, core, and vascular bundles (47). Painting of ^{45}Ca on the skin of apple fruit showed that ^{45}Ca penetrates the fruit to the main vasculars and then moved out of the fruit through the pedicel to the adjacent leaves (21). Leaching of ^{45}Ca out of apple fruit during periods of frequent rainfall has also been reported (48).

The objectives of this chapter are to investigate: (1) the absorption of ^{45}Ca by 'Anjou' pear fruit skin, which may be used as measure for the effectiveness of Ca sprays: (2) the competition between the leaves and the fruits for the Ca moving up the stem by injecting ^{45}Ca in the stem; (3) the differences in ^{45}Ca movement between the sun exposed and shaded sides of the tree; and (4) the effect of reduced moisture stress on the movement of Ca by injecting ^{45}Ca in trees with over-tree sprinkler irrigation compared to non-sprinkled trees.

Materials and Methods

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

Branch injection. Five similar branches with single fruit

on each were selected on the sun-side of the tree (south side) and similarly five fruiting branches were selected on the shaded side (north side). Five branches were similarly selected on a tree under sprinkler irrigation automatically set to provide cooling if the air temperature excluded 130°C. Radioactive Ca was applied by injecting 0.5 ml (37.35 μc) of $^{45}CaCl_2$, with a hypodermic syringe equipped with a fine needle (21) inserted through the bark (between the phloem and the xylem). The solution was forced into the branch slowly by applying pressure from a rubber band tied around the plunger and the needle lock of the syringe. The injection zone was located 20 cm below the attachment of the fruit on the branch.

Fruit injection in the flesh. Five branches with single fruit were selected for each treatment as described above. The radioactive Ca was applied by injecting 0.5 ml $^{45}CaCl_2$ (37.35 μc), using one ml hypodermic syringe with a fine needle inserted deep into the fruit flesh in the calyx end of the fruit (21). The solution was forced into the fruit by gentle manual pressure on the plunger.

Fruit injection in the carpel cavity. Five branches with one fruit on each were selected for each treatment as described above. Radioactive Ca was applied by injecting 0.5 ml (37.35 μc) of $^{45}CaCl_2$, into the carpel cavity through the calyx similar to fruit flesh injection.

Painting on the fruit surface. Five branches with single fruit on each were selected on the north side of the tree. All the leaves

that could possibly touch the fruit were removed from the branch to avoid contamination. A solution of radioactive Ca (0.5 ml $^{45}CaCl_2$ 37.35 μc) was painted on the fruit surface using a camel hair brush.

Painting on the leaf surface. Five non-fruiting branches (about 75 cm length) were selected on one tree and four to five leaves were selected on a non-fruiting spur so that the treated leaves could not possibly touch the branch. All the leaves that could touch the treated leaves were removed to avoid contamination. Radioactive Ca was applied by painting 0.5 ml $^{45}CaCl_2$ (37.35 μc) on the upper surface of the leaf by the same procedure as for fruit painting.

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

Fruit painting and leaf painting experiments were repeated on September 3, 1976 in different 'Anjou' pear trees and harvested on September 20, 1976 for a treatment duration of 17 days. Fruit injection, and branch injection treatments were tried earlier on 'Bartlett' pear trees on June 25, 1976 with the same procedures as described above. The branches were harvested after three days to study the short term movement of ^{45}Ca. The 'Bartlett' trees were at the Lewis-Brown farm (Oregon State University, Corvallis).

Sampling. The branches were cut and divided into subsamples immediately. The subsamples for each treatment are shown in

Table 1. In the branch injection treatments, the application zone is the part of the branch where the ^{45}Ca was injected including 3 cm above and below the injection location. The part of the branch below the application zone was not used in sampling but was removed as a potential (but trivial, we found) source of radioactivity.

Each subsample was dried in a forced air oven at 80°C, weighed and then ground in a Wiley Laboratory mill to pass 20 mesh. Fruit samples and leaf samples from the painting experiments were washed twice with water before drying to remove the remaining unadsorbed ^{45}Ca, and the fruits were cut into quarters (43). The powder from each subsample was thoroughly mixed for uniformity.

Counting the radioactivity. A portion of the powder from each composite sub-sample was transferred to a pre-weighed planchet (5 cm diameter), weighed, and the total dry weight calculated (18). All the planchets were filled to a fixed height by leveling the surface of the ground sample with the edges of the planchets (28). The dried plant powder was then covered with a collodion liquid which upon drying forms a very thin film to fix the powder into planchets. The collodion liquid was prepared by mixing 30 mg collodion (U.S. Products) with 20 ml diethyl ether and 20 ml of 100% ethanol. Untreated branches were harvested from the same orchard and handled similarly as controls.

Radioactivity in the dried plant material was assayed directly

(28) in the planchets at infinite thickness (18). Counting was done with NMC gas-flow proportional counter interfaced with an automatic scaler. Samples were counted to a total of 10, 000 counts or for ten minutes. Background counts were counted for one hour before and after counting the samples, and were averaged and subtracted from the average gross count (20, 44). All counts less than five per minute above the control were reported as zero (18). Counting data were corrected for machine efficiency, by using a ^{36}Cl standard (44), (two machines were used and their efficiencies were 0.59 and 0.57). The results are expressed as: SA (relative specific activity = dpm/g of dry weight); RTA (Relative Total Activity), which is the SA times the total dry weight in grams: and as % Tdpm (percentage of grand total dpm). Grand total dpm is obtained by adding the total dpm in each subsample of a branch (18).

Results

Branch injection. When ^{45}Ca was injected into the branch 20 cm below the attachment of the fruit spur, during the second week of August, it distributed in an irregular pattern to all parts of the branch including the fruit (Table 1). There was no significant difference in the distribution pattern of ^{45}Ca between the branch injection in the sun side and shaded side of the tree. Perhaps the unusually wet and humid summer in the Medford area during the time of the

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

Treatments and subsamples	SA	STDV*	RTA	% Tdpm
BRANCH INJECTION IN SUN				
Fruit	393	24	10967	2.067
Application Zone	43864	2068	58896	11.102
Leaves between Application Zone and Fruit	70259	9613	117586	22.166
Leaves above Fruit	31581	1648	149600	28.201
Stem between Application Zone and Fruit	25214	1311	122034	23.004
Stem above Fruit	15307	1410	71396	13.459
BRANCH INJECTION IN SHADE				
Fruit	307	26	9558	1.873
Application Zone	40878	2189	43565	8.538
Leaves between Application Zone and Fruit	58744	2048	115182	22.574
Leaves above Fruit	24960	1204	90149	17.668
Stem between Application Zone and Fruit	42722	2989	142966	28.019
Stem above Fruit	47083	2125	108826	21.328

Table 1. (Continued)

Treatments and subsamples	SA	STDV*	RTA	% Tdpm
FRUIT INJECTION UNDER SKIN (in the flesh) IN SHADE				
Fruit	22976	1406	692838	99.940
Leaves 20 cm below Fruit	28	13	63	0.009
Leaves above Fruit	16	6	39	0.006
Stem 20 cm below Fruit	0	0	0	0.000
Stem above Fruit	189	15	310	0.045
FRUIT INJECTION IN CALYX END (Carpel Cavity) IN SHADE				
Fruit	17161	1618	364191	97.134
Leaves 20 cm below Fruit	1581	179	3790	1.011
Leaves above Fruit	1351	131	4073	1.086
Stem 20 cm below Fruit	386	85	826	0.220
Stem above Fruit	1933	358	2056	0.549
FRUIT INJECTION UNDER SKIN (in the flesh) IN SUN				
Fruit	11181	1928	350737	99.707
Leaves 20 cm below Fruit	21	12	89	0.025
Leaves above Fruit	65	22	612	0.174
Stem 20 cm below Fruit	105	36	328	0.093
Stem above Fruit	0	0	0	0.000

Table 1. (Continued)

Treatments and subsamples	SA	STDV*	RTA	% Tdpm
FRUIT INJECTION IN CALYX END (Carpel Cavity) IN SUN				
Fruit	18247	1874	671097	99.864
Leaves 20 cm below Fruit	60	18	133	0.020
Leaves above Fruit	74	19	542	0.081
Stem 20 cm below Fruit	30	15	110	0.016
Stem above Fruit	35	14	126	0.019
PAINTING ON FRUIT SURFACE				
Fruit	4988	384	198931	59.403
Leaves 20 cm below Fruit	4307	204	31535	9.417
Leaves above Fruit	7470	911	59760	17.845
Stem 20 cm below Fruit	960	46	8344	2.492
Stem above Fruit	6579	628	36316	10.844
PAINTING ON LEAF SURFACE				
Application Zone	311412	15533	311412	89.838
Leaves above Application Zone	1333	121	6729	1.941
Stem 20 cm below Application Zone	670	37	1574	0.454
Stem above Application Zone	10029	493	26924	7.767

Table 1. (Continued)

Treatments and subsamples	SA	STDV*	RTA	% Tdpm
BRANCH INJECTION UNDER SPRINKLER				
Fruit	142	29	4392	1.065
Application Zone	57241	2633	90941	22.049
Leaves between Application Zone and Fruit	27959	1184	93665	22.709
Leaves above Fruit	17959	1052	102510	22.758
Stem between Application Zone and Fruit	38093	1323	98440	24.854
Stem above Fruit	10831	982	22501	5.455
FRUIT INJECTION IN CALYX END (Carpel Cavity) UNDER SPRINKLER				
Fruit	17346	1315	221494	99.678
Leaves 20 cm below Fruit	11	6	28	0.013
Leaves above Fruit	23	7	87	0.039
Stem 20 cm below Fruit	130	20	328	0.148
Stem above Fruit	146	33	272	0.122

*STDV is the standard deviation of the SA.

(z) Treatments were applied on August 11, 1976 and harvested on September 20, 1976.

treatments diminished any water potential gradients which might otherwise have exerted a more pronounced effect. About 2% of the total ^{45}Ca moved into the fruit from the branch injection treatments in both the sun side and the shaded side of the tree. About 89% of the ^{45}Ca moved out of the application zone after 40 days in the branch injection in the sun side and about 92% of ^{45}Ca moved out of the application zone for the same period in the branch injection treatment on the shaded side of the tree. Twenty-two percent of the activity was detected in the leaves between the application zone and the fruit in both the sun and shaded side of the tree. The highest activity (28.2%) was detected in the leaves above the fruit in the branch injection in the sun side, compared to only 17.7% in the shaded side of the tree. Lower activity (36.5%) remained in the stem above the application zone in the sun side than the shaded side (49.4%).

When ^{45}Ca was injected in the branch under sprinkler irrigation, only 78% of the activity moved out of the application zone, and only 1% of the activity translocated to the fruit after 40 days. Most of the activity (45.5%) was detected in the leaves above and below the fruit, and only 30.3% was detected in the stem above the application zone (Table 1).

Branch injection of 'Bartlett' pear tree early in the season (June 25, 1976), after removing the terminal meristem showed a different distribution of ^{45}Ca compared to branches with the growing

Table 2. Distribution of activity after translocation for 17 days in parts of branches of 'Anjou' pear tree, treated with ^{45}Ca. The activity is expressed the same as in Table 1. The treatments were applied on September 3, 1976. Each value is the mean of five subsamples.

Treatments and subsamples	SA	STDV*	RTA	% Tdpm
PAINTING ON FRUIT SURFACE				
Fruit	928	53	30700	92.403
Leaves 20 cm below Fruit	226	34	470	1.415
Leaves above Fruit	616	68	1691	5.090
Stem 20 cm below Fruit	67	9	137	0.412
Stem above Fruit	70	11	226	0.680
PAINTING ON LEAF SURFACE				
Application Zone	274071	24232	320668	92.855
Leaves above Application Zone	4275	391	13417	3.885
Leaves 20 cm below Application Zone	5300	276	9369	2.713
Stem 20 cm below Application Zone	195	22	520	0.151
Stem above Application Zone	525	32	1368	0.396

*STDV is the standard deviation of the SA.

point intact (Table 3). About 28% of the ^{45}Ca moved out of the application zone during three days if the growing point was removed compared with only 17.5% if the growing point was left intact. After three days of translocation 20.9% of the ^{45}Ca was detected in the stem of the branch with the growing point removed, compared with 13.3% in the stem of the branch with the growing point intact. The leaves in the branch with the growing point removed accumulated 8.6% of ^{45}Ca after three days, compared with 1.9% in the leaves of the branch with the growing point intact. The SA of ^{45}Ca in the fruit on the branch with the growing point removed is three times higher than the SA of the fruit on the branch with the growing point left intact (Table 3). In general, the short term translocation (3 days) of ^{45}Ca showed that most of the activity was present in the stem while the long term (40 days) translocation of ^{45}Ca showed that most of the activity had moved to the leaves.

Fruit injection. Injection of ^{45}Ca in the carpel cavity or in the fruit flesh of 'Bartlett' pear early in the season (June 25, 1976) showed that a very small amount of ^{45}Ca moved out of the fruit within three days. The growing points were one of the main sites for ^{45}Ca accumulation (Table 3).

When ^{45}Ca was injected in the fruit flesh on August 11, 1976, in the shaded side of the tree, only 0.06% of the ^{45}Ca moved out of the fruit, while in the sun side of the tree, about 0.3% of the ^{45}Ca

Table 3. Distribution of activity after translocation for three days in parts of branches of 'Bartlett' pear tree, treated with ^{45}Ca. The activity is expressed the same as in Table 1. The treatments were applied June 25, 1976. Each value is the mean of five subsamples.

Treatment and subsamples	SA	STDV*	RTA	%Tdpm
BRANCH INJECTION WITH GROWING POINT REMOVED				
Stem 20 cm below Application Zone	51	12	310	0.064
Application Zone	37836	2262	350501	72.331
Leaves between Application Zone and Fruit	4429	385	17937	3.702
Stem between Application Zone and Fruit	10468	1084	77905	16.077
Leaves between Fruit and Growing Point	826	43	12931	2.668
Stem between Fruit and Growing Point	1504	246	23472	4.844
Fruits with Stems	75	18	1525	0.315
BRANCH INJECTION WITH GROWING POINT NOT REMOVED				
Stem 20 cm below Application Zone	0	0	0	0.000
Application Zone	38704	1747	301209	82.525
Leaves between Application Zone and Fruit	406	34	2516	0.689
Stem between Application Zone and Fruit	4591	154	16759	4.592
Leaves between Fruit and Growing Point	324	32	4528	1.241
Stem between Fruit and Growing Point	1943	179	31888	8.737
Fruit with Stem	21	7	364	0.100
Growing Points with Folded Leaves	1755	181	7729	2.118

Table 3. (Continued)

Treatment and subsamples	SA	STDV*	RTA	% Tdpm
FRUIT INJECTION IN THE CALEX END (Carpel Cavity)				
Growing Point with Folded Leaves	21	7	63	0.018
Leaves between Fruit and Growing Point	8	5	259	0.076
Stem between Fruit and Growing Point	23	6	636	0.186
Fruit with Stem	18699	1673	340685	99.704
Leaves 20 cm below Fruit	6	4	54	0.016
Stem 20 cm below Fruit	0	0	0	0.000
FRUIT INJECTION UNDER THE SKIN (in the Flesh)				
Growing Point with Folded Leaves	81	16	205	0.053
Leaves between Fruits and Growing Point	0	0	0	0.000
Stem between Fruit and Growing Point	6	4	110	0.028
Fruit with Stem	19876	1440	388392	99.919
Leaves 20 cm below Fruit	0	0	0	0.0
Stem 20 cm below Fruit	0	0	0	0.0

*STDV is the standard deviation of the SA.

moved out of the fruit (Table 1). Injection of ^{45}Ca in the carpel cavity showed that 2.9% moved out of the fruit in the shade and 0.14% moved out of the fruit in the sun side of the tree. Most ^{45}Ca that moved out of the fruit tended to accumulate in the leaves above and below the fruit. Injection of ^{45}Ca in the carpel cavity under sprinkler irrigation showed that 0.4% of the ^{45}Ca moved out of the fruit. There was no significant difference in ^{45}Ca movement between the shaded side, sun side and under sprinkler irrigation. This is presumably due to unfavorable weather conditions which normally would have been expected to provide markedly different drought stress conditions.

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

Painting ^{45}Ca on the fruit surface just preceding harvest time (September 3, 1976) and left for 17 days resulted in low absorption of radioactivity by the fruit (Table 2). The SA for the late painting was 928 dpm/g of dry weight compared with 4988 dpm/g of dry weight for the early painting (August 11, 1976). Only 7.6% of the total ^{45}Ca

absorbed, moved out of the fruit within 17 days, in the late treatment compared with 41% within 40 days in the early treatments. Most of the ^{45}Ca that moved out of the fruit in the late painting accumulated in the leaves above the fruit (Table 2).

Painting on the leaf surface. When ^{45}Ca was painted on the leaf surface, on August 11, 1976, it was absorbed by the leaf and moved out of the leaf and accumulated in the leaves and stem above the application zone (Table 1). Leaf painting treatment was repeated on September 3, 1976, as harvest time approached and left for 17 days showed similar results (Table 2). About 10% of the absorbed ^{45}Ca by the leaf, moved out during 40 days, in the early leaf painting, while in the late leaf painting 7.6% of the ^{45}Ca moved out of the leaf during 17 days. In the late leaf painting, the ^{45}Ca tended to accumulate more in the leaves above the application zone than in the leaves below the application zone (in the ratio of five to one).

Discussion

Branch injection. Translocation of ^{45}Ca after 40 days from branch injection treatment showed that most of the activity accumulated in the leaves (50.4%), and only 2% of the activity was detected in the fruit. These results showed that the leaves are the major competing organs for the ^{45}Ca that is moving up the stem, as suggested by many authors (4, 5, 6, 21, 22, 53). Accumulation of ^{45}Ca in leaves in the shaded side of the tree (40.3%) was lower than that accumulated by the leaves on the sun side (50.4%). That difference

may be due to the higher transpiration rate of the sun side leaves as suggested by some workers (5, 39, 53). After 40 days of translocation, 36.5% of the ^{45}Ca was retained by the stems in the sun side while 49.4% was retained by the shaded side stems and 11% and 8% was retained by the respective application zones (Table 1). These results may agree with the suggestions of many authors (15, 40, 41, 52), that Ca is immobilized on exchange sites in the xylem. The slow movement of ^{45}Ca upward in the stem, as shown in our data may add further evidence to the concept that Ca translocation in the xylem is by an exchange mechanism, as suggested by many researchers (2, 6, 13, 32, 40, 41, 52). The results in Table 1 show that ^{45}Ca is still moving into the fruit late in the season (August 11, 1976 to September 20, 1976) but at a slow rate, which disagrees with some results with apple, that Ca movement into the fruit ceased late in the season (33). Millikan (22), and Wieneke and Fuhr (51), also showed that only a negligible amount of ^{45}Ca was moved to the apple fruit during the second half of August. Our data showed that 2.1% of grand total activity in the sun side and 1.9% of grand total activity in the shaded side was detected in the fruit from a late season application of ^{45}Ca. This amount of ^{45}Ca may be enough to make the difference between a cork spot and normal fruit. Branch injection of 'Bartlett' pear early in the season show that

^{45}Ca arrived at the fruit within three days of translocation. The total activity in the fruit was 364 dpm if the growing point in the branch was not removed, compared with 1525 dpm in the fruit if the growing point was removed. A total of 7729 dpm was accumulated in the growing point after three days, which suggests that the growing points are one of the major organs competing with fruit for the Ca moving up the stem early in the season. These findings agree with the suggestions of some authors (22, 51) that the young fruits, meristematic tissue, and young leaves act as strong sinks. The growing points may have strong effects on the translocation and distribution of Ca by acting as a source of hormones which may control the translocation (12, 38). These results may explain the advantage of summer pruning in increasing the Ca content of the fruit and reducing bitter pit in apple (W. J. Bramlage, communication by letter).

