

AN ABSTRACT OF THE THESIS OF

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Title: Preharvest Fruit Analysis as a Predictor of D'Anjou Pear
and Yellow Newtown Apple Physiological Disorders, Storage
Quality and Ripening Behavior

Abstract Approved: _____
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Regression equations associating the preharvest mineral status of fruit with the occurrence of physiological disorders were used to predict the occurrence of bitter pit (BP) and internal breakdown (IB) on Newtown apples and cork spot on D'Anjou pear fruits up to 60 days before harvest in two years. Equations for a given year were applied to data from the other. Twenty and ten orchards in 1982 and 1983, respectively, were sampled and analyzed for Ca, P, K, N, Mg, Mn, Fe, Zn, B, S, Na and Se. There were higher bitter pit and cork spot incidence after storage than at harvest.

In Newtown apples, fruit Ca, fruit weight, and wt/Ca ratio accounted for the most consistent predictive factors. Bitter pit could be predicted 20 days before commercial harvest. Internal breakdown could be predicted from fruit sampling data 60 days before harvest. Fruit Ca and the ratios wt/Ca and N/Ca accounted for the

most consistent predictive tools for cork spot in D'Anjou pears.

Cork spot could be predicted 40 days before harvest.

Respiration, ethylene evolution and internal C_2H_4 of D'Anjou fruits at harvest, after 3 and 7 mo. in storage varied with different Ca levels. Higher Ca levels were related to lower respiratory activities of the fruits. High Ca in the fruits delayed the onset of C_2H_4 evolution and reduced C_2H_4 production. Low fruit Ca resulted in greater total CO_2 production and an earlier climacteric peak.

D'Anjou pear normally requires 60-70 days of $-1^\circ C$ storage to initiate C_2H_4 production and ripening processes. Fruit Ca concn. affected this process. Low Ca fruits initiated C_2H_4 production within 5 days at $20^\circ C$ without cold treatment. Medium and high fruit Ca initiated C_2H_4 production only after 50 and 70 days, respectively, in cold storage. Fruit Ca might affect the conversion of 1-aminocyclopropane-1-carboxylic acid (ACC) to C_2H_4 or at least delay it when present in high levels.

D'Anjou flesh firmness was highly significantly correlated with fruit Ca at harvest, and after 3 and 7 mo. in storage. Fruit Ca was also positively correlated with fruit chlorophyll retention and TA (84) at the end of long term storage. IB and fruit rot incidence were highly significant and negatively correlated with Ca. Vacuum infiltration and dipping the fruits in $CaCl_2$ solutions increased fruit Ca concn. High fruit Ca levels were associated with reduced incidence of rots caused by *Botrytis* and *Penicillium*. Vacuum treatments induced flesh injury in D'Anjou fruits. Dipping the fruits in

6% CaCl_2 solution substantially reduced storage rots without flesh injury.

Fruits to be stored satisfactorily for long periods of time should have high amounts of Ca. Once fruit Ca concn. is known, it would be possible to estimate their storage potential.

Preharvest Fruit Analysis as a Predictor of D'Anjou Pear
and Yellow Newtown Apple Physiological Disorders,
Storage Quality and Ripening Behavior

by

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This thesis is dedicated to my family
Valeria, Daniella and Fabio,
and to the memories of my father "Dico"
and my friend "Quito"

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PREFACE

This thesis is presented as a series of six papers written in the format required by the Journal of the American Society for Horticultural Science.

Preharvest Fruit Analysis as a Predictor of D'Anjou Pear and
Yellow Newtown Apple Physiological Disorders,
Storage Quality and Ripening Behavior

Chapter I

INTRODUCTION

In the Pacific Northwest, particularly in the Hood River Valley, Oregon, the apple and pear industry represents approximately \$33 million annually. From this, more than 80% is represented by Yellow Newtown apples and D'Anjou pears. Although the highly developed technologies of delaying fruit senescence such as controlled atmosphere, and many other postharvest advances in actual use in the Valley, there are still considerable postharvest losses every year. These losses involve the loss of the whole fruit itself due to physiological disorders such as bitter pit, internal breakdown, water core, cork spot and others or due to a decrease in fruit quality such as premature yellowing, softening and decrease in titratable acidity during storage as well as the incidence of rot which in consequence decreases the fruits market value.