Fruit injection. A negligible amount of ^{45}Ca moved out of the fruit from fruit injection treatments early in the season (June 25), which agrees with the suggestions that the young fruits are a strong sink (22, 51). Injection late in the season resulted in up to 2.9% of the total activity located outside the druit, mainly in the leaves (Table 2). This suggests that the leaves are not only competing with the fruit on the ascending Ca but also are "pulling" soluble Ca from the fruit.

Painting on the fruit surface. Table 1 shows that 41% of the

^{45}Ca that was absorbed by the fruit moved out of the fruit, mainly to the leaves above the fruit. These results agree with the findings of Martin (21), that painting ^{45}Ca on the skin of apple fruit allows ^{45}Ca moved out of the fruit to the adjacent leaves, but the author did not show the amount of ^{45}Ca that moved out of the fruit. Wilkinson (54) suggested during August and September the total Ca per fruit may decline, indicating that part of the fruit Ca moved out. Wilkinson (54) also suggested that some of the Ca (1 to 1.5 mg) late in the season is in a free mobility between tree and fruit, which is sufficient to affect storage behavior. Our results from 'Anjou' pear frut confirm these hypotheses. Our data suggests that Ca movement out of the fruit late in the season could be the main cause of cork spot in 'Anjou' pear fruit. Wilkinson (54) suggested that among the factors which may affect Ca movement into or out of apple fruit late in the season is the competition between shoot and fruit. The results in Table 1, show that most of the ^{45}Ca that moved out of the fruit accumulated in the leaves. It has been shown that water movement out of the fruit occurred mostly through the xylem (19), which may suggest that the fruit loses its Ca via the xylem to the mainstream of transpiration.

The absorption of ^{45}Ca by the fruit from surface painting decreased near harvest (September 3, 1976) as shown in Table 2. It was reported that the penetration of Ca into apple fruit from spot application of ^{45}Ca on the skin decreased with progressive development of

the fruit (45). This may be due to the heavier cuticular wax development accompanying maturation.

We do not have a good explanation for the low activity of ^{45}Ca found out of the fruit in the fruit injection treatments compared with the high activity out of fruit in the fruit painting treatments (Tables 1 to 3). One possible explanation is that, in the painting treatments, ^{45}Ca is distributed on a larger surface area of the fruit and may have penetrated the skin to the ends of the vascular bundles, and then may have moved out of the fruit with the water that is leaving the fruit in the xylem to the leaves especially under moisture stress conditions. Support for this hypothesis is that the development of cork spot lesions typically appears close to the ends of the vascular bundles. The wounding injury from the needle may have caused a localized high respiration rate and released more CO_2 which may have caused the precipitation of Ca into $CaCO_3$ or vacuolar organic acids may have chelated the ^{45}Ca and reduced its movement out of the fruit. Also ^{45}Ca was found to be fixed on the core of apple fruit but not in the flesh (47).

Painting on the leaf surface. Leaf painting treatment on August 11, 1976 showed that 10% of the absorbed ^{45}Ca moved out of the leaf and accumulated in the leaves and stem above the application zone within 40 days (0.25% per day) from application. Later season

(September 13, 1976) painting on the leaf surface showed that 7.6% of the absorbed ^{45}Ca moved out of the leaves within 17 days (0.45% per day) from application. These results agree with the results of Martin (21) who found that painting of ^{45}Ca or the application of ^{45}Ca to the leaf surface of apple trees after harvest moved back to the spurs and reappears in the next season's leaves and fruits. Our data showed that the later the leaf painting the faster the movement of ^{45}Ca out of the leaf. This may be due to the aging and senescence of the leaf late in the season and reduced sink activity. These results suggest that Ca is translocated back to the stem from the leaves even though the treated leaves were harvested one month (September 20, 1976) prior to natural leaf fall. Calcium may have moved out of the leaves via the phloem as suggested by many authors (21, 23-25, 29, 30, 49). Our data showed that at least part of the Ca is mobile in 'Anjou' pear tree, but no attempt was made to characterize the relative amounts of ^{45}Ca movement in the phloem or xylem.

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CHAPTER III

RELATIONS BETWEEN CALCIUM, RESPIRATION, AND ETHYLENE EVOLUTION OF 'ANJOU' PEAR FRUIT

Literature Review

I. Effect of Calcium on Senescence and Abscission

Calcium is important for the maintenance of cellular integrity of both cell walls and membranes. Abscission of leaves, flowers, and fruits results from weakening of the cell walls and breakdown of membranes in the abscission zone. Calcium is important as calcium pectate, the cementing substance between cells (33). Calcium is also involved in the cell walls by forming linkages between the galacturonic acid chains, as well as between the galacturonates and the carboxyl groups of other components such as protein. Addition of $CaCl_2$ to the petiole of bean was shown to inhibit leaf abscission while other macronutrients were ineffective (31). The inhibition of abscission may result from a retardation of localized senescence due to Ca treatment (7, 31). The Ca concn in the abscission zone was increased and abscission was delayed if bean leaves were debladed under a $CaCl_2$ solution (33). Treating the leaves with ethephon or ethylene promoted abscission of bean leaves, and there was a corresponding decrease in Ca in the abscission zone just before abscission occurred

(33). Treating bean plants with $CaCl_2$ and then exposing the plants to ethylene or treating them with ethephon showed that $CaCl_2$ completely inhibited the abscission-enhancing effect of ethephon or ethylene in intact bean leaves (31, 33, 34). Calcium also inhibited the abscission-enhancing effect of NAA in bean plant (31). The inhibition of abscission caused by Ca may be due to the reduction of ethylene synthesis (31). Poovaiah and Leopold (32) followed the ethylene formation as a function of $CaCl_2$ concn and found a reduction of ethylene biosynthesis over a range of Ca concn from 10^{-4} to 10^{-2} M $CaCl_2$. The senescence of leaf discs was delayed by Ca treatment, and the effect was additive to the cytokinin effect on delaying senescence (32). Calcium treatment completely prevented the increase in apparent free space associated with senescence. Calcium treatment in leaf discs also deferred the increase in hydraulic permeability associated with senescence (32). The authors suggested that Ca delayed senescence of leaf discs by maintaining cellular membranes.

II. The Effect of Calcium on Membranes

One of the major functions of Ca in plants is the maintenance of cell membranes. Submicroscopic studies by Marinos (22) on barley shoot apices showed that Ca-deficiency became macroscopically evident within two days from the time the plants were transferred to the water culture. The effect of Ca-deficiency on cell ultrastructure

appeared as structural abnormalities, resulting in the breakup of the nuclear envelope and the plasma and vacular membranes and "structureless areas" appeared in cells, followed by the disorganization of the mitochondria and Golgi apparatus, and eventually the chloroplasts disintegrated (22). The cell walls stain darker and gaps may appear with the progress of Ca deficiency. The author suggested that Ca is essential for the maintenance and formation of cell-membrane systems, on which the functional integrity of cell metabolism is dependent. Marinos also suggested that the effects of Ca on cell walls was secondary. It was found later that the addition of Ca can restore the damaged membranes (2, 9) which adds further evidence that Ca is essential to the maintenance of plasma membranes. A substantial break-up of the endoplasmic reticulum and other cytoplasmic membranes in the cells of corn root apices was observed (17) under a divalent-ion-free root environment. It was suggested (9), that the action of Ca is not only to prevent the injury to cell membranes, but also Ca contributes actively to the formation of cytoplasmic organelles. Calcium deficiency caused a reduction in the amount of mitochondria and decreased the function of root mitochondria (9, 17). There is a large volume of evidence in the literature (2, 9, 17, 22, 30, 38, 41, 42) which shows that Ca participates in building up and maintaining cytoplasmic membranes in a functional condition. Calcium deficiency first leads to leaky membranes long

before breakdown (2). Removal of Ca from tissue by EDTA causes membranes to become leaky and highly permeable (42). Treating the isolated or in situ plasma membranes of soybean hypocotyls with 0.5 M $CaCl_2$ for 20 min caused the membranes to be 15 to 20% thicker than controls (30). The effect of $CaCl_2$ was abolished by EDTA. Exchange of Ca by either Mg, K, or H increased the general permeability in plant tissues from roots, leaves and fruits (3). Low Ca tomato fruits had a higher tissue permeability preceding the occurrence of blossom-end rot (41). Plasma membranes in pitted tissue of apples and in tomatoes with blossom-end rot are severely distrubed if not destroyed (2). Higher permeability of cell membranes may cause the acids as well as phenols to permeate from the tonoplast into the cytoplasm and destroy or deactivate enzymes, mitochondria, and other subcellular particles and thus damage the cell and the tissue. This might happen in a Ca deficient fruit and in bitter pit and cork spotted tissue. Calcium may affect membrane permeability and stabilization in two ways: (a) Ca may bind to the phosphate end-groups of the phospholipid molecules in the membrane and/or (b) Ca may bind to the protein molecules in the membrane. This binding of Ca may alter the size of the pores in the membranes and reduce passive permeability (2).

III. The Effect of Calcium on Respiration

Calcium ions have been shown to alter some aspects of senescence development in corn leaf discs (32), and apple fruit (4, 6, 20, 26, 28). Adding $CaCl_2$ (10^{-3} to 10^{-1} M) to the incubation solutions of apple fruit tissue slices decreased respiration yet increased protein synthesis (4, 8, 13, 15). Calcium not only depressed respiration but also delayed the onset of the climacteric rise of avocado fruits treated with 0.1 M $CaCl_2$ (40). Respiration of apple fruit was negatively correlated with Ca concn of flesh (8, 15). Respiration was higher with high N concn, and the high Ca counteracted the effect of high N and kept the respiration at a low level (15). Apple fruits with low Ca lost 30 to 70% of their ability to synthesize proteins and nucleic acids (15). One of the possible explanations for the high respiration in the tissue with low Ca is that higher permeability of the membranes may increase substrate availability to the respiratory enzymes in the cytoplasm and mitochondria (2). Respiration was increased during the development of bitter pit in apple (14, 16), and in 'Anjou' pear affected with cork spot (44). It is not known whether the high respiration of low-Ca fruit is the cause of bitter pit or whether it is only the result of leaky membranes.

IV. The Effect of Calcium on Ethylene Evolution

Calcium can inhibit the abscission-enhancing effect of ethylene (31, 33, 34), probably by the retention of membrane integrity in abscission layer cells and thereby preventing or delaying the onset of senescence, and thus the responsiveness of the abscission layer to ethylene (34). The authors suggested that Ca can overcome the effect of ethylene by preventing or reducing the sensitivity of the plant cells to ethylene. However Ca was reported to have a synergistic effect with kinetin on increasing ethylene production by mung bean hypocotyl segments (18, 19). In that experiment Ca increased the uptake and accumulation of kinetin either to the injury level which may have increased ethylene production or the high level of kinetin could have maintained a concn of endogenous auxin (IAA) which increased ethylene production. Calcium depressed the peak of ethylene production and delayed ripening in avocado fruit infiltrated with 0.1 M $CaCl_2$ (40). The rate of ethylene production increased at the first visible sign of the development of bitter pit in apple. Calcium concn was two-fold higher in healthy tissue than pitted tissues (14). 'Anjou' pear fruit affected with cork spot produced more ethylene than normal fruit (44). It is not known whether the high ethylene produced by pitted apples and cork spotted pears is the result of low calcium or due to the injure and death of the tissues.

V. The Effect of Calcium on Fruit Firmness

Several investigators have found Ca to be relatively effective in promoting firmness and delaying softening of apple fruit during prolonged storage (4, 6, 11, 12, 24, 27, 36). Treating apple fruits with $CaCl_2$ solutions prior to storage decreased the softening significantly (4). Increasing the uptake of Ca by adding surfactant and a thickener (Keltrol) to $CaCl_2$ solutions increased the fruit firmness up to 1.0 kg above the control (24, 27, 36). The increase in fruit firmness by $CaCl_2$ treatment depends on apple cultivars. In 'Spartan' apple an increase of 0.4 kg in the pressure test was obtained from a post-harvest dip in $CaCl_2$, while the increase in 'Jonathan' firmness was 0.7 kg (27). The increase in 'McIntosh' apple firmness was 0.52 kg (24), while 'Golden Delicious' apple firmness increased 1.0 kg by $CaCl_2$ and Keltrol dip (36). The rate of softening of 'McIntosh' apple was decreased by dipping the fruits in $CaCl_2$ plus Keltrol (24). The increase in fruit firmness caused by Ca could be explained on the basis of increasing the turgidity of the cells by improving the integrity of the cell membranes and reducing the permeability. Calcium treatments may also increase fruit firmness by strengthening cell walls.

VI. Postharvest Application of Calcium

The use of postharvest dips in Ca-containing solutions is another approach to correct the Ca-deficiency in the fruit. Many workers (4-6, 8, 11, 12, 23-25, 27, 29, 36, 37) have tested Ca-solutions dips on apple fruits, and found them to be effective. Dipping 'McIntosh', 'Cortland', and 'Baldwin' apples in solutions of 4% $CaCl_2$ with and without wetting agent raised Ca in fruit flesh about 15%, regardless of variety or wetting agent (6).

Dewey at Michigan State University (11, 12) studied the postharvest application of Ca for improving the quality of apple, and a summary of his results is as follows: Postharvest application of $CaCl_2$ controlled the internal breakdown of 'Jonathan' apple by increasing the Ca concn in the fruit. Dipping 'Jonathan' and 'McIntosh' fruits in 4% $CaCl_2$ alone doubled the Ca content of the fruit cortex and reduced the incidence of breakdown from 3.3 to 0.2%, and reduced 'Jonathan' spot from 16.3 to 1.3%. Fruit injury at the lenticels and at the calyx cavity was increased by increasing Ca concn. The effect of certain additives including adjuvants, spreaders, fungicides and scald inhibitors was tested by Dewey. He concluded that diphenylamine (DPA), Triton X-100 and the fungicide Mertect 260 were highly effective in reducing lenticel and calyx cavity injury. Dewey did not indicate whether these additives affected the penetration or uptake of

Ca by the fruit.

Martin and co-workers in Australia (23) studied the effect of postharvest Ca treatments on the development of bitter pit and internal breakdown in apples, and they reached the following conclusions: postharvest dips in a combination of $Ca(NO_3)_2$ and diphenylamine (DPA) inhibited the development of storage bitter pit significantly. Combination of 2% $Ca(NO_3)_2$ and DPA (2000 ppm) not only reduced bitter pit but also reduced scald, and inhibited internal breakdown. Different varieties of apple behave differently regarding uptake and permeability of Ca thru the skin. In varieties with a lower permeability, addition of Tween-20 (0.1%), glycerol (1%) or DPA (2000 ppm) increased the rate of penetration. Penetration rate of Ca in the fruit is greater at higher humidities (85% RH) than at lower (75% RH) humidities.

Bangerth (1, 4), studied the effect of $CaCl_2$ dips on the incidence of bitter pit in apple and showed that the treatment is effective if high concn of $CaCl_2$ (3-5%, depending on the apple variety), increased dipping time (2-10 min) and high relative humidity (90-95%) in the storage rooms were used. Treating 'Cox's Orange Pippin' apples by the above treatment reduced bitter pit development in storage from 29.4% to 1% (1). Dipping 'Jonathan' apples while still on the tree twice in $CaCl_2$ reduced lenticel spot (a disorder similar to cork spot) from 19.9% to zero (1).

Uptake of Ca by apple fruit from $CaCl_2$ solution dips occurred mainly during storage from residues left on the apple (6). It was suggested that the dipping time and the solution temperature did not affect Ca uptake. Furthermore, the concn of $CaCl_2$ was suggested to be the most influencing factor on the uptake of Ca by the fruit. Absorption of Ca from the solution was mainly through natural openings in the fruit surface (open lenticels, open calyx), and this might explain the great differences observed between apple varieties (6). It was also suggested that the humidity in storage is an important factor for Ca uptake, and 95% RH was recommended to keep the $CaCl_2$ residue from drying out, so that the apples can continue to absorb Ca for several months.

Mason and co-workers in British Columbia (24, 25, 27, 29), developed economical dip procedures to prevent breakdown and bitter pit in 'Spartan' and 'McIntosh' apples. The dip solutions contained 4% $CaCl_2$. Nine hundred pound (408.6 kg) bins of apples were moved by lift trucks and dipped as soon as the bins arrived at the packing house from the orchard. After the bin was dipped, it was left to drain before it was moved into cold storage. The cost of the dip was about 65¢/bin. Calcium uptake from the dip was about 80 ppm Ca in the flesh of the apple fruit, while Ca uptake from the orchard spray was about 30 ppm (29). They also studied the effect of thickeners added to the dip solution on Ca uptake by the fruit. Their results

showed that addition of arrowroot gum thickener to the dip solution increased Ca uptake to 217 ppm, compared with 78 ppm without thickener. Addition of the thickener "Kelzan" increased Ca uptake to 352 ppm more than the control (4% $CaCl_2$ without thickener). The thickener "Keltrol" which is used as a commercial pie filling can be used also. The addition of Keltrol (0.3%) to $CaCl_2$ solutions (4%) increased Ca concn in the flesh of 'McIntosh' apple 535 ppm above the water control and 405 ppm above the $CaCl_2$ alone (24), while Ca concn in the peel was increased 1509 ppm above the $CaCl_2$ treatment alone.

The literature indicates considerable promise for postharvest dipping of apples in calcium solutions to improve storage life. Therefore the object of study in this chapter was to re-investigate these treatments to evaluate their effectiveness on increasing the Ca concn in 'Anjou' pear fruit. The second objective was to study the effects of Ca and N on respiration and ethylene evolution, which may explain the abnormal ripening and senescence of the disordered fruits.

Materials and Methods

I. Postharvest Applications of Calcium

A. Dipping the fruits in $CaCl_2$. 'Anjou' pear samples were harvested from the Naumes orchard (with history of no cork spot at

harvest) on September 4, 1976, placed in cold storage at 0°C, dipped on September 20, 1976, and returned to storage. Samples were dipped 15 minutes in distilled water (control), or in solutions of 2% or 5% $CaCl_2$ with and without Keltrol as a thickener (0.125% or 0.25%) (4, 24, 25). Each treatment was replicated five times, using 50 fruit for each replication. The dipped fruits were enclosed in perforated polyethylene film liners and stored six months at 0°C prior to analyses for Ca uptake. The fruits were enclosed in the plastic liners before they were allowed to drain, to keep the relative humidity high, and prevent the $CaCl_2$ residue from drying out. The fungicide Mertect 140-F was included (7 g/gal) in all dip treatments. The fruits were then taken from storage and washed carefully with distilled water on March 5, 1976, and samples of five fruits from each replicate were prepared for mineral analyses as described in Chapter I. The rest of the fruits were used for respiration and ethylene evolution studies.

B. Vacuum infiltration with $CaCl_2$. Samples of 25 'Anjou' pear fruits were placed in a large desiccator with a vacuum outlet, containing $CaCl_2$ solution (1% or 2%), with and without fungicide (Mertect 7 g/gal). The controls were distilled water with and without fungicide. Vacuum from a water aspirator was applied (30 to 15 mm Hg) for 1 min or 5 min, starting after the air bubbles appeared on the fruit surface. Each treatment was replicated five times. Fruits were then stored as described above. Treatments without fungicide

included in the solution, were dipped in fungicide prior to storage. The pears were then taken from storage on March 5, 1976, washed carefully with distilled water, and samples of five fruits from each replicate were prepared for mineral analysis as described in Chapter I. The remaining fruits were used for respiration and ethylene evolution studies.

II. Respiration

Samples of five fruits (about 1 kg) each, for respiration determination were put in one-gal glass jars and sealed with large rubber stoppers containing inlet and outlet tubes. Air flow rate was maintained at 200 ml per minute per container with fresh air drawn from outdoors using an air pump and fine metering valves (35, 43, 45). The experiment was conducted in a ripening room maintained at 20°C. Rate of respiration was measured daily using the Claypool-Keefer method (10) for the colorimetric determination of CO_2. Additional samples of fruits with cork spot and some normal fruits from orchards with low Ca were used to get a wide range of Ca concentration. At the end of the experiment the fruits were used for mineral analyses as described in Chapter I.

III. Ethylene Evolution

One ml gas samples were taken from the outlet tubes of the

samples described in the respiration section (35, 45), by hypodermic syringe for ethylene determination. Ethylene was monitored daily by a flame ionization gas chromatograph (Varian Aerograph Model 1200) using a 5 ft x 1/8 inch stainless steel column packed with 60/80 mesh alumina. Flow rates were: H_2 = 30 ml/min; N_2 = 30 ml/min; and air 300 ml/min. Temperatures were: injector 70°C, column 30°C, and detector 60°C (45).

Respiration and ethylene evolution experiments were performed on fruit samples after six months in storage. The respiration experiment was also done on fruits after harvest (September 19, 1976), but we were not able to do the ethylene measurements due to an equipment malfunction.

IV. Measurements of Ethylene in the Internal Atmosphere of 'Anjou' Pear Fruit

Samples of 'Anjou' pear fruit were dipped in 5% $CaCl_2$ solution plus 0.125% Keltrol thickener and fungicide (Mertect) and stored as described earlier. Samples of 20 fruits from the treatments and control were enclosed in plastic buckets fitted with the appropriate tubes and an air tight cover (45); were treated in the same way as the one-gal jars in the respiration section. Another sample of fruits with light cork spot was used for comparison. Treatments and control were replicated five times.

Ethylene in the internal atmosphere of fruits was extracted daily by taking two fruits from each bucket and placing one fruit at a time in a small vacuum desiccator filled with NaCl-saturated water (39) at 20°C. A small funnel with a rubber septum fitted at the end of the stem was placed over the fruit carefully so there was no air trapped in the funnel. A vacuum (30 to 15 mm Hg) was applied on the desiccator from a water aspirator until the stem of the funnel was filled (about 4 ml) with the gas extracted from the fruit. The vacuum was then stopped and the desiccator cover was removed. One ml gas samples were drawn from the funnel through the septum by using one ml syringe fitted with hypodermic needle. The syringe was closed by pushing the needle into a soft rubber stopper (21). Ethylene concn in the gas samples was determined by gas chromatography as described previously.

The fruits were then used to measure the flesh firmness (24) by using Magness-Taylor type pressure tester with 8 mm plunger. The firmness was determined daily to measure the rate of fruit softening during ripening in relation to Ca concentration and the rate of ethylene evolution.

Results

I. Postharvest Uptake of Ca by 'Anjou' Pear Fruit

A. Uptake from $CaCl_2$ dips. Dipping the fruits for 15 min in 2% or 5% $CaCl_2$ after harvest increased the Ca concn in thefruit from 7.11 mg/100 g fresh weight to 9.20 and 9.57 mg/100 g of fresh weight respectively (Table 1). In the absence of thickener there was no significant effect on the accumulation of Ca by the fruit upon increasing the Ca concn in the dip solution from 2% to 5%. Adding Keltrol (0.125%) thickener to the dip solution increased Ca uptake 2.85 mg/100 g of fresh weight above the 2% $CaCl_2$ dip alone, and 4.16 mg/100 g of fresh weight above the 5% $CaCl_2$ dip alone. Addition of the thickener to the dip solution increased Ca uptake significantly from both concentrations. Elevating the thickener concn from 0.125% to 0.25% in the 5% $CaCl_2$ increased Ca uptake by 4.37 mg/100 g of fresh weight. The increases in Ca uptake are significant at the 1% level using Duncan's multiple range test (Table 1).

B. Vacuum infiltration with $CaCl_2$. The increase in Ca concn in the fruit after vacuum infiltration for 1 min in 1% or 2% $CaCl_2$ was 1.52 and 3.49 mg/100 g of fresh weight respectively (Table 1). The 5 min vacuum infiltration period increased Ca uptake from the 1% $CaCl_2$ solution by 3.41 mg/100 g of fresh weight above the water control. Infiltrating the fruit for 5 min in 2% $CaCl_2$ increased Ca

Table 1. Postharvest uptake of calcium by 'Anjou' pear fruit treated with $CaCl_2$ solutions.

	Treatment	Mean Ca concn mg/100 g of f.w.
1.	Dipped in water + Keltrol (0.125%)	7.11a
2.	Dipped in 2% $CaCl_2$	9.20b
3.	Dipped in 5% $CaCl_2$	9.57b
4.	Dipped in 2% $CaCl_2$ + Keltrol (0.125%)	12.05c
5.	Dipped in 5% $CaCl_2$ + Keltrol (0.125%)	13.73d
6.	Dipped in 5% $CaCl_2$ + Keltrol (0.25%)	18.10e
7.	Vacuum infiltration with water for one min.	7.00a
8.	Vacuum infiltration with 1% $CaCl_2$ for one min.	8.52b
9.	Vacuum infiltration with 2% $CaCl_2$ for one min.	10.49c
10.	Vacuum infiltration with water for five min.	7.25a
11.	Vacuum infiltration with 1% $CaCl_2$ for five min.	10.66b
12.	Vacuum infiltration with 2% $CaCl_2$ for five min.	11.13b

Duncan's $(LSR)_{.01}$ = 1.48 and .05 = 1.244

NOTE: Mean separation by different letters is significant at the 1% level.

uptake 3.88 mg/100 g of fresh weight. These increases in Ca uptake are significant at the 1% level.

II. Respiration

Respiration is expressed as mg CO_2 evolved per kg of fruit per hr during ripening at 20°C. The climacteric rise in respiration of fruits with different Ca concn immediately after harvest and after six months in storage is shown in Figures 1 and 2, respectively. The peak of CO_2 evolution during the climacteric rise in respiration is referred to as "CO_2 peak." Figure 1 shows that cork spotted fruits with low Ca were able to ripen right after harvest, while the normal 'Anjou' pear did not ripen even after 15 days at 20°C. Figure 2 shows that the higher the Ca concn in the fruit the lower the peak of CO_2 evolution. Increasing Ca concn in the fruit to 10.49 mg/100 g of fresh weight by vacuum infiltaration in 2% $CaCl_2$ diminished the peak of CO_2 evolution during the respiratory climacteric during ripening at 20°C.

Figure 1 shows that 'Anjou' pear fruit with very low Ca have no chillling requirement for ripening and those fruits were able to ripen on the tree and even drop on the ground before harvest.

A. Simple linear correlations between respiration, Ca, N and N:Ca ratio (r-values). The results are expressed as correlation coefficients (r-values) (Tables 2 and 3). There is a highly significant

Fig. 1. Respiratory climacteric of 'Anjou' pear fruits in relation to Ca concn in the fruit, after harvest (September 19, 1976). Each point represents an average of four samples.

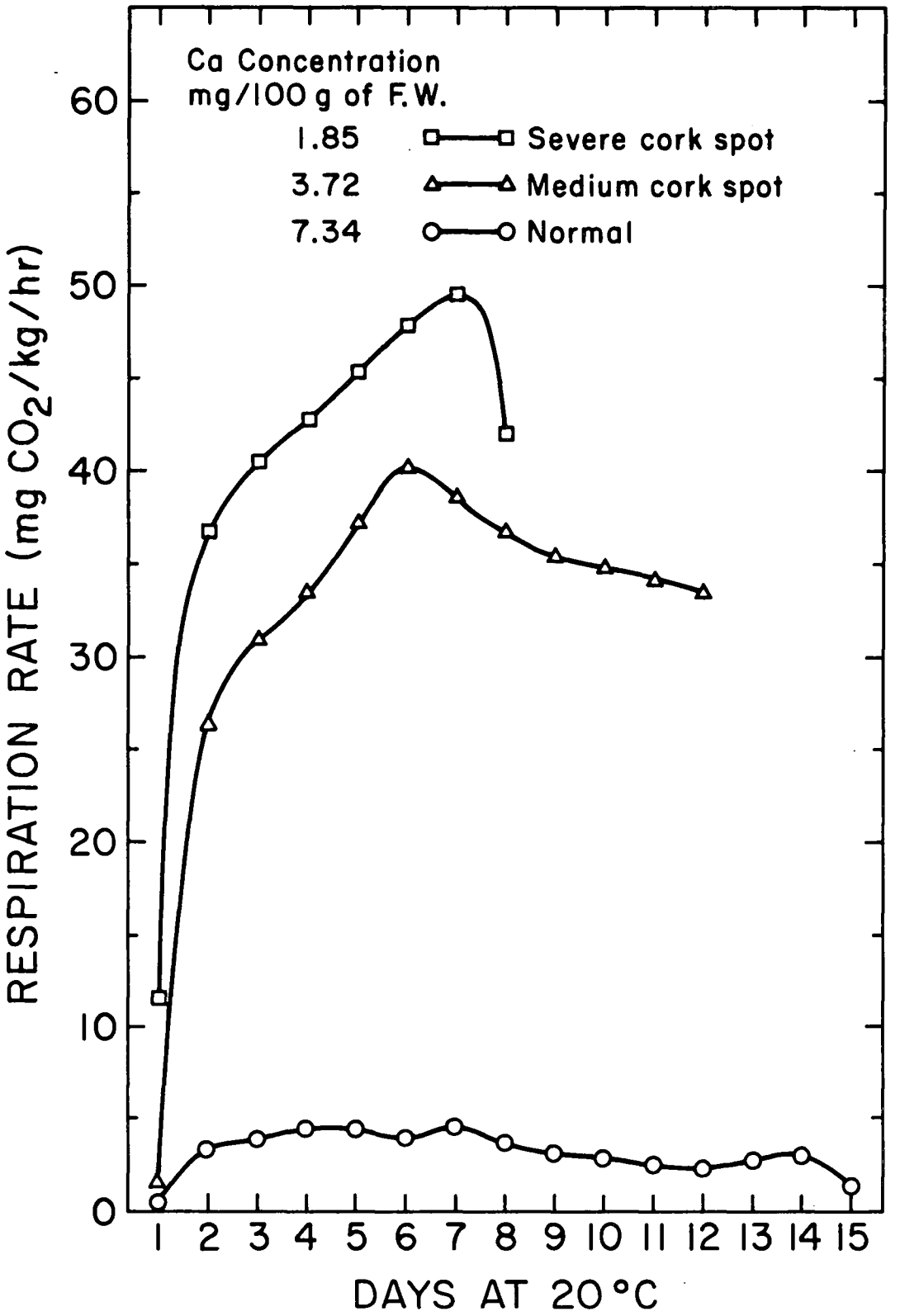

Ca Concentration
mg/100 g of F.W.
1.85 Severe cork spot
3.72 Medium cork spot
7.34 Normal
RESPIRATION RATE (mg CO2/kg/hr)
0
10
20
30
40
50
60
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
DAYS AT 20 °C

Fig. 2. Respiratory climacteric of 'Anjou' pear fruits in relation to Ca concn in the fruit, after six months in storage. Each point represents an average of five samples.

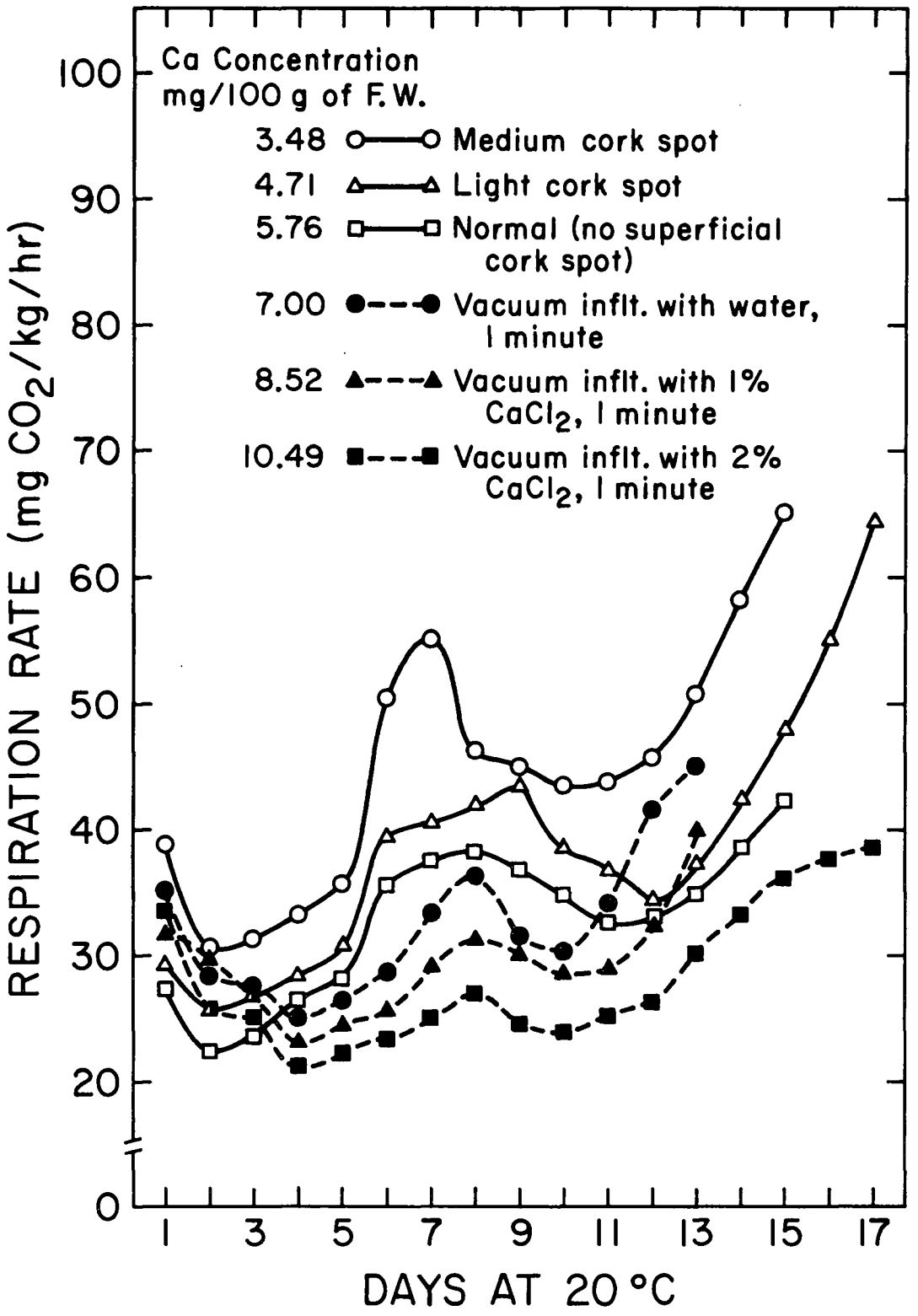

Ca Concentration
mg/100 g of F.W.
3.48 Medium cork spot
4.71 Light cork spot
5.76 Normal (no superficial cork spot)
7.00 Vacuum inflt. with water, 1 minute
8.52 Vacuum inflt. with 1% CaCl2, 1 minute
10.49 Vacuum inflt. with 2% CaCl2, 1 minute
RESPIRATION RATE (mg CO2/kg/hr)
DAYS AT 20 °C
0
20
30
40
50
60
70
80
90
100
1
3
5
7
9
11
13
15
17

Table 2. Simple linear correlations between respiration, and calcium, nitrogen, and nitrogen:calcium ratio, after harvest (on September 19, 1976). Correlation coefficients (r-values) are shown. df = 23

Independent variables	Dependent variables	
	Initial CO_2	CO_2 peak
Total Ca	-0.46*	-0.87**
Water soluble Ca	-0.45*	-0.91**
Nitrogen	-0.30 NS	-0.08 NS
N:Ca ratio	0.77**	0.59**

**Significant at the 1% level; *significant at the 5% level; NS - not significant.

Table 3. Simple linear correlations between respiration, ethylene evolution and calcium, nitrogen, and nitrogen:calcium ratio, after six months storage. Correlation coefficient (r-values) are shown. df = 57

Independent variables	Dependent variables			
	Initial CO_2	CO_2 peak	Initial ethylene	Ethylene on 8th day
Total Ca	-0.64**	-0.83**	-0.81**	-0.87**
Water soluble Ca	-0.59**	-0.76**	-0.74**	-0.83**
Nitrogen	0.20 NS	0.21 NS	0.02 NS	0.26*
N:Ca ratio	0.57**	0.72**	0.59**	0.74**

**Significant at the 1% level; *significant at the 5% level; NS - not significant.

negative correlation between both Ca (acid extract) and water soluble Ca and the peak of CO_2 evolution in the respiration experiment right after harvest (September 19, 1976) (Table 2). The correlation coefficient (r-value) between water soluble Ca and CO_2 peak is greater (-0.91) than the correlation with total Ca (-0.87). Initial CO_2 and both total Ca and water soluble Ca are significantly correlated at the 5% level. Nitrogen and both initial CO_2 and CO_2 peak are not significantly correlated but the correlations with N:Ca ratio are highly significant and positive (Table 2).