Preharvest as well as postharvest factors are known to affect fruit quality. A loss in fruit quality not only means a reduced potential cash crop, but also waste of manpower, capital and less food availability for human consumption.

Many biochemical factors interact to determine the fruit's storage potential, as is the case of ethylene production, a natural

plant hormone, which if present in the fruits at certain levels will induce ripening in climacteric fruits and hasten fruit senescence during storage. Metabolic reactions leading to fruit respiratory activities also determine advances or delays in fruit senescence, therefore affecting the length of storage.

The main objective of this thesis was to investigate the role of fruit Ca on the postharvest behavior of Newtown apples and D'Anjou pears regarding to physiological disorders, and fruit quality aiming to contribute to the reduction in postharvest losses.

The first manuscript (Chapter III) deals with the development of a predictive model for the incidence of bitter pit and internal breakdown on Newtown apples based upon preharvest fruit mineral analysis. In this paper, the optimum sampling date as well as the characterization of the nutritional elements most closely correlated with the disorders is described. By predicting the future potential incidence and severity of BP and IB in the fruits far ahead of harvest would allow the use of alternative ways to reduce the problems.

The second manuscript (Chapter IV) describes the attempt to develop a similar preharvest fruit sampling model for the prediction of cork spot (CS) in D'Anjou pear fruits at harvest and during storage. To develop this study, simple and multiple correlations of mineral nutrients were developed with harvest and storage cork spot. Three different sampling times were also involved. The predictions of CS and its incidence relative to storage potential is also discussed.

The third manuscript (Chapter V), discusses the physiologic effects of calcium on respiration rate, ethylene production and occurrence of cork spot in D'Anjou pears. Evidence is presented that calcium influences the time of occurrence of the climacteric as well as the magnitude of CO_2 and C_2H_4 production.

The fourth manuscript (Chapter VI) examines the interaction between chilling requirements for D'Anjou pear fruit ripening, ethylene synthesis, and respiration and fruit calcium concentration. The effect that Ca has on ethylene synthesis and fruit ripening is also speculated.

The fifth manuscript (Chapter VII) describes the relationship of fruit calcium to firmness, internal breakdown, incidence of rot, green color retention and storability of D'Anjou pear. The main objective was to examine the effect of Ca nutrition on major quality aspects of D'Anjou pear fruit. The nature of the relationships are discussed.

The sixth manuscript (Chapter VIII), the relationship of calcium infiltration of D'Anjou pears and postharvest fruit decay caused by Penicillium expansum and Botrytis cinerea, was carried out with three main objectives: to determine the effects of postharvest Ca treatments on decay caused by those fungi on D'Anjou pears, to determine the optimum method of treatment and to determine optimum concentration of CaCl_2 solution that would be used in the postharvest treatment of D'Anjou pear fruits.

Chapter II

LITERATURE REVIEW

Bitter Pit, Internal Breakdown and Cork Spot

Introduction

In the Pacific Northwest, in spite of highly developed technology of environmental modifications for delaying ripening and senescence of pear and apple fruits, substantial losses can occur during and after storage. It is now recognized that the mineral nutrient status of the fruit is a substantial factor in these losses, which have a significant economic impact on the industry.

In the past, fruit nutrition research attempted to optimize tree growth and cropping. More recently, emphasis has turned to the effects of fruit nutrient status on quality maintenance, and has focused on the important relationship between calcium and quality retention.

There is evidence that calcium occupies a central position in fruit nutrition, and that the importance of other mineral elements on fruit quality occur largely through their interaction with calcium in the fruit cells.