The correlations between both total Ca and the water extractable Ca and the peak of CO_2 evolution in the respiration experiment after six months in storage were negative and highly significant (Table 3). Correlations with initial CO_2 were also highly significant and negative (Table 3). No significant correlations existed between N and initial CO_2 or CO_2 peak, but N:Ca ratio was significantly correlated with both dependent variables (Table 3).

III. Ethylene Evolution

Figure 3 shows ethylene evolution from 'Anjou' pear fruit at 20°C after six months in storage, in relation to Ca concn in the fruit. Figure 3 also shows that high ethylene is associated with low Ca even in the absence of injured or senescent tissue (i.e., normal fruits with low Ca showed high ethylene evolution).

Although we have no data on ethylene evolution at harvest (because of GC malfunction), one would assume that only the low Ca fruit would have high ethylene production, because of the development of cork spot.

A. Simple linear correlations between ethylene evolution and Ca, N, and N:Ca ratio (r-values). There is a highly significant correlation coefficient between the initial rate of ethylene and both total Ca and water soluble Ca (r-values are -0.81 and -0.74, respectively) (Table 3). Ethylene evolution after eight days at 20°C is highly correlated with total Ca and water soluble Ca in the fruit (r-values are -0.87 and -0.83) (Table 3). The correlations between Ca fractions and both initial ethylene and ethylene evolution were negative and highly significant (Table 3). The correlation between N and initial ethylene is not significant, but the correlation with ethylene evolution after eight days at 20°C is significant at the 5% level. Both initial ethylene and ethylene evolution after 8 days have positive and highly significant correlations with the N:Ca ratio (Table 3).

B. Multiple correlation between ethylene evolution and Ca, N, and N:Ca ratio. The results are expressed as coefficient of determination (r^2-values). The best two values that have an effect on the initial ethylene are Ca and N, which are responsible for 67% of the variations in the initial ethylene evolution (r^2=0.67) (Table 5).

Table 4. Multiple correlations between respiration and calcium, nitrogen, and nitrogen:calcium ratio after harvest (September 19, 1976). Coefficient of determination (r^2-values) are shown. df = 23

Dependent variables	Independent variables (1)	r^2 Value
Initial CO_2	N/Ca - N	0.64**
Initial CO_2	N/Ca - N + Ca	0.70**
Initial CO_2	N/Ca - N + Ca - Ca (water soluble)	0.73**
CO_2 peak	N/Ca - Ca (water soluble)	0.87**

**Significant at the 1% level

(1): (-) or (+) indicate sign of partial correlation.

Table 5. Multiple correlations between respiration, ethylene evolution and calcium, nitrogen, and nitrogen:calcium ratio, after six months storage. Coefficient of determination (r^2 values) are shown. df = 57

Dependent variables	Independent variables (1)	r^2 Value
Initial CO_2	N/Ca - Ca	0.42**
CO_2 peak	N/Ca - Ca	0.71**
Initial ethylene	- Ca - N	0.67**
Initial ethylene	N/Ca - Ca - N	0.68**
Ethylene on 8th day	N + Ca	0.77**

(1): (-) or (+) indicate sign of partial correlation; **significant at the 1% level.

The same two variables are responsible for 77% of the variation in ethylene evolution after eight days at 20°C (Table 5). The importance of the N:Ca ratio and the water soluble Ca for initial ethylene and ethylene evolution after eight days is masked by total Ca, which may be responsible for 66% and 76% of the variation in both dependent variables respectively (Table 5).

IV. Concentration of Ethylene in the Internal Atmosphere of Stored 'Anjou' Pear Fruit

There was a rapid rise in the rate of ethylene evolution from the internal atmosphere of stored fruits with light cork spot and the normal fruits with low Ca concentration, while the rate of increase in the fruits with high Ca concentration (dipped in 5% $CaCl_2$ + Keltrol) was slower (Fig. 4). The increase in ethylene concn in the internal atmosphere of 'Anjou' pear fruit during ripening at 20°C follows the same pattern as the climacteric rise in the external ethylene, however, the concn in the internal atmosphere reaches the peak much earlier than the external atmosphere (compare Figures 3 and 4). The peak of ethylene concn in the internal atmosphere is much higher (122 ppm ethylene) in the fruit with a high Ca concn rather than low Ca concn (70 ppm ethylene), but the fruits with high Ca concn reached the peak four days later than those low in Ca (Figure 4).

Fig. 3. Ethylene evolution of 'Anjou' pear fruit in relation to Ca concn in the fruit, after six months in storage. Each point represents the average of five samples.

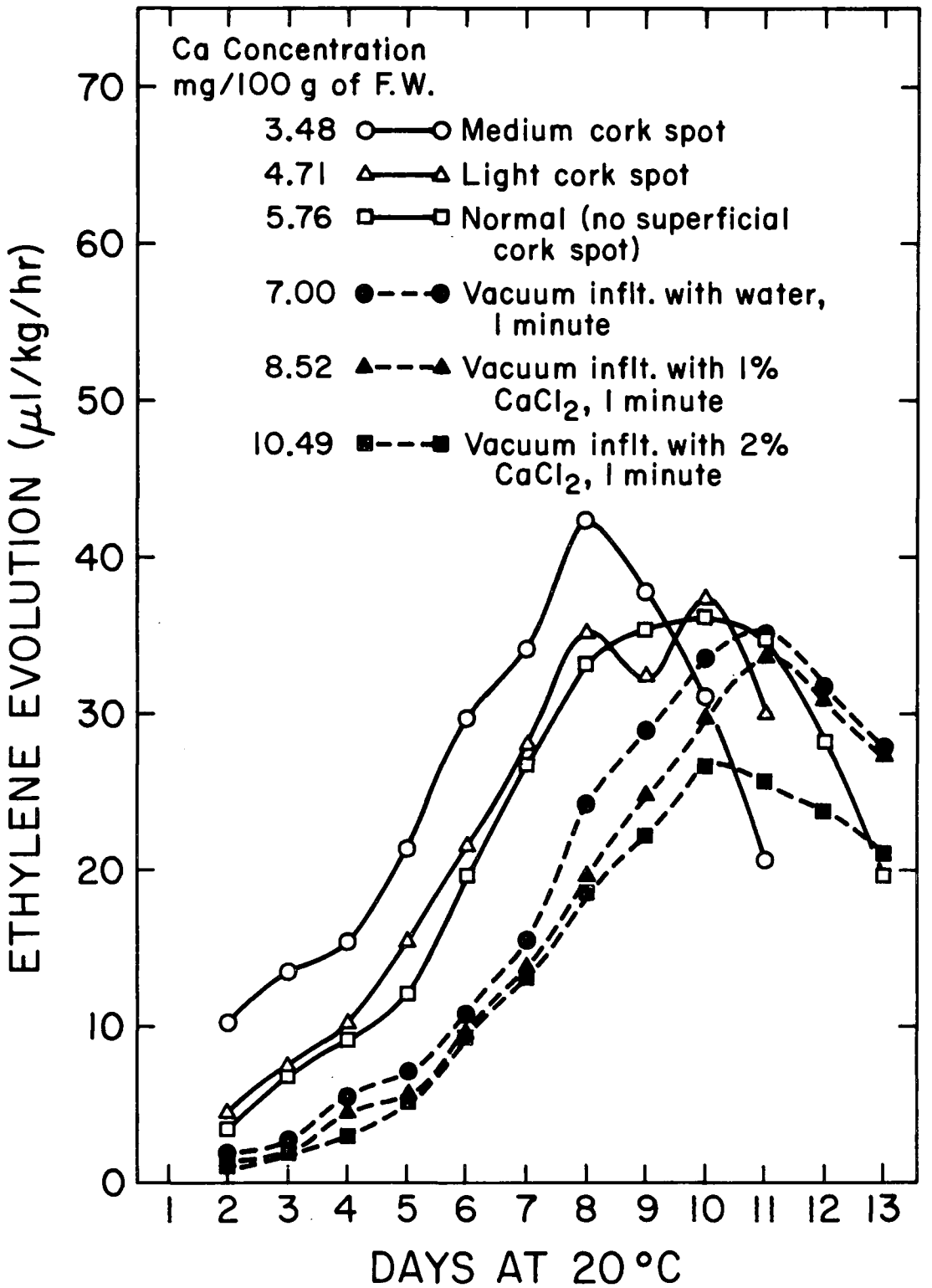
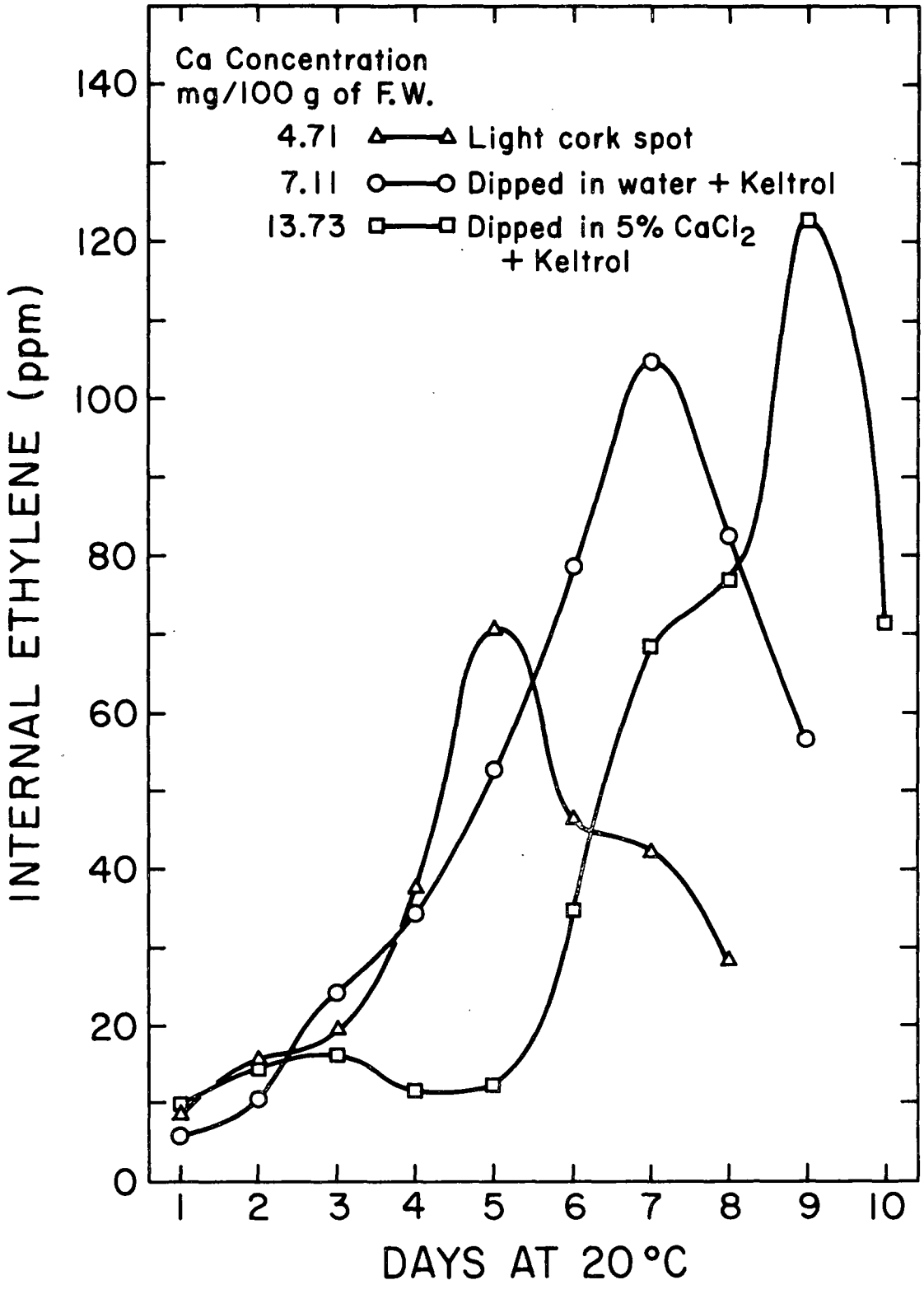

Ca Concentration
mg/100 g of F.W.
3.48 Medium cork spot
4.71 Light cork spot
5.76 Normal (no superficial cork spot)
7.00 Vacuum inflt. with water, 1 minute
8.52 Vacuum inflt. with 1% $CaCl_2$, 1 minute
10.49 Vacuum inflt. with 2% $CaCl_2$, 1 minute
ETHYLENE EVOLUTION (µl/kg/hr)
0
10
20
30
40
50
60
70
1 2 3 4 5 6 7 8 9 10 11 12 13
DAYS AT 20 °C

Fig. 4. Relation between ethylene concentration in the internal atmosphere of 'Anjou' pear fruit during ripening and the concn of Ca in the fruit. Each point represents an average of ten fruits.


Ca Concentration
mg/100 g of F.W.
4.71 Light cork spot
7.11 Dipped in water + Keltrol
13.73 Dipped in 5% CaCl2 + Keltrol
INTERNAL ETHYLENE (ppm)
0
20
40
60
80
100
120
140
1
2
3
4
5
6
7
8
9
10
DAYS AT 20 °C

V. Rate of Fruit Softening in Relation to Calcium Concentration

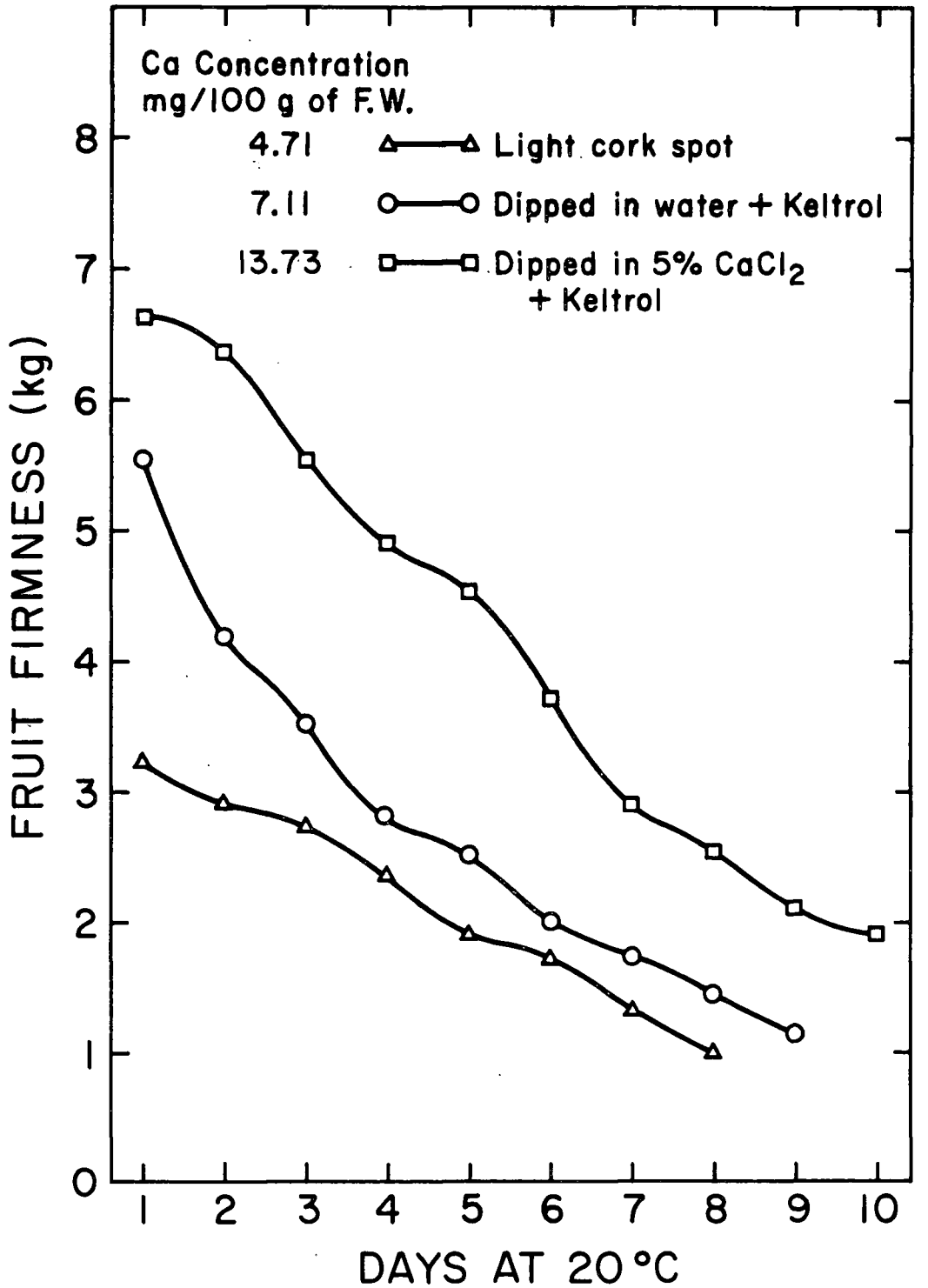
Fruit firmness was measured during ripening at 20°C and the results are shown in Figure 5. After six months in storage, pears with a high Ca concn were considerably firmer (6.63 kg) than those with low Ca concn (3.21 kg). After eight days in the ripening room at 20°C, the firmenss of the fruits with high Ca were about 2.5 times greater than that of the fruits low in Ca (Figure 5). Fruits with an intermediate Ca concn (7.11 mg/100 g of fresh weight) also softened much faster than fruits with a high Ca conc. Pears with different Ca concn all ripened satisfactorily by the end of the experiment.