Although numerous investigation over 200 years have been done around the world, the physiological disorders known as bitter pit and

internal breakdown on apple, and cork spot on pear, remain a major source of waste of apple and pear fruits.

Numerous factors interact to create conditions whereby the fruit may become deficient in one or more mineral elements such as calcium.

Beginning at the soil-water-root level, soil pH is known to restrict calcium availability by tying up calcium as insoluble complexes. Since Ca is generally less soluble than many other divalent and monovalent cations, insufficient soil moisture can also reduce available Ca. Even when soil moisture is high, if there is inadequate oxygen, many of the divalent cations, including Ca, which are at least in part dependent on active metabolically-linked transport for root uptake might not enter the plant even if available in the soil water solution. Low soil temperatures would also retard the metabolically-linked active uptake of Ca. Competing ions, especially NH_4^+ and K^+ , present in the soil could also reduce Ca uptake. Whereas NO_3^- , for example, seems to promote Ca uptake, implying that forms of N-fertilizers could have effects on Ca loading.

The distribution and morphology of roots also can be a significant factor in that Ca is preferentially absorbed by the finer, young root hairs in contrast to some of the other elements. Once Ca enters the root there may be further control on translocation at the Casparian strip, a site where active transport across cellular membranes may exert ion selectivity. There are also implications of phloem-translocated substances from the top of the plant which can

also influence how much and how rapidly Ca is loaded into conducting tissues, whether xylem or phloem. Some of these substances may well be organic acids which specifically (relatively speaking) assist in Ca movement. Thus, rootstocks, and interstocks, can and do have important interactions in how the scion variety receives and partitions absorbed nutrients.

A further set of factors, many manageable by cultural practices, relate to flowering, fruit set, and ultimately to crop load and how individual fruits interact with competing sinks such as growing shoots, leaves, and buds. Drought stress conditions magnify the competition for nutrients between leaves and fruits. If stress is severe enough, not only might nutrients such as Ca preferentially translocate to more rapidly transpiring leaves and shoots, but fruit serve as emergency water reservoirs and fruit solutes can actually be lost to move toward the leaves as will be later discussed. When only a few large fruits are on the tree, considerable mineral nutrients can be lost, whereas if there are many fruits, the stress on individual fruits is less. Adequate root moisture supply, or over-tree sprinkler irrigation can substantially reduce the stress and preserve mineral elements in the fruits from being retranslocated. Excessive nitrogen fertilizer, rampant growth of water sprouts caused by heavy pruning, or over-thinning of fruit can all aggravate the fruit-shoot competition for nutrients. Thus, many interacting factors can lead to Ca deficiency disorders in some fruits.

Description of the Disorders

Bitter Pit of Newtown Apples. Bitter pit of apples is a disorder in which small, brown, dry areas disfigure the flesh. These pockets of brown tissue are roughly circular, and range from about 1 to 4 mm in diameter (122). The location of the pits is usually just below the skin, but in severe cases the pit may extend throughout the cortex (340). The skin over these pits, or depressed lesions, often takes on a deeper green color than the surrounding skin before the brown, desiccated pit develops in storage. The pits may be few in number and more prevalent in the calyx end of the fruit or they may be numerous, extending over much of the surface of the fruit.

Bitter pit is initiated while the fruit is still on the tree and visual symptoms may show up just before harvest, but usually do not become evident until the fruit is in storage or sometimes not until a few days after removal from storage (336).

Under the microscope, the pitted areas are seen to consist of dead, collapsed cells with apparently normal cell walls (122). The affected tissue of bitter pit is softer than the surrounding tissue (109, 122, 332). The affected tissue is often bitter in taste, largely because of increased polyphenolic synthesis in the lesioned area.

Internal Breakdown of Newtown Apples. Many types of flesh breakdown can only be described visually; they are therefore difficult to

define and sometimes given different names in different parts of the world (122).

Carne (65) made a distinction between primary and secondary breakdowns. Under primary breakdown he included those induced by overmaturity or by storage conditions. The secondary breakdowns are forms of senescent breakdown. Such distinctions were also adopted by Fidler et al. (122) and Martin and Lewis (215).

A. Low temperature breakdown. According to Fidler et al. (122) this breakdown of apples is seen in the cortical tissues as a general browning of the flesh. The vascular tissue is picked out as dark brown specks. The boundaries between healthy and affected tissues are diffuse, and there is frequently a zone of 2 or 3 mm clear tissue immediately below the skin. For this reason the apple might have quite serious internal damage before it is obvious on the outside. As the disorder progresses, the skin eventually becomes discolored and apparently waterlogged, giving a dark translucent appearance.

Tissue affected by this disorder is likely to be firmer and more moist and darker in color than tissue affected by senescent breakdown (344).

B. Senescent breakdown. This disorder has been called "mealy breakdown" in the past. The early stages of mealiness usually precedes any browning of the flesh. The cells of the flesh become soft and crumbling and eventually turn brown (344).

The problem is symptomatic of old age and over-storage. The

problem is worse in large fruits, in fruit from high-nitrogen and low-calcium trees and after delayed storage (344).

Typically, in Cox's Orange Pippin it appears first on the outside as a dull darkening of the skin, and is very often first seen at the calyx end of the fruit where it is usually most severe at any time (122).

Cork Spot of D'Anjou Pears. Cork spot is a physiological disorder very similar to bitter pit in apples that occurs mainly in D'Anjou pears, but it also can occur in '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 grayish-brown spot (109). The brown spot can be anywhere between the skin and core, but in most cases it is close to the surface of the fruit just beneath the skin (109, 340). A depression develops above the internal spots as the fruit enlarges due to the reduced growth in the affected tissues. As in apples, the spots are usually more frequent at the calyx end of the fruit.

History

Bitter pit has been recognized for a very long time. The disorder had been previously described by Jagger (165) in 1869, but the name "bitter pit" was used for the first time in 1895 by Cobb (79)

because the abnormal tissue tasted bitter. Before the turn of the century, however, it was called by many other names (109), including 'dry rot' (87), 'brown rot' (173), 'Baldwin spot' (78), 'fruit spot' (51), and 'fruit pit' (163).

An association between bitter pit and the fungus Alternaria was reported in 1914 by Reed (295). However, attempts to isolate pathogens from the spots failed in 1914 (352). Thus, it is likely that Alternaria may have been a secondary infection in the disordered tissue. Association between bitter pit and water relations (drought stress) was reported in 1920 (52, 53).

Cork spot on pears was reported for the first time in 1921 (237). Early picking was reported to increase storage pit in 1924 (3), and cold storage at 0°C retarded the appearance of it. The first mineral analysis of apple fruit was reported in 1926 (54). In 1936 (88) Ca was reported to be lower than normal in fruits with bitter pit. In 1956 attempts were made to increase Ca concentration in the fruit by foliar sprays with Ca salts, reducing the occurrence of the disorder from 40% to 10% (24). Many reports on the successful control by Ca sprays have been made thereafter (122, 129, 213, 220, 322, 324, 326, 331, 355, 420).

The term "low-temperature breakdown" was first used by Kidd and West (183) in 1928, and was applied to a form of injury which they first described for Bramley's Seedling and Pippins in 1922 (182). They considered it to be identical with that described earlier as occurring in Yellow Newtowns (275).

Others to describe the disorder were Carne and Martin in 1935 (66) and Trout et al. in 1940 (368). Some of the earliest low-temperature breakdown reports were concerned with the effects of storage humidity as noted in 1923 (262) in Yellow Newtown apples, which developed more breakdown under conditions of low relative humidity. However, with the widespread use of calcium sprays as a means of controlling bitter pit, several sources observed that the amount of senescent breakdown was also reduced (34, 74, 327). This has led to investigations into a possible connection between the amount of calcium in the fruit and susceptibility to breakdown (122).