Discussion

I. Postharvest Uptake of Ca by 'Anjou' Pear Fruit

A. Uptake from $CaCl_2$ dips. The dip treatments increased Ca uptake by the fruit significantly. These results agree with results from studies with $CaCl_2$ dips in apples (1, 6, 11, 12, 23). Increasing $CaCl_2$ concn in the dip solution and addition of Keltrol thickener increased Ca uptake as suggested by many authors (1, 6, 8). The addition of Keltrol as a thickener increased Ca uptake from the high concn of $CaCl_2$ significantly. These findings are in agreement with the results of many workers with apples (24, 25, 27, 29). High

Fig. 5. Relation between the rate of fruit softening and Ca concn during ripening of 'Anjou' pear fruit. Each point represents an average of ten fruits.


Ca Concentration
mg/100 g of F.W.
4.71 Light cork spot
7.11 Dipped in water + Keltrol
13.73 Dipped in 5% CaCl2 + Keltrol
FRUIT FIRMNESS (kg)
0
1
2
3
4
5
6
7
8
1 2 3 4 5 6 7 8 9 10
DAYS AT 20 °C

relative humidity is an important factor to keep the $CaCl_2$ residue from drying out so that the fruit can continue to absorb Ca for several months during storage (6). In our work the humidity around the fruits was kept high by enclosing the fruits in plastic liners before they were allowed to drain. Moreover the addition of the thickener (Keltrol) left a thick layer of residue of $CaCl_2$ especially at the high Ca concn (5% $CaCl_2$), and the thickener retained more water which prevented the $CaCl_2$ residue from drying out. Postharvest Ca dips of unpacked fruits would be an economical way to prolong storage life and reduce storage disorders.

B. Vacuum infiltration with $CaCl_2$. The vacuum infiltration treatments increased Ca concn in the fruit significantly. This treatment caused no injury to the fruit compared with the dip treatment at high concn (5% $CaCl_2$) which caused some lenticel injury. Vacuum infiltration treatments have been reported later on apple (37), and proved to be an effective method for reducing bitter pit of 'Gravenstein' and 'Cox's Orange Pippin' apples stored for three weeks at room temperature in New Zealand. The authors did not measure the increase in fruit Ca concn caused by the vacuum infiltration. In our work, we were able to increase Ca concn significantly and successfully store fruits for six months. At the end of the experiment the fruits were green and firm and could likely have been held in storage for a much longer time. We did not attempt vacuum infiltration with

high $CaCl_2$ concn in order to avoid possible injury; however, it was recently reported that apple fruits can be vacuum infiltrated with high concn of $CaCl_2$ (up to 10%) without injury if the fruits are rinsed with water after the infiltration (37). Vacuum infiltration treatment is a very promising method for postharvest Ca treatment and it may have a positive effect on storage life of pear fruit, providing an economical method can be worked out.

II. Respiration

A. Simple linear correlations between respiration and Ca, N, and N:Ca ratio.

The negative and highly significant correlation coefficient between Ca and both rate of initial CO_2 and CO_2 peak right after harvest and after six months in storage means that Ca deficiency in the fruit is a major cause of high respiration (Tables 2 and 3). Our results agree with those of other studies with apple fruit tissue slices (4, 13), and intact apple fruit (4, 8, 15), and avocado fruit (40), in which treating the fruit with Ca as $CaCl_2$ caused a significant decrease in respiration. Respiration of apple fruit has also been negatively correlated with Ca concn of the flesh (8, 15). With 'Anjou' pear fruit the higher the Ca concn in the fruit the lower the peak of CO_2 during the respiratory climacteric (Figure 2). The same had been observed for avocado fruit treated with $CaCl_2$ (40). Calcium not only depressed respiration but also delayed the onset of the

climacteric rise of avocado fruits infiltrated with $CaCl_2$ solution. In our work with 'Anjou' pear fruit, increasing Ca concn in the fruit by vacuum infiltration almost abolished the peak of CO_2 during the climacteric rise, but did not delay the onset of the climacteric rise significantly (Figure 2). These results also show that this fruit can still ripen normally even with a high concn of Ca. Figure 1 shows that the fruits with severe cork spot and medium cork spot already went through the climacteric rise on the tree (before harvest), and most of these fruits dropped to the ground before harvest. However, the normal fruits immediately after harvest did not show any climacteric rise even after 15 days at 20°C. 'Anjou' pear fruit normally need to be stored at least 60 days at 0°C before they are able to ripen, but calcium deficiency caused the fruit to ripen much earlier, even on the tree. Respiration was increased during the development of bitter pit in apple (14, 16) and in 'Anjou' pear affected with cork spot (44). One of the possible explanations for the high respiration in the tissue with low Ca, is the higher permeability of the membranes, which may allow larger amounts of substrates to reach the respiratory enzymes in the cytoplasm and the mitochondria (2). It is not known at this time whether the high respiration of low-Ca fruit is a cause or a result of bitter pit and cork spot or whether it is only the result of leaky membranes. A further effect might be related to inhibition of ethylene synthesis or antagonism to the

response of fruit tissue to ethylene.

B. Multiple correlation between respiration and Ca, N, and N:Ca ratio (coefficient of determination, or r^2-value). The results show that the CO_2 peak after harvest is mainly affected by the water soluble Ca and the N:Ca ratio, which are responsible for 83% of the variations in the CO_2 peak (Tables 2 and 4). This means that a deficiency of the water soluble Ca in the fruit is the main cause of the high respiration in the fruit affected with cork spot at harvest. Some of our preliminary results show that the water soluble fraction of Ca decreased in the fruit as harvest approached, and the data in Chapter II showed that 40% of the radioactive Ca moved out of 'Anjou' pear after painting on the fruit surface. These data support the hypothesis that the water soluble calcium moves out of the fruit late in the season, especially under moisture stress conditions. The data reported here show that the deficiency of water soluble Ca is strongly associated with high respiration in the fruit at harvest. Data from Chapter I showed that the deficiency of water soluble Ca was a major cause of severe cork spot at harvest.

After six months in storage total Ca (acid extractable) and the N:Ca ratio are the main variables which may affect both initial CO_2 and CO_2 peak at the climacteric rise in respiration (Table 5). The importance of the water soluble Ca during storage is masked by the total Ca, suggesting that more of the total Ca became water soluble

during storage.

III. Ethylene Evolution

A. Simple linear correlation between ethylene evolution and Ca, N, and N:Ca ratio (r-values). The correlation coefficient between Ca and both initial ethylene and ethylene evolution after eight days at 20°C following six months storate are negative and highly significant (Table 3). The results mean that Ca deficiency is strongly associated with high ethylene evolution. Figure 3 shows that the higher the Ca concn in the fruit the lower the initial ethylene and the lower the ethylene evolution during the climacteric rise. The "dip" in the ethylene curve for the light cork spot fruits (Figure 3) is likely due to two or more of the five fruits reaching their peak ethylene evolution two days apart. Increasing Ca concn in 'Anjou' pear fruit by vacuum infiltatation reduced ethylene production significantly as shown in Figure 3. The same results were reported with avocado fruit infiltrated with Ca Cl_2 (40). Figure 3 also shows that the fruits with medium cork spot produced more ethylene than normal ones. The rate of ethylene production reportedly increased at the first visible sign of the development of bitter pit in apple (14). Ethylene production by 'Anjou' pear fruit affected with cork spot was reported to be four times higher than normal fruits (44). Calcium completely inhibited the abscission-enhancing effect of ethylene, and also reduced ethylene

biosynthesis over a range of Ca concn from 10^{-4} to 10^{-2} M of $CaCl_2$ (31, 33, 34). The authors suggested that Ca can overcome the effect of ethylene, probably by the retention of membrane integrity which would prevent or delay the onset of senescence.

The multiple correlation results showed that N and Ca are the most important variables which may control the initial ethylene and ethylene evolution after eight days at 20°C (Table 5). Seventy-seven percent of the variation in the ethylene evolution could be accounted for by N and Ca (r^2=0.77). The effect of N on ethylene evolution is not known, and the problem needs further investigation.

IV. Concentration of Ethylene in the Internal Atmosphere of 'Anjou' Pear Fruit

The rate of increase in ethylene concn in the internal atmosphere of 'Anjou' pear fruit is much faster than the rate of increase of ethylene in the external atmosphere, but the shape of the curve is similar to that of the climacteric rise in ethylene concn in the external atmosphere. The higher the Ca concn in the fruit the higher the peak of internal ethylene, but the time to reach the ethylene peak is longer in the fruits with high Ca (Figure 4). The results reported in this section are surprising and hard to explain, due to the paucity of studies on the internal ethylene in the fruits. The first possible explanation for the low internal ethylene in the fruit with cork spot

is that the fruit can not accumulate high concentration of ethylene due to the rupture of the cuticle and the epidermis, which allows ethylene to move out of the fruit freely or permit other gases to move into the fruit and dilute ethylene concn in the internal atmosphere of the fruit. Low Ca concn in the fruit tissues may result in leaky membranes which allow ethylene and other gases to move out of the cells freely which may reduce ethylene concn in the internal atmosphere but not in the external atmosphere. On the other hand, the fruit with high Ca has high membrane integrity and intact cuticle and epidermis, which may restrict the free movement of ethylene and other gases out of or into the fruit. This may cause ethylene to accumulate in the internal atmosphere of the fruit. The second possible explanation for the low ethylene in the internal atmosphere of low Ca fruit (light cork spot) is that the fruits may have gone through the climacteric rise in ethylene or they may have already spent some of their C_2H_4 synthesizing capacity during storage and before the start of the experiment, but this does not explain why the normal fruit with 7.11 mg Ca/100 g of fresh weight would have a lower internal ethylene peak than the fruit with high Ca (13.73 mg Ca/100 g of fresh weight). Although ethylene peak was higher in the fruits with high Ca, total C_2H_4 released (integrated areas under the curves) was about equal (454 ppm in the fruits with 7.11 mg Ca/100 g f.w. and 435 ppm ethylene in the fruits with 13.73 mg Ca/100 g f.w.).

Since the high Ca (13.73 mg Ca/100 g of fresh weight) was experimentally introduced in the fruit, it might have generated some injury. In fact all the Ca dip treatments which increased Ca concn in the fruit above 12 mg/100 g of fresh weight have also increased respiration and ethylene evolution (data were not included). Treating plasma membranes of soybean hypocotyls with $CaCl_2$ increased the thickness of the membranes 15 to 20% above the control (30). This may reduce the permeability of the membranes to gases including ethylene.

V. Rate of Fruit Softening in Relation to Calcium Concentration

Dipping 'Anjou' pear fruit in 5% $CaCl_2$ + 0.125% Keltrol increased the uptake of Ca and reduced the rate of softening during storage and during ripening. The firmness of the fruit with high Ca (13.73 mg Ca/100 g of fresh weight) after six months in storage was 1 kg higher than the control and 3.42 kg higher than the fruits with low Ca (4.71 mg Ca/100 g of fresh weight). After seven days at 20°C the firmness of the fruit with high Ca was 1.2 kg higher than the control and 1.6 kg higher than the cork spotted fruit. These results suggest that doubling Ca concn in the fruit slowed the rate of softening during storage but did not inhibit the ripening of 'Anjou' pear fruit. Our data are in agreement with the results from apple, which suggests

that treating apple fruits with $CaCl_2$ solution prior to storage increased the firmness of the fruit in the range of 0.4 kg up to 1 kg depending upon apple cultivars (4, 24, 27, 36). Calcium treatments have reduced the rate of respiration and ethylene evolution, and also reduced the rate of fruit softening. These results may suggest that Ca treatments are able to delay fruit senescence during storage.

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CHAPTER IV

RELATION BETWEEN CALCIUM CONCENTRATION, RESPIRATION, RESPIRATORY CONTROL RATIO, AND ADP:O RATIO OR MITOCHONDRIA ISOLATED FROM NORMAL AND CORK SPOTTED 'ANJOU' PEAR FRUIT

Literature Review

Introduction

One of the major functions of Ca in plants is the maintenance of cell membranes. The effect of Ca-deficiency on cell ultrastructure in the shoot apex of barley appeared as structural abnormalities resulting from the break-up of nuclear envelope and the plasma and vacuolar membranes and "structureless" areas appear in the cells, followed by the disorganization of the mitochondria (28). The effects of Ca on cell walls were secondary. Calcium deficiency caused a general degradation of the endoplasmic reticulum, mitochondria and the chloroplasts in the green alga, Scenedesmus (30). Calcium deficiency first leads to leaky membranes long before a visible breakdown (3). Removal of Ca from the tissue by EDTA caused the membranes to be leaky and highly permeable (45). Addition of Ca can restore the damaged membranes (3, 5). Added Ca caused isolated and in situ plasma membranes to be 15 to 20% thicker than controls and theeffect of Ca was abolished by EDTA. The results of many

studies (28-30, 45) suggested that Ca is essential for the maintenance and formation of cell-membrane systems, on which the functional integrity of cell metabolism is dependent.

The action of Ca is not only to prevent injury to cell membranes but also contributes actively to the formation of cytoplasmic organelles (5, 19, 25). Calcium deficiency in barley shoot apex caused the disintegration of the mitochondria and the chloroplasts (28). Florell (13) reported fewer mitochondria in wheat roots grown in nutrient solution without Ca. Increasing Ca concentration in the nutrient solution to 10^{-4} M, increased the amount of mitochondria by 54% (13), and increased the dry weight of the mitochondrial protein 47% (25) over the control. Calcium deficiency not only decreased the synthesis of mitochondria, but also reduced their functional capability (5). Wheat roots in a nutrient solution low in Ca produced mitochondria with shorter lifetimes (faster turnover) (25). Calcium deficiency caused the dissolution of the lamellar system and the breakdown of membranous structures of the mitochondria (5, 19).

Studies on the effect of Ca deficiency on plant mitochondria in the USSR was reviewe recently (42). The Russian workers showed that calcium deficiency decreased the P:O ratio in the mitochondria, which suggests that Ca deficiency may have reduced the structural integrity of the mitochondrial membranes or at least affected coupled oxidative phosphorylation. Electron microscopic investigation of the

mitochondria from Ca-starved plants showed that acute Ca starvation caused swelling of the mitochondria, loss of electron impermeability of the matrix, separation and vesiculation of the cristae, and the outer membranes became indistinguishable from the inner membrane (42). By studying the effects of other factors on mitochondria the authors concluded that the changes in mitochondrial structure and function described for Ca were specific. They also found that mitochondria isolated from plants at early stages of Ca deficiency, before any sign of growth retardation and detection of deficiency symptoms by electron microscopy cannot withstand stress such as high temperature, and lost their structural and functional integrity long before those of the control plants. This finding suggests that mitochondria formed under low Ca conditions are defective or easily become defective.

Mitochondria can accumulate very large quantities of Ca in the presence of an energy source and inorganic phosphate (8, 10, 11, 16, 32, 44). Isolated animal mitochondria can accumulate large amounts of Ca, up to several hundred times the initial Ca content, during electron transport in vitro (22, 23). The "massive loading" of Ca in isolated animal mitochondria is a response to the following conditions: (a) high Ca concn in the reaction medium, (b) the presence of ATP or ADP, (c) electron transport is required for Ca uptake, (d) Ca uptake is promoted by certain neutral salts as NaCl and

KCl. Uncoupling agents inhibit Ca uptake. When Ca is accumulated, no oxidative phosphorylation of ADP occurs. Phosphate is required, and it is not known whether phosphate or Ca was accumulated first (22, 23). Lehninger et al. (23) found that inorganic phosphate is accumulated from the medium with Ca as $Ca_3(PO_4)_2$ and $CaHPO_4$. Phosphate accumulation had the same requirements as Ca accumulation and was inhibited by the same inhibitors. The presence of high-affinity and specific Ca-binding sites are evidence for the occurrence of a specific carrier for Ca in the mitochondrial membranes. The high-affinity Ca-binding sites in animal mitochondria can bind Ca in the absence of eelctron transport or hydrolysis of ATP (22). It was found later (6), that there are both high and low affinity Ca-binding sites in the inner membrane and only the low affinity Ca-binding sites in the outer membrane of animal mitochondria. Lehninger (22) postulated that the accumulation of tricalcium phosphate ($CA_3(PO_4)_2$) as micro-packets in the matrix of animal mitochondria could play an important role in calcification of bones.

Plant mitocondria can also accumulate Ca (8, 10, 11, 16, 32, 42, 44), but ion accumulation is not unique to Ca (3), since phosphate and divalent cations such as Sr^{2+} and Ba^{2+}, can also be accumulated in the plant mitochondria (32, 44). The accumulated Ca in corn mitochondria is released from the mitochondria when respiration ceases (10). This suggests that the accumulation of Ca and possibly

other ions is only temporary in the plant mitochondria and not permanent deposits. Mitochondria possibly control the movement and concentrations of Ca within the cells through temporary accumulation (44).

Infection of cucumber leaves with powdery mildew increased the accumulation of ^{45}Ca in the mitochondria of the relatively resistant variety, but the mitochondria of the sensitive variety did not accumulate ^{45}Ca (1). Infection of the sensitive variety uncoupled the oxidative phosphorylation, disturbed the osmotic properties of the mitochondria and decreased the ATP content (1). The structural and functional integrity of the mitochondrial membranes of the relatively resistant variety, did not break down during infection (1).

Chen and Lehninger (8), studied Ca transport in isolated mitochondria from 14 different higher plants and fungi and reached the following conclusion: Additions of Ca to isolated plant mitochondria caused little or no stimulation of State IV respiration. Uptake of Ca was inhibited by respiratory inhibitors and uncouplers. Of all the plants which have been studied only the mitochondria from sweet potato show both high-affinity and low-affinity Ca binding sites (as in animal mitochondria), while the mitochondria from all other plants studied showed only low-affinity Ca binding sites.

Endogenous Ca concn in isolated mitochondria was measured in mitochondria from animals (23) and plants (8), and the results

show that Ca concn in the isolated mitochondria depends on the species. Endogenous Ca concn in rat liver mitochondria is 10.8 nmoles per mg of protein (23), while in avocado fruits is 73.3 nmoles per mg of protein, and in turnip roots is 181 nmoles per mg of protein (8). Endogenous Ca concn in isolated plant mitochondria also depends on the type of tissue used for isolation, for example Ca concn is 10 nmoles per mg of protein in mitochondria isolated from mung bean roots, while Ca concn is 1.7 nmoles in mitochondria isolated from mung bean stems (8). The content of Ca in the mitochondria also depends on the conditions of isolation, as suggested by Lehninger et al. (23). The authors also suggested that cations such as Mg are tightly bound to membranes and the reported metal content is unlikely to reflect the free cationic composition of the true intramitochondrial aqueous phase.