Changes in the Affected Tissue

Histological Changes. Electron microscope studies of bitter pit showed browning and rupture of cell walls followed by the appearance of round inclusions (55). There have been reports (343) of a proliferation of cells in the pitted areas and arising from the injured cells, and of pectin protuberances. In the affected parts of the fruits, the cells were found to be thinner and the nuclei prominent (238), suggesting that the cells are large and loosely connected.

Mahanty and Fineran (208) compared cells of pitted normal areas in apple fruits and reported that in pitted areas the golgi bodies are rarely observed; endoplasmic reticulae profiles were usually swollen into vesicles and tubules; mitochondria were fewer and their internal structure was generally less recognizable; thylakoids were

swollen and broken; liposomes with inclusions were seen; and the ground plasm was coarsely granular and poorly preserved. Ultrastructural studies by Buchloch et al. (55) revealed that the middle lamella of cells in the bitter pit area differentiated normally but the finely laminated suberin layer could not be found. In some places, the walls of neighboring cells were separated and islands of pectin could be seen. Walls of healthy cells, on the other hand, appeared more homogeneous (55, 109).

Several observations lead one to assume that the cause of bitter pit occurs while the fruit develop in the orchard, and that the susceptibility toward development of the disorder depends on the conditions occurring during the development of the fruit on the tree. In this respect, Simons et al. (342) found abnormalities adjacent to necrotic vascular bundles in the outer cortical region to be microscopically apparent early in the life of apple fruit, i.e., by 14 days after full bloom. Later Simons et al. (341) reported that the development of the disorder was characterized by breakdown of the article, and changes in the cells of the hypodermis and cortex of the fruit.

More recently, in 1978, Woodridge and Terblanche (419), using scanning electron microscope (SEM) and energy dispersive x-ray analysis (EDX), showed that in bitter pit-affected tissues, the cell walls were thinner, there was cellular disorganization, lack of conducting tissue and the presence of high concentrations of starch; confirming work done forty years earlier. Interestingly, amylase requires Ca^{++}

as a cofactor, so starch accumulation might depend on low calcium concentration.

Biochemical Changes. Starch content of the discolored pit tissues of bitter pit is much higher than in unaffected tissue (16, 106, 238, 345, 419). It has been known for more than two decades that starch remains unconverted in the pits (55, 122, 309). The enzyme α -amylase that degrades starch requires Ca as a cofactor. No reports have appeared specifying whether starch accumulation is related to α -amylase concentration or activity, or whether it is related to Ca.

During the maturation process, starch is hydrolyzed to glucose, converted to fructose, and subsequently to sucrose, which is the chief storage carbohydrate in mature apples (109). It is interesting to note that the pitted tissue was low in sucrose but high in glucose and fructose (17, 356), indicating, perhaps, that metabolic processes resulting in sucrose synthesis may be disrupted, thus causing an accumulation of glucose and fructose, which in turn may slow down starch hydrolysis (109).

Pitted areas have also been found to be relatively high in total nitrogen and protein nitrogen (120, 153), implying a high metabolic rate.

Citric acid replaced malic as the principal acid in the pits of 'Cox's Orange Pippin' apples (153) and was the only acid found in the pits of 'Golden Delicious' and 'Ontario' apples. The pattern of

fatty acids was studied (55) and there was a decrease in oleic acid and an increase in linoleic acid in the bitter pit-affected tissues.

Large quantities of some mineral elements have been found to move into the affected areas of bitter pit (109). The principal elements that moved into the affected area were Mg, Ca and B (115). Ca characteristically is translocated into metabolically active tissue. It is possible that the metabolic activity in cork spot tissue is great enough to mobilize Ca (109).