The possibility of changes in mitochondrial population due to the formation of new mitochondria during fruit ripening was suggested by some workers (21, 33, 35, 38), and also during aging of tissue slices of beet root (45). The respiratory control ratios and ADP:O ratio were increased during the ripening of avocado fruit (21). Incorporation of ^{14}C L-valine and ^{14}C L-leucine into protein was higher during the early stages of climacteric rise during ripening of avocado fruit (33). The incorporation of amino acids into protein may conceivably lead to biosynthesis of a new mitochondrial protein.

Indeed Romani and Fisher (35) measured the incorporation of ^{14}C-leucine in isolated mitochondrial protein and found it to be maximum just before the climacteric peak in respiration during the ripening of 'Bartlett' pear.

Since Ca is suggested to be required for the formation of new mitochondria (5, 13, 19, 25, 42), the newly formed mitochondria during fruit ripening may be expected to be defective or fewer in number in fruits with Ca deficiency. This may explain the development of cork spot in low Ca fruits during storage and ripening. Calcium deficiency in mature fruits late in the season and during storage may have a primary effect on the cytoplasmic membranes and cytoplasmic organelles such as the mitochondria, while the effects of Ca deficiency on cell walls could be a secondary effect.

The objectives of the studies in this section are: (a) to measure the endogenous Ca in mitochondria from 'Anjou' pear fruits affected with cork spot (Ca-deficient) in comparison to normals (no Ca deficiency), and (b) to study the effect of Ca on the integrity of the mitochondria by measuring their activities as a function of their integrity.

Materials and Methods

Isolation of mitochondria. Mature green 'Anjou' pear fruits were stored at 0°C until December, 1975 when the experiment started. Mitochondria were isolated from normal fruits and fruits

with cork spots by the following procedure: Fruits were held for 4 to 5 days at 20°C to soften, then chilled at 0°C, peeled and cored, and 100 g of tissue was gently grated into 300 ml of isolation medium, using an apparatus similar to that described by Romani et al. (39). Isolation was carried out in containers surrounded by crushed ice (0 to 4°C) in a pre-cooled apparatus, and the isolation medium was cooled to 0°C before it was used. The pH was maintained at 6.7 to 6.9 during the isolation, with additions of 1N KOH. The isolation medium was similar to that used by Romani et al. (36), except for the additional use of 3 mM $MgCl_2$ to prevent aggregation of the mitochondria during isolation (14, 49). The isolation medium consisted of 0.25 M sucrose, 0.05 M potassium phosphate buffer (pH 7.2), 5 mM EDTA, 0.2% polyvinylpyrollidone (PVP, 40, 000 MW), 0.1% bovine serum albumin (BSA), 5 mM β-mercaptoethanol, and 3 mM $MgCl_2$. The suspension was squeezed through two layers of muslin fabric (mesh 50 strands/cm) (31). The pH of the filtrate was adjusted to 6.7 to 6.9 by dropwise addition of 1 N KOH. The filtrate was centrifuged in pre-cooled, thin-walled polyallomer centrifuge tubes in a refrigerated centrifuge (Sorvall model RC2-B) held at -2 to 0°C.

Several centrifugation procedures were evaluated (4, 11, 14, 17, 20, 31, 36-38, 40, 41, 48, 49), and the following procedure was found to yield active mitochondria and more mitochondrial protein than the others, from 'Anjou' pear fruit. The flow diagram for the centrifugation is as follows (Figure 1).

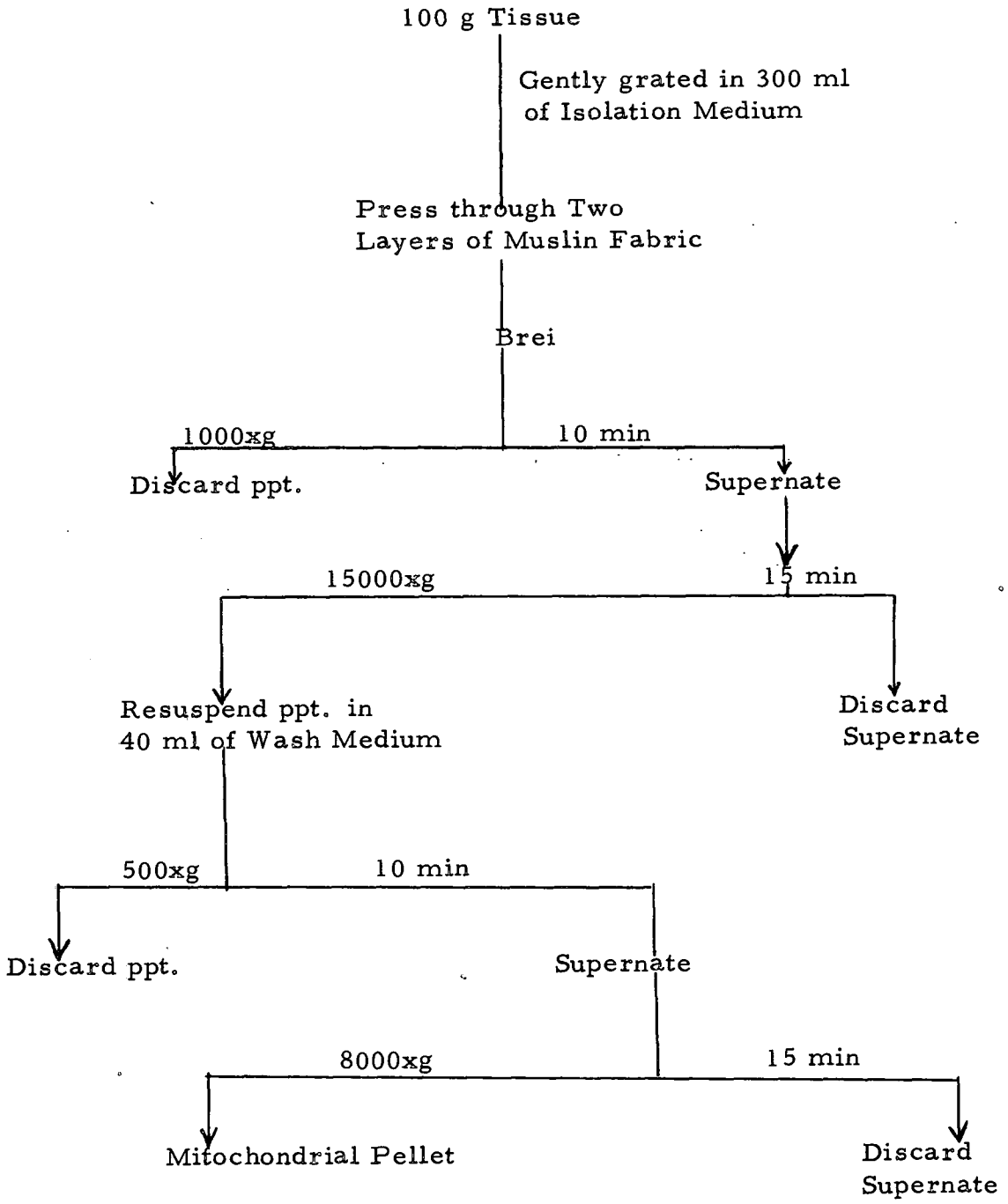


Figure 1. Scheme for centrifugation and isolation of mitochondria from 'Anjou' pear fruit.

The wash medium is the same as that used by Romani et al. (36) except β-mercaptoethanol was not used as suggested by other authors (4, 9, 12, 47, 48), while 3 mM $MgCl_2$ was used as suggested earlier. The wash medium consisted of 0.25 M sucrose, 0.05 M potassium phosphate buffer (pH 7.2), 0.1% BSA, and 3 mM $MgCl_2$. The pellet was suspended in 40 ml of wash medium after the first high speed centrifugation by using a pre-cooled Teflon tissue homogenizer. After the final centrifugation step, the mitochondria were resuspended in an equal volume of wash medium and held at ice temperature before assaying. Protein was estimated by the Lowry method (27) using BSA as the standard.

Mitochondria were assayed for oxygen consumption in a medium (3 ml) consisting of: 0.75 mmole sucrose, 200 μmoles Pi (pH 7.2), 30 μmoles α-ketoglutarate, 0.3 μmole NAD, 0.1 μmole thiamine pyrophosphate, 3 μmoles $MgCl_2$, 0.01 μmole CoA, 3 mg bovine serum albumin, 100 μg chloramphenicol and 3 to 6 mg of mitochondrial protein. This reaction medium is the same as that suggested by Romani et al. (37). Oxygen consumption was measured polarographically (12) at 25°C, using a Clark oxygen electrode (Yellow Springs Instruments). Respiratory control (RC) and ADP:O ratio were calculated by the method of Chance and Williams (7).

Measurement of Calcium in the Mitochondria

Mitochondria were isolated as described above, except 300 g of fruit tissue was grated in 900 ml of isolation medium. After the final centrifugation step, the mitochondria were resuspended in an equal volume of wash medium (without BSA) and referred to as the mitochondrial suspension which was used for determination of endogenous calcium.

Determination of total Ca in the mitochondria. Quantitative colorimetric determination of total Ca in the mitochondria was performed using the "Pierce Calcium Rapid Stat Kit," this is a kit containing a dye reagent and a base reagent. Details and principles of the procedure for Ca determination in blood serum are described in the instruction sheet supplied by the Pierce Co. (2). The modified procedure used in this work for Ca determination in the mitochondria is as follows: Working reagent was made by mixing equal volumes of the dye reagent, base reagent, and deionized water and stored in a plastic container. Reagent blank was prepared by adding 3 ml of the working reagent to 0.5 ml deionized water in polystyrene (17x100 mm) test tubes. The absorbance of the spectrophotometer was adjusted to zero with the reagent blank at a wavelength of 612 nm. A sample of 0.5 ml of the mitochondrial suspension was mixed with 3 ml of working reagent and the absorbance was read immediately in

Table 1. Respiratory activity of mitochondria isolated from normal 'Anjou' pear fruit and from fruits affected with cork spot.*

Treatment df = 14	Rate of O_2 uptake in "State III," nanoatoms O_2/mg of protein/min	Rate of O_2 uptake in "State IV" nanoatoms O_2/mg of protein/min	RC ratio	ADP:O ratio
Normal fruit	170.385	85.692	2.154	2.263
Fruit with cork spot	84.904	62.081	1.361	1.493
$LSD_{0.05}$	21.695	15.066	0.382	0.427
$LSD_{0.01}$	30.109	20.910	0.530	0.592

*For reaction medium see text.

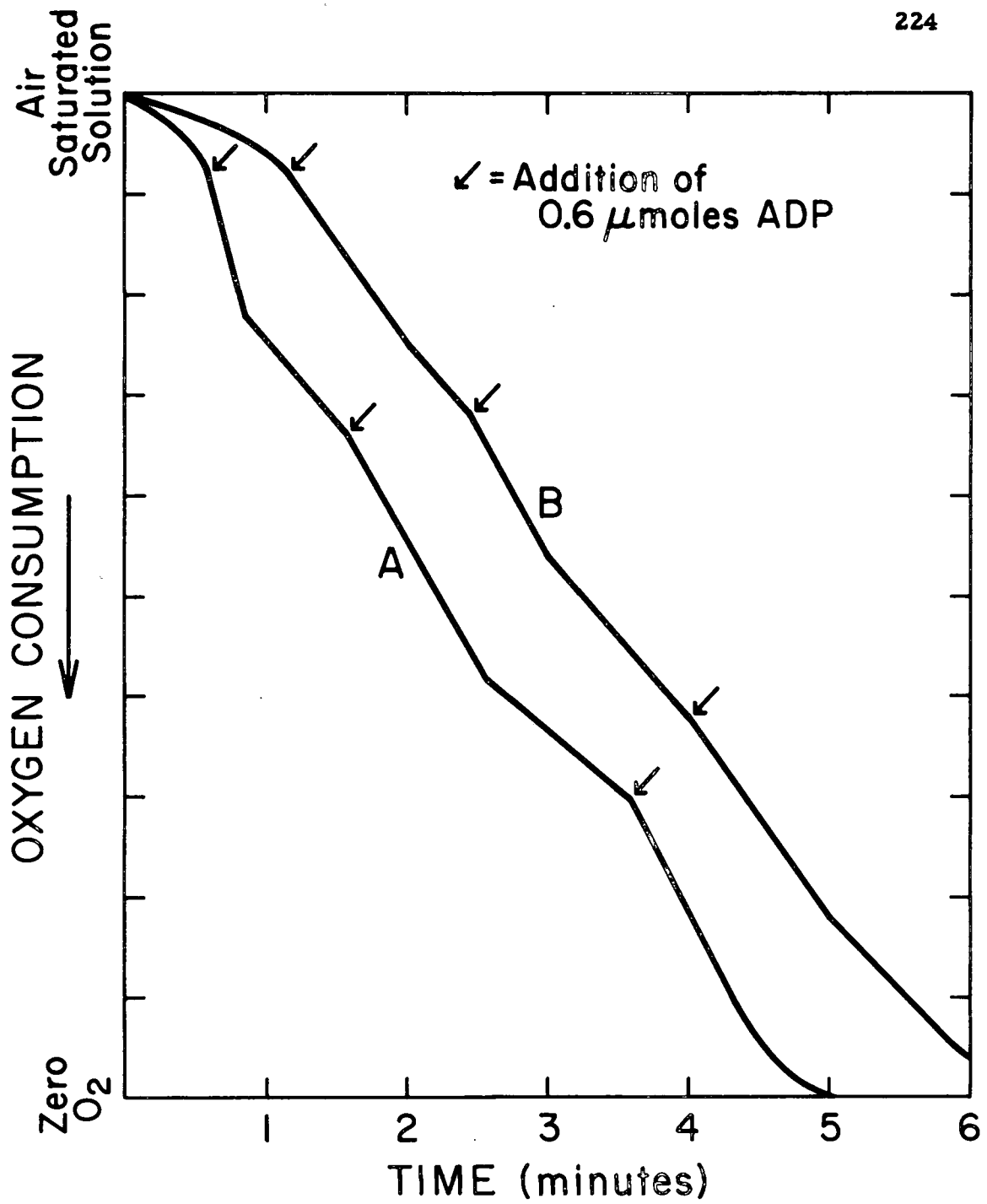
a Beckman DB spectrophotometer. The calibration graph was prepared by reading a series of Ca carbonate standards in the range of 0.0 to 50 ppm.

Determination of acid soluble Ca in the mitochondria. Calcium was extracted from the mitochondria by mixing the mitochondrial suspension with an equal volume of 5% HCl, and 5% trichloroacetic acid (15). The mixture was shaken well (manually) and centrifuged at 13, 000 x g for 5 min to remove mitochondrial fragments. $SrCl_2$ was added to the supernatant (final concn 3%), and Ca was measured in a Perkin-Elmer Atomic Absorption spectrophotometer, model 303.

Results

Isolation and assay. The summary of the results is shown in Table 1. The rate of O_2 uptake in "State III" of the mitochondria isolated from normal fruits is twice that of mitochondria isolated from fruits with cork spot (Ca-deficient). The rate of O_2 uptake in "State IV" of mitochondria isolated from normal fruits is about 86 nanoatoms O_2/mg of mitochondrial protein/min, compared with 62 nanoatoms O_2/mg of mitochondrial protein/min of the mitochondria isolated from cork spotted fruits. The difference in O_2 uptake in "State IV" is also statistically significant at the 1% level (Table 1). Respiratory control ratios (RC) of mitochondria from the normal fruit is about 2.0 compared with 1.4 for the mitochondria isolated

Fig. 2. Polarographic measurement of oxygen consumption by mitochondria isolated from normal (A) and cork spotted (B) 'Anjou' pear fruit. For experimental details and reaction medium see text.


Air Saturated Solution
↙ = Addition of 0.6 μmoles ADP
B
A
OXYGEN CONSUMPTION
Zero O_2
1
2
3
4
5
6
TIME (minutes)

from fruits with cork spot. The difference in RC is significant at the 1% level. The value of ADP:O ratio in the mitochondria isolated from normal fruit is about 2.3 while it is about 1.5 in the mitochondria from cork spotted fruits (Table 1). The difference in ADP:O ratio is also significant at the 1% level.

Calcium concentration in the mitochondria. Both total Ca and acid soluble Ca were significantly higher in the mitochondria isolated from normal fruits compared to the mitochondria isolated from fruits with cork spot (Table 2). Total Ca was about 14.7 μg/mg of mitochondrial protein in mitochondria isolated from normal fruits compared with about 6.1 μg Ca/mg of mitochondrial protein. Total Ca in the mitochondrial suspension was not measured by the Atomic Absorption Spectrophotometer, because the mitochondrial suspension blocks the capillary tube used for the sample aspirator. Acid soluble Ca was about 7.1 μg/mg of mitochondrial protein in the mitochondria isolated from normal fruit compared with 3.3 μg Ca/mg of mitochondrial protein in mitochondria isolated from cork spotted fruits (Table 2). Acid extractable Ca was not measured by the colorimetric assay due to the interference from hydrochloric acid and trichloroacetic acid on the dye and base reagents used in the colorimetric assay. Although the comparisons are noteworthy, the absolute values of Ca by the two methods are not comparable.

Table 2. Calcium concentrations in mitochondria isolated from normal 'Anjou' pear fruit, and from fruit with severe cork spot.

Treatment df = 38	Calcium concn µg/mg of protein	
	Total Ca (colorimetric assay)	Acid extractable Ca (Atomic absorption assay)
Normal fruits	14.668	7.055
Fruits with severe cork spot	6.081	3.259
$LSD_{0.05}$	1.625	1.039
$LSD_{0.01}$	2.180	1.394

Discussion

Isolation and assay. The results in Table 1 show that the mitochondria isolated from normal fruit have a higher rate of O_2 uptake in both State III and IV, higher RC ratio, and higher ADP:O ratio than the mitochondria isolated from cork spotted fruits. The results presented above support the concept that the structural and functional integrity of mitochondria isolated from normal fruit is much better than that of mitochondria isolated from Ca-deficient fruits. The higher RC ratio and ADP:O ratio in mitochondria isolated from normal fruit shows that oxidation and phosphorylation are coupled, but not in the mitochondria from Ca-deficient fruits. Our results agree with one report that Ca deficiency decreased P:O ratio in the mitochondria (42).

Fruit ripening apparently is associated with an increase in the mitochondrial population (21, 33, 35, 38), and the newly formed mitochondria in Ca-deficient fruits may be expected to be defective.