In cork spot, acetate is apparently the major substrate for respiration rather than glucose (109). Respiration, though tightly coupled, can be uncoupled by 2,4-dinitrophenol in disordered tissue to about the same degree as respiration in the unaffected tissue (109). Based on histochemical studies it was proposed that bitter pit was caused by failure of the dehydrogenase system to function (14). The increased respiration in the cork spot tissue is preceded by an increased production of ethylene (109). This rise is detectable at the very earliest sign of the disorder, and by the time the disorder has fully developed ethylene production is 50-60 times that of the unaffected tissues (109). Faust and Shear (109) identified the sequences of events occurring in the development of cork spot tissue in detail. At the first visible sign of the disorder, the rate of ethylene production increased in the affected tissue. Respiration also increased. Protein synthesis, pectin synthesis, and movement of inorganic ions into the affected tissue followed. At the

final stage of development, the tissue became brown and appeared as a firm brown spot in the flesh of the fruit.

Apples with internal breakdown accumulate toxic volatiles, including acetaldehyde (77) and acetic acid (410). In these fruits there is also leakage of phenolic precursors for the browning reactions and cellular death (116).

Effect of Nutritional Elements on the Development of Bitter Pit, Cork Spot, and Internal Breakdown in Apple and Pears

Calcium

A. Root Uptake and Ca Mobilization. The Ca content of a plant is genetically controlled and is little affected by the Ca supply in the root medium provided its availability is adequate for normal plant growth (243). Generally the Ca content of the soil solution is higher than other nutrients cations, i.e. potassium. However, the rate of Ca uptake is lower than that of K. Clarkson and Landerson (76) explains this based on the fact that low Ca uptake occurs because Ca can only be absorbed by young root tips. The Ca uptake can also be competitively depressed by the presence of other cations such as K and NH_4 which are rapidly taken up by the roots (243). In both uptake and transport it is believed that extensive Ca movement is associated with exchange sites (130, 160).

The ability of absorption and translocation is different for different parts of the plant roots. Movement of calcium across the root into xylem is restricted where the endodermis becomes suberized

(11, 305). It seems that Ca uptake and transfer to the xylem will be restricted to younger parts of the roots (118).

The transport of Ca from the cortex to the stele is restricted to the apoplastic or free space pathway which is only accessible in non suberized young roots (75).

The root xylem sap has consistently been shown to be electro-negative to the outside solution, therefore, the electropotential gradient tending to favour the inward movement of cations (118). This could lead to the assumption that root uptake would produce the Ca levels found in the sap (118). Ferguson (118) compared calcium concentration in xylem sap with that in the soil solution, to assess the contribution of root uptake to calcium transport. In kiwi fruit, the maximum calcium concentration was 1.7 mM at the time of intensive bleeding, and averaged 3.8 mM over the season. In sap from apple trees Ca concentration ranged from 1.4 to 3.5 mM (174) or a maximum of 4.5 mM (43). A value for Ca concentration in the soil solution would be about 1 mM (26).

Calcium uptake appears mainly to be a passive process (243). The same holds true for the translocation of Ca within the plant. The mechanism of Ca translocation in the xylem is described as via an exchange mechanism by many authors (31, 117, 339, 363). The xylem cylinder of the stem may operate as an exchange column for the upward translocation of Ca in the stem of bean plant (31). Lignin was suggested to be one of the possible exchange sites for Ca translocation (338). Ca might ascend in the xylem by mass flow if all

exchange sites are occupied by ions which cannot be replaced by Ca (363). Translocation of Ca in the xylem is dependent upon Ca concentration and can be influenced by transpiration. If Ca is available to the xylem its translocation is proportional to water uptake and transpiration (113) only to a certain extent. The Ca uptake into leaves and fruits sharply declines with the organs age although a constant transpiration rate is maintained (188).

There is evidence that Ca is translocated preferentially towards shoot apex even though the transpiration rate is much lower than in older organs (243). It is suggested that this preferential movement is related to cell division. The high IAA content in the faster developing tissues promotes a proton efflux pump which increases the formation of new cation exchange sites so that the growing tissues become a center for Ca accumulation (211, 243).

The establishment of high concentrations of ions in xylem sap by root uptake early in the season when transpiration is still low would require substantial root metabolic activity. However, Head (150) and Atkinson (18) found little root activity early in the season when soil temperature were still below 7°C.

It is suggested (396) that the secondary translocation of Ca that had been deposited in the wood and bark during the previous growth period is the main source of Ca supply to young developing tissue and that this movement of Ca occurs early in the season (118). Most of the Ca enters the fruit early in the season and the maximum

Ca concentration is reached at about the time that cell division ceases in various parts of the fruit (157).

The form of N has been shown to influence the translocation and distribution of Ca within the plant (113, 335). If NH_4^+ is used as source of N, Ca accumulates in the young leaves but if NO_3^- is used as source of N, then Ca accumulates in the mature leaves (335). Plants that were fertilized by NH_4^+ as source of N had lower Ca content than those fertilized with NO_3^- as source of N (335, 336). Ammonium ions may interfere with both uptake and translocation of Ca.

B. Uptake of Ca by the Fruit. It is generally considered that calcium taken up by the roots is consistently transported to the leaves and fruits possibly via the xylem, in which the element is relatively mobile. Very little Ca supply to the fruits happens via the phloem, in which calcium moves much less readily than, for instance potassium (367, 397). Most of calcium eventually present in the fruits reaches them during their first 4 or 5 weeks of development (338, 401) while the cell walls are being formed; at a later stage, there is very little further uptake, in fact there may even be a loss of calcium from the fruit, particularly during periods of drought (401). Migration of calcium from the fruit has been confirmed by studies in which ^{45}Ca applied to the fruit surface could later be found in leaves and shoots (213). Kohl (122) also confirmed that as the weight of apple fruit increases, the absolute mineral content of K keeps pace with it whereas the absolute Ca content remains almost static so that its concentration decreases, the

greatest decrease being found during the most rapid growth period in August. Similar results were obtained by Van Goor (380) and by Millikan (246).

A theory put forward by Wiersum (397) and developed by Bangerth (20) links the development of bitter pit to changes in the mode of water supply to the fruit, where a stress condition during the growing season would result in a reduced root growth ceasing therefore the Ca uptake by the young plant roots. Since the leaves transpire at a faster rate than the fruits under drought conditions, there is water movement from the fruit to the leaves to compensate for the water loss. With the water moving out of the fruits, some Ca is also carried out, reducing therefore the fruit's Ca content, a condition prone to bitter pit development.

Young apple fruits have a relatively large surface area and highly permeable cuticle, and thus also have a high rate of transpiration. They therefore have a high water requirement. Water supply is mainly via the xylem, in which Ca moves comparatively freely (296). With increasing fruit size, transpiration diminishes and there is much greater phloem translocation of assimilates from the leaves with a corresponding reduction in the xylem flow (296). Some other mineral elements, eg. K and Mg are mobile in the phloem stream, but Ca is not.

When water supply is low, fruits have to compete with leaves for water, and the leaves are stronger competitors by 10 to 11-fold on an

area basis. Under such conditions, migration of Ca from the fruits to the leaves may occur.

Wieneke (395) found that ^{45}Ca was readily taken up by the roots of apple seedlings. Radio-autographs showed that upward transport of Ca took place in the xylem but was almost absent from the phloem.

Van Goor (380) also discusses the implications of a changeover in nutrient transport to the fruit resulting from a switch in translocation from partial supply by the xylem to almost exclusive phloem transport, occurring when the fruit weights about 30g.

Stebbins and Dewey (353), however, provided some evidence for an active translocation of Ca in the phloem, in young apple trees. Thus the nutrient distribution pattern in juvenile trees may not be the same as that in older plants.