All the attempts to isolate active mitochondria from mature green, but unfipened, 'Anjou' pear fruits failed. Hence it was not possible to compare mitochondria from normal fruits with those from cork spotted fruits at the mature green stage. The main problem likely associated with the inability to use green fruit is that gentle grating cannot be achieved due to the physical nature of the fruits while

severe blending caused complete loss of activity (data not shown). The use of mortar and pestle was not an improvement (48). The control of the pH during maceration was very important due to the acid nature of the fruits (17, 18, 20, 39, 43). The pH cannot be controlled during maceration if the usual techniques (such as blender) are used unless more elaborate modification can be implemented. Another problem with the use of green fruits is the release of phenolic compounds during maceration, which tends to inhibit the activity of the mitochondrial enzymes (17, 18, 24, 26). The use of PVP to suppress phenolics is not very effective if the pH is not maintained between 6.7 and 6.9 (18, 26, 39). In addition to mechanical damage to the mitochondria from isolation, phenolic compounds may specifically uncouple oxidative phosphorylation (26), which may explain the low activity of the mitochondria isolated from green fruits which have higher levels of free phenolics than ripe fruits. Polyclar AT was reported to be very effective in binding phenolics during the isolation of enzymes (26). It was suggested (W. D. Loomis, personal communication) that 1 g of Polyclar AT per g of fresh weight of fruit tissue be used to be effective. Mixing this compound with the medium in the blender during maceration resulted in production of fine particles in the isolation medium, which was not possible to remove from the mitochondrial fraction by differential centrifugation.

Ripe fruits are easy to macerate in an apparatus similar to that

described by Romani et al. (39), which permits the control of the pH during maceration, and causes less damage to the mitochondria. Starch reportedly (41) inhibited mitochondrial activity, and ripening may remove starch by converting it to sugar. Ripening may result in some changes in the metabolism of phenolics and cause some "detoxification" by changing them into inactive polymeric forms such as by glycosidation, esterification, and lignification. Ripening also may cause an increase in total mitochondrial population, due to the synthesis of new mitochondria (21, 33, 35, 38), which may increase the apparent mitochondrial activity in the ripe fruit.

Calcium concentration in the mitochondria. The results in Table 2 show that both total Ca and acid soluble Ca is lower in mitochondria isolated from fruits with cork spot than the Ca concn in mitochondria isolated from normal fruits. The results in Chapter I showed that cork spotted fruits are Ca-deficient. The evidence presented above confirms that Ca is deficient on the subcellular level in the fruits with cork spot. Adding the results in Table 2 to those in Table 1, we can conclude that there are some relations between Ca deficiency in the mitochondria and its functional integrity. This conclusion is supported by several reports (13, 25, 42), on mitochondria from different plant sources.

The acid soluble fraction of Ca (Table 2) may represent the free Ca content of the intramitochondrial water phase, while total

Ca may represent the water phase and the Ca which is tightly bound to the mitochondrial membranes. The colorimetric assay for total Ca has a high affinity for Ca (2) and may react with the membrane bound Ca.

Since Ca in this study was measured by two different methods and we don't know what the relative solubilities of membrane Ca are in 5% HCl vs. the Pierce Rapid Stat method nor do we know what the specific dye binding affinity constant for Ca means in relation to comparative sensitivity of dye binding vs. atomic absorption, it is difficult to make reasonable comparisons between acid soluble Ca and "total Ca." It was not possible to measure Ca in the acid extract by the colorimetric method and was also not possible to measure Ca directly in the mitochondrial suspension due to the difficulties stated earlier.

Extracting Ca from the mitochondrial suspension with the acid denatured the mitochondrial protein and formed aggregates, which may have enclosed Ca inside the membranes and caused it to precipitate with the mitochondrial fragments during centrifugation and reduced its concentration in the supernatant (acid extract)

Our data showed that acid extractable Ca from pear mitochondria is slightly higher than what was reported in avocado mitochondria (8). Calcium concn in avocado reportedly was 73.3 nanomole Ca per mg of protein, and converting the data in Table 2, we found that Ca

concentration in pear mitochondria is about 81 and 176 nanomoles Ca per mg of mitochondrial protein. It was reported that endogenous Ca concn in isolated mitochondria vary, depending on the species, the type of tissue used for isolation, and the conditions of isolation (8, 23). The reported endogenous Ca concn is mostly measured in mitochondria isolated by the discontinuous sucrose gradient method, extracted by acids and measured by emission spectroscopy (23). The mitochondria in our work were not isolated by the discontinuous sucrose gradient which may have helped to maintain higher levels of mineral ions.

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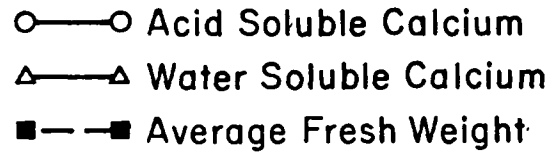
APPENDIX

APPENDIX A

Seasonal uptake of Nitrogen and calcium by 'Anjou' pear trees in relation to the development of cork spot.*

*Data presented here are in preparation for publication in the Journal of American Society for Horticultural Sciences.

Figure 1. Seasonal changes in acid soluble Ca and water soluble Ca concn in 'Anjou' pear fruit in relation to the increase in fresh weight starting one week before bloom until leaf fall. Each point is the average of duplicate sample analysis.



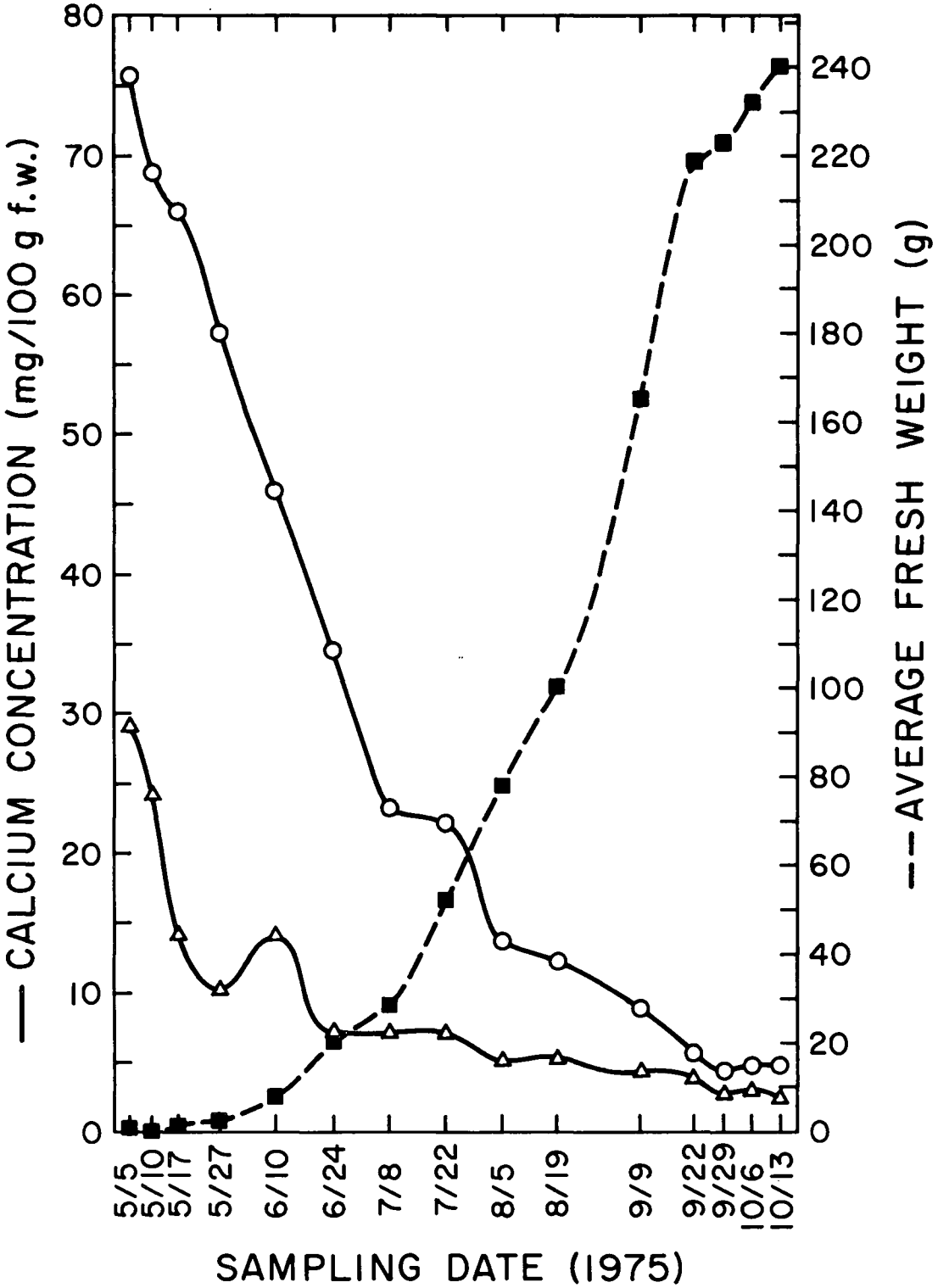
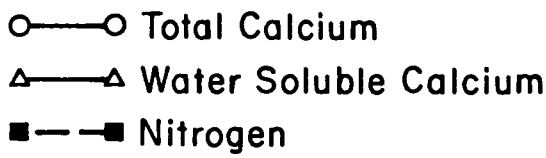

80
70
60
50
40
30
20
10
0
240
220
200
180
160
140
120
100
80
60
40
20
0
— CALCIUM CONCENTRATION (mg/100 g f.w.)
--AVERAGE FRESH WEIGHT (g)
5/5
5/10
5/17
5/27
6/10
6/24
7/8
7/22
8/5
8/19
9/9
9/22
9/29
10/6
10/13
SAMPLING DATE (1975)

Figure 2. Seasonal changes in the amount of total Ca (acid soluble and water soluble) in mg per fruit and in N concn (mg/ 100 g of fresh weight) of 'Anjou' pear fruit starting one week before bloom until leaf fall. Each point is the average of duplicate sample analysis.



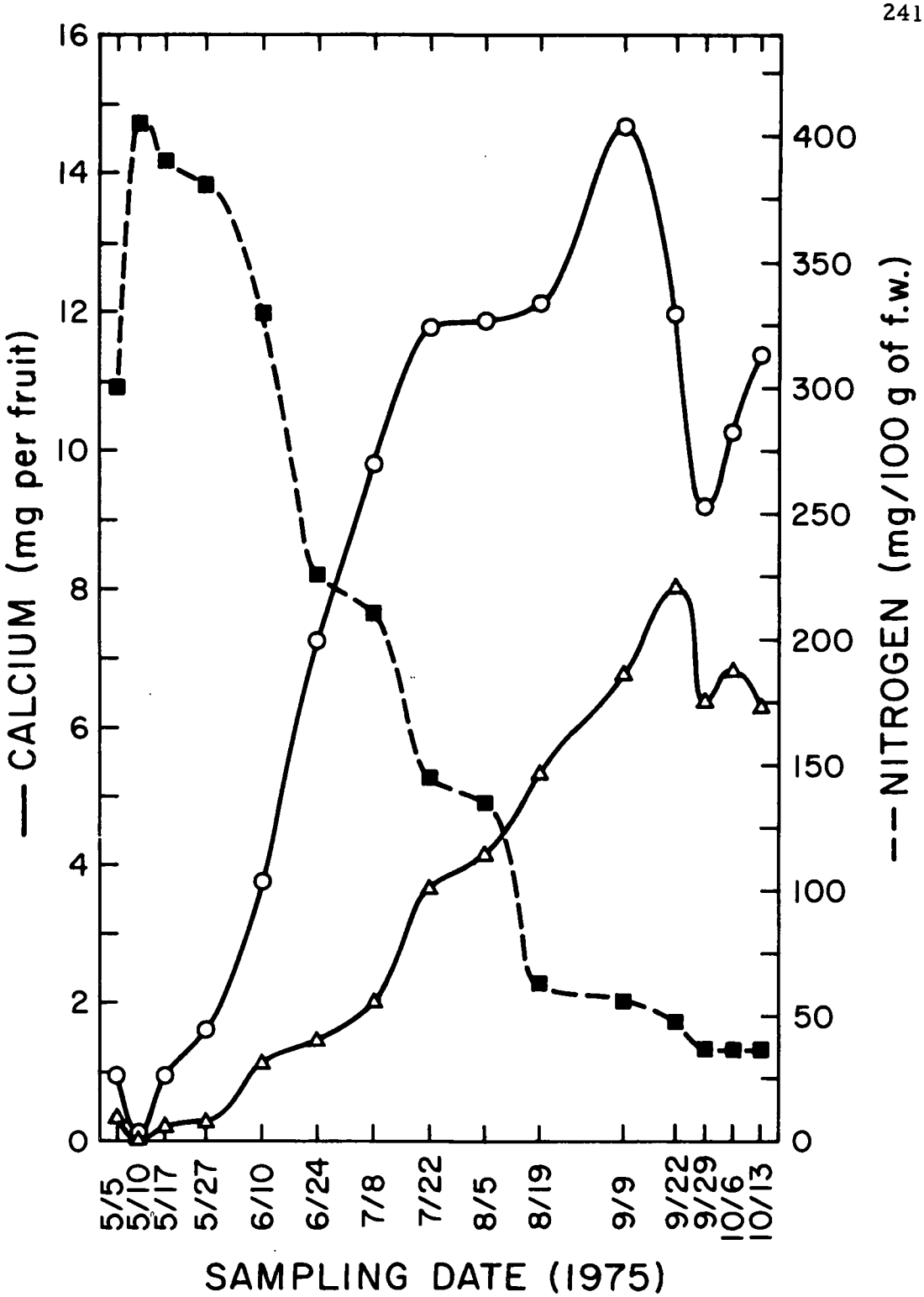

16
14
12
10
8
6
4
2
0
— CALCIUM (mg per fruit)
400
350
300
250
200
150
100
50
0
-- NITROGEN (mg/100 g of f.w.)
5/5
5/10
5/17
5/27
6/10
6/24
7/8
7/22
8/5
8/19
9/9
9/22
9/29
10/6
10/13
SAMPLING DATE (1975)

Figure 3. Seasonal changes in water soluble Ca:water nonsoluble Ca ratio and water soluble:acid soluble Ca ratio in the fruit of 'Anjou' pear starting one week before bloom until leaf fall. Each point is the average of duplicate sample analysis.

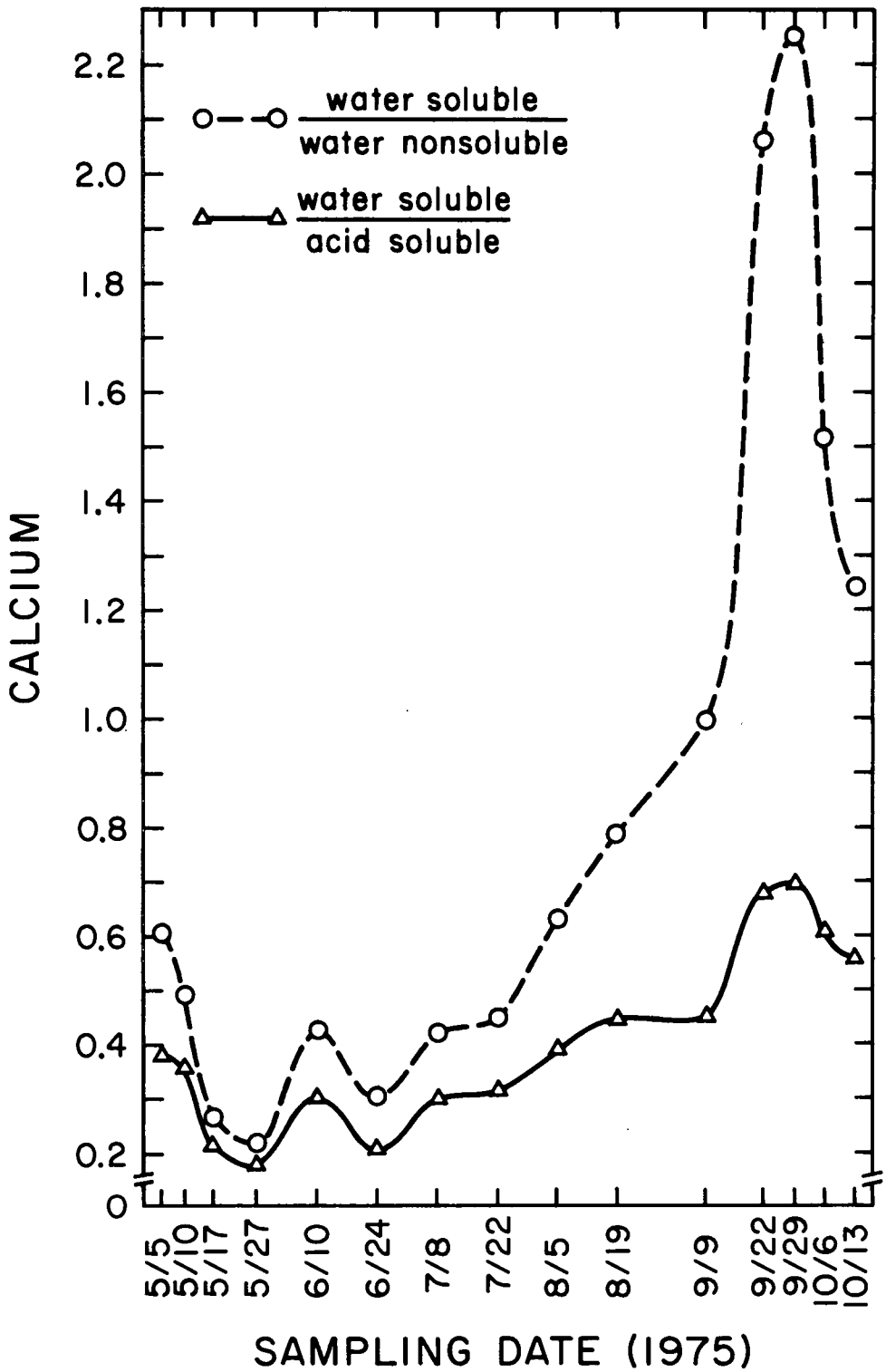
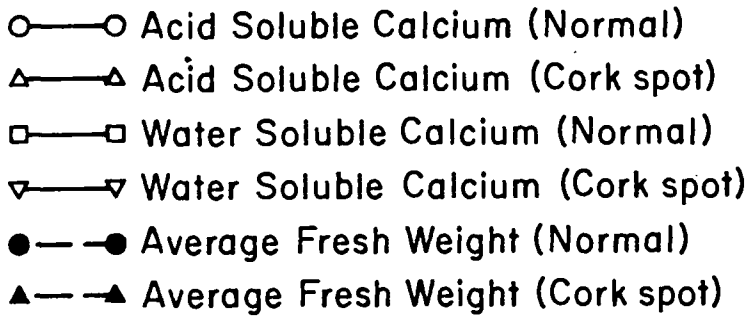

water soluble / water nonsoluble
water soluble / acid soluble
CALCIUM
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
5/5
5/10
5/17
5/27
6/10
6/24
7/8
7/22
8/5
8/19
9/9
9/22
9/29
10/6
10/13
SAMPLING DATE (1975)

Figure 4. Seasonal changes in the concn of acid soluble Ca and water soluble Ca in 'Anjou' pear fruit in relation to the increase in fresh weight of normal and cork spotted fruits. Each point is the average of 10 to 15 samples.