Shear and Faust (335) used ^{45}Ca applied to young and mature apple leaves and confirmed the observations of Stebbins and Dewey (353). The growth regulator, Kinetin, was found to increase the movement of ^{45}Ca into mature leaves (335). Stem sections from the seedlings were eluted with solutions containing salts of various cations to study the exchange of ^{45}Ca taken up via the roots, and it was found that solutions containing Ca, Mg, or Ba were effective in exchanging ^{45}Ca from sites that it had occupied. These results supported the concept of Bell and Biddulph (31) that Ca ascent was due to exchange processes rather than to transpiration-induced mass flow, and that the removal of Ca ions from the exchange sites is governed by the metabolic requirements of individual tissues.

Faust and Shear (113) distinguished two types of Ca movement within the apple tree. When ^{45}Ca was administered to the intact roots it was transported through the phloem, whereas if administered to the cut end of the stem it was transported through the xylem. In the former case Ca moved mainly into the developing leaves and in the latter it reached all the leaves including the mature ones.

When the roots of a plant were killed by hot water, Ca transport was shifted from the phloem to xylem (296). Interestingly, the same effect could be obtained by removing the terminal bud. Transport towards the fruit appeared to take place via the phloem, but once inside the fruit the mode of transport was similar to that observed in xylem (112, 118). They suggest that the changeover from phloem to xylem transport takes place at the point of attachment of the pedicel to the bark, since applied ^{45}Ca did not move into the fruit unless the pedicel was cut above this point.

The formation of Ca oxalate crystals in apple pedicels (200, 354, 396) has been suggested as a possible factor in impeding the movement of Ca into the fruit and generally tying up Ca in an inaccessible crystalline form. Cox's Orange Pippin, a variety prone to bitter pit, had a much higher fraction of Ca fixed as oxalate in the pedicel than did the less susceptible cultivar Golden Delicious (200). Studies on leaf petioles showed oxalate crystals to occur in the phloem tissues rather than in the xylem (354).

Relationship of Ca with Bitter Pit, Cork-Spot and Internal Breakdown

A. Bitter pit and cork spot. In many countries, bitter pit is the most serious physiological disorder of apples. Since the investigations of De Long in 1936 (88) and Garman and Mathis in 1956 (129) it has been known that deficiency of Ca and/or imbalance of Mg/Ca or $Mg^{+}K/Ca$ ratio is associated with this disorder.

The disorders are essentially initiated and generated during development under the orchard conditions. Trees subject to marginal Ca deficiency (180, 248, 261) developed severe bitter pit and cork spot. Ca content of the fruit (88, 89, 129, 152, 184, 226, 249, 261, 273, 280, 301, 418) has been associated with bitter pit and cork spot. In years when Ca content of fruit was high, no pitting occurred; in other years, when Ca content was low, severe pitting developed (109).

Sprays of Ca compounds were found to be beneficial in reducing the incidence of pitting in apples (29, 33, 74, 86, 122, 132, 147, 161, 162, 169, 222, 224, 228, 263, 291, 314, 322, 324, 355, 359, 420) and pears (233, 289, 304, 416, 418). Calcium nitrate (29, 34, 55, 74, 86, 132, 161, 162, 224, 289), calcium chloride (29, 220, 301, 156, 292, 311, 355), Ca-lactate (312), Ca-acetate (17, 55) and bordeaux mixture (237) have been used to decrease the occurrence of bitter pit. Martin et al (220) were able to reduce bitter pit in the apple cultivar 'Cleopatra' from 35% to 7% by spraying with $Ca(NO_3)_2$. Spraying with $Ca(NO_3)_2$ or $CaCl_2$ are equally effective in reducing

bitter pit in apples (213, 217, 220, 223), but CaCl_2 was recommended when the fruit nitrogen concentration is already high. Low concentrations of $\text{Ca}(\text{NO}_3)_2$ (5 lbs/100 gal.) were recommended for earlier sprays and high concentrations of CaCl_2 (222, 224, 226) (10 lbs/100 gal) were recommended for later spraying (213, 217, 220). The degree of control depends on the number of applications and there is little possibility that single well-timed applications will correct the problem. Al-Ani (8) reported a reduction of cork spot in D'Anjou pears from 49.8 to 16.5% and an increase in fruit Ca of 20% over