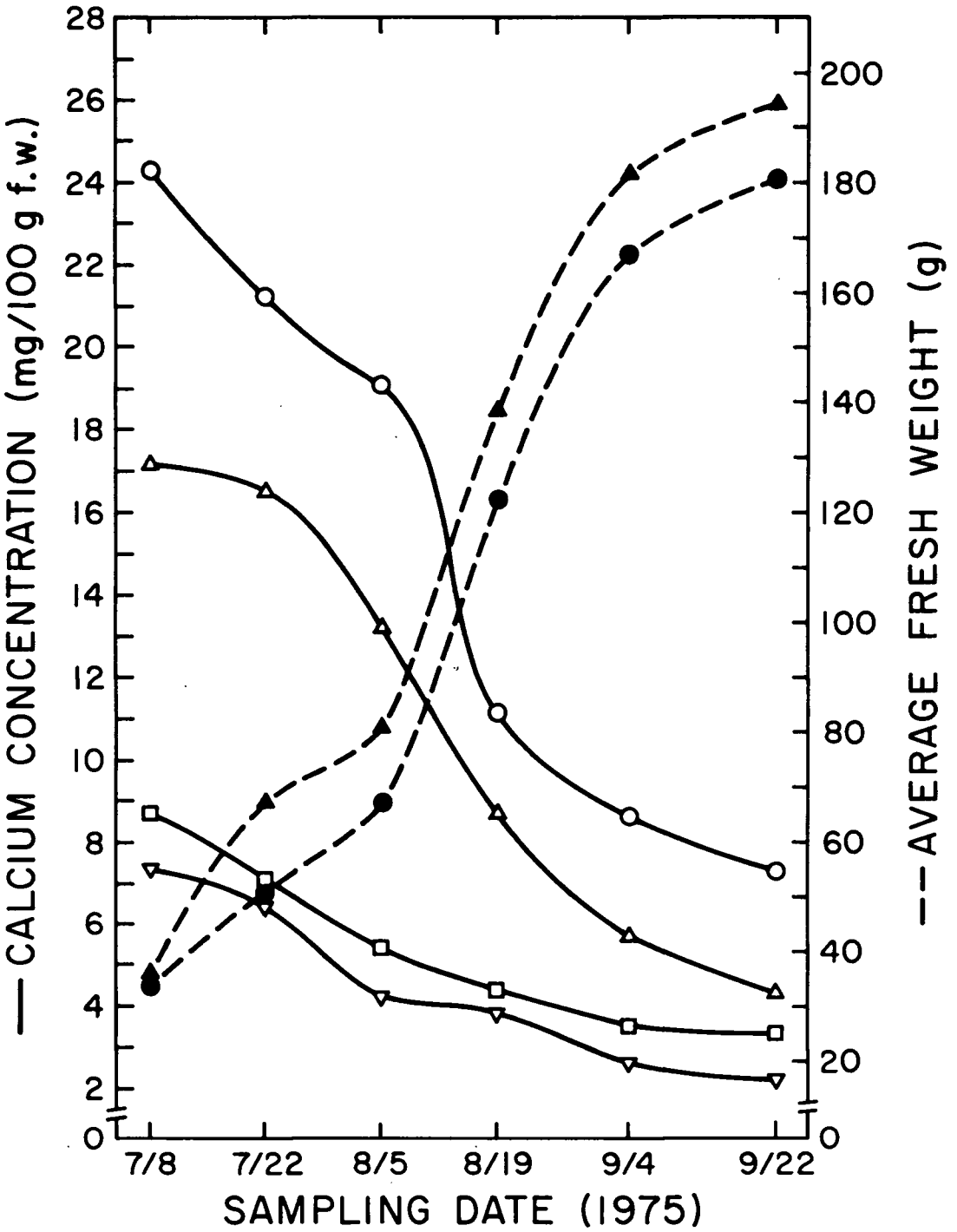
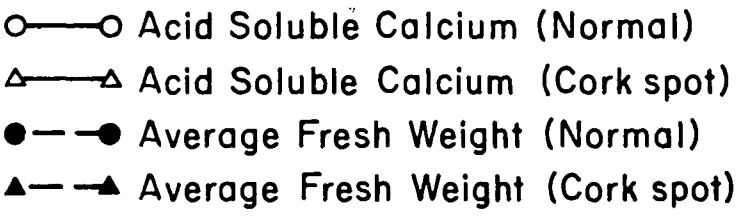

28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
200
180
160
140
120
100
80
60
40
20
0
7/8
7/22
8/5
8/19
9/4
9/22
SAMPLING DATE (1975)
— CALCIUM CONCENTRATION (mg/100 g f.w.)
-- AVERAGE FRESH WEIGHT (g)

Figure 5. Seasonal changes in Ca concn in normal 'Anjou' pear fruit compared with cork spotted fruits in relation to increase in fresh weight in 1976. Each point is the average of 10 to 15 samples.



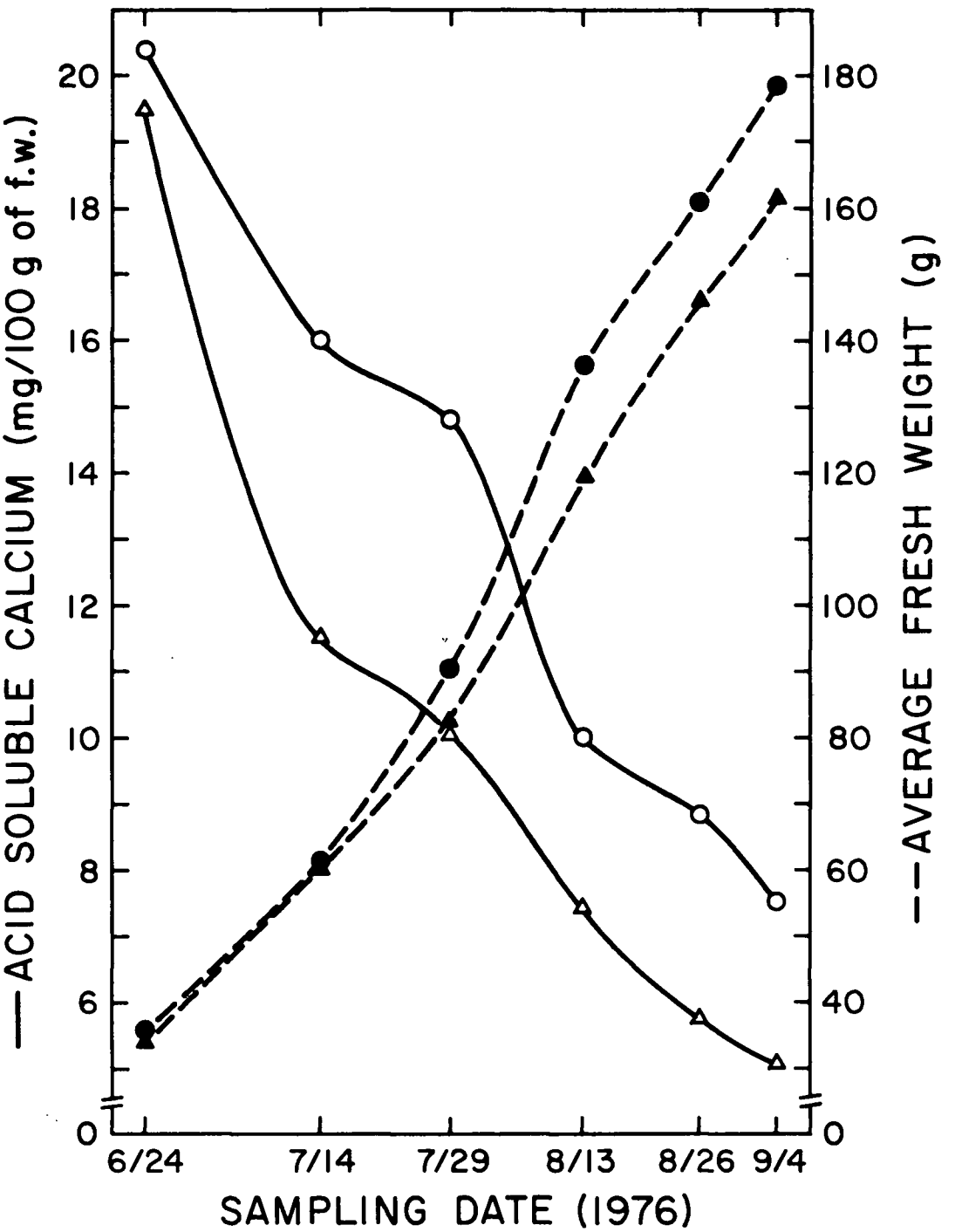
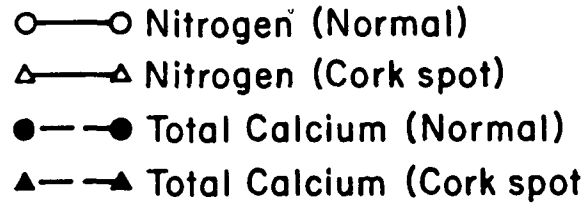

—ACID SOLUBLE CALCIUM (mg/100 g of f.w.)
--AVERAGE FRESH WEIGHT (g)
20
18
16
14
12
10
8
6
0
180
160
140
120
100
80
60
40
0
6/24
7/14
7/29
8/13
8/26
9/4
SAMPLING DATE (1976)

Figure 6. Seasonal changes in amounts of Ca (mg per fruit) and N concn in normal and cork spotted 'Anjou' pear fruit during development in 1975. Each point is the average of 10 to 15 samples.



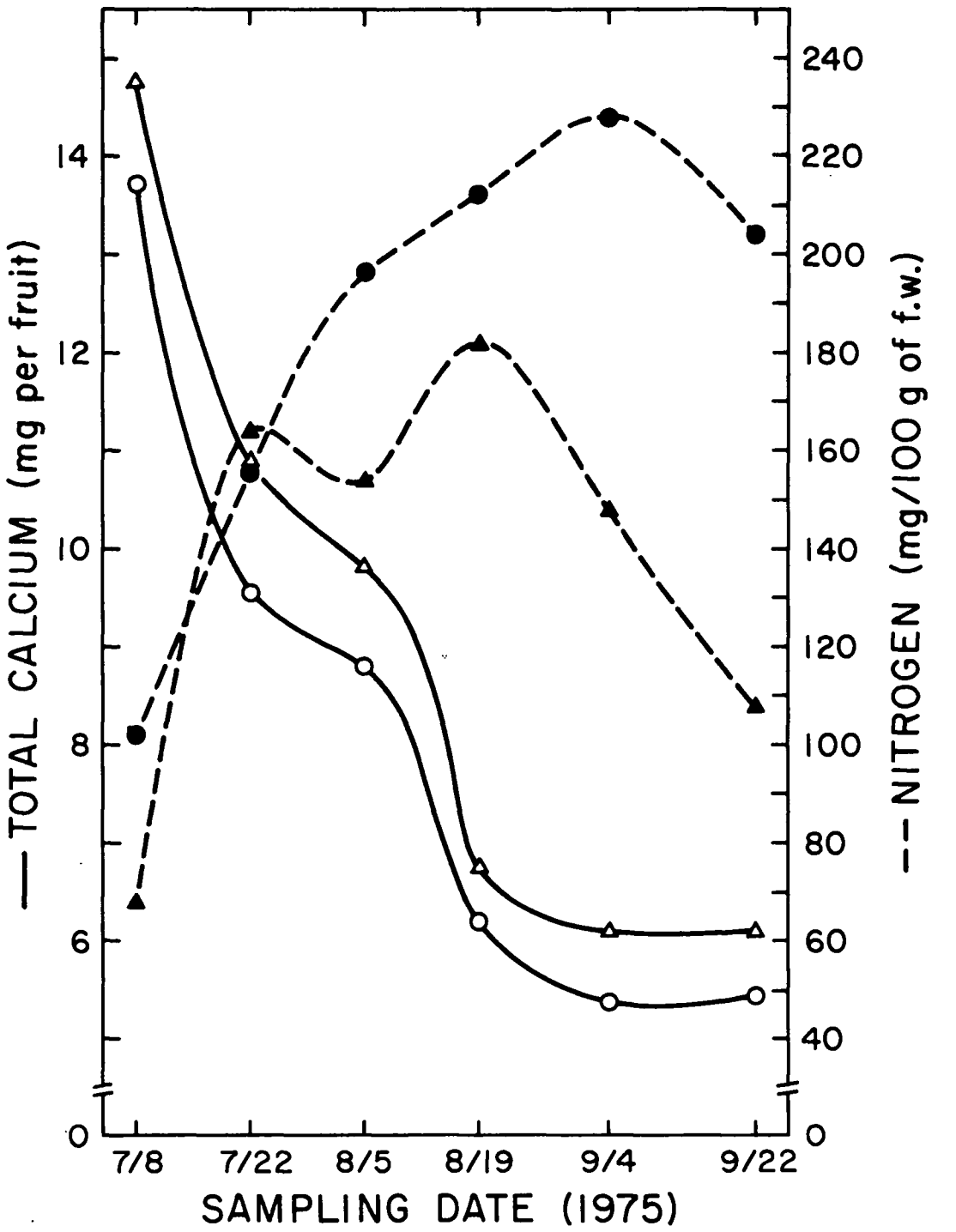
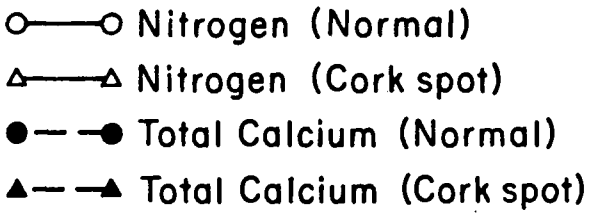
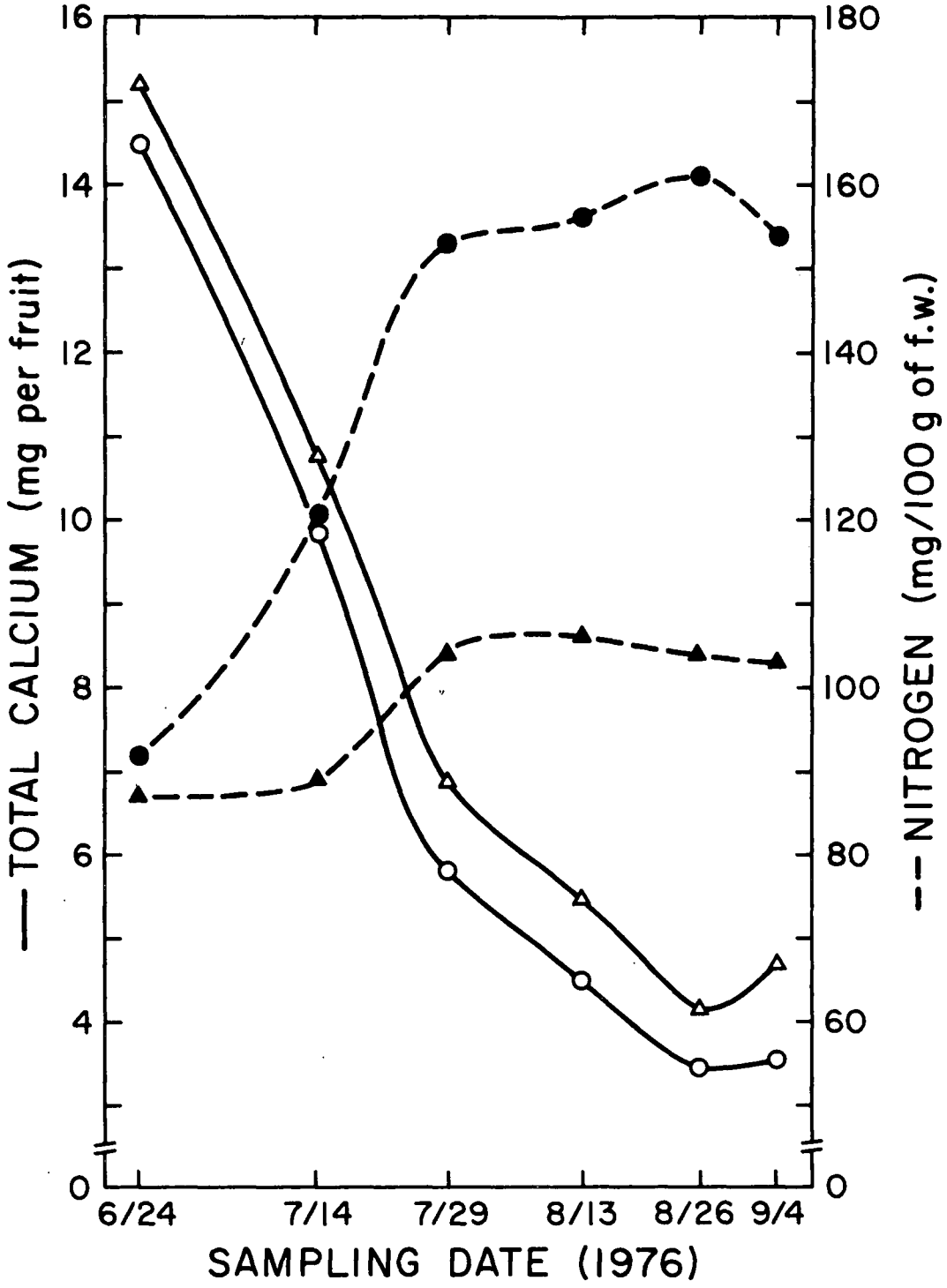

— TOTAL CALCIUM (mg per fruit)
-- NITROGEN (mg/100 g of f.w.)
0
6
8
10
12
14
40
60
80
100
120
140
160
180
200
220
240
7/8
7/22
8/5
8/19
9/4
9/22
SAMPLING DATE (1975)

Figure 7. Seasonal changes in amounts of Ca (mg per fruit) and N concn in normal and cork spotted 'Anjou' pear fruit during development in 1976. Each point is the average of 10 to 15 samples.




16
14
12
10
8
6
4
0
180
160
140
120
100
80
60
0
TOTAL CALCIUM (mg per fruit)
NITROGEN (mg/100 g of f.w.)
6/24
7/14
7/29
8/13
8/26
9/4
SAMPLING DATE (1976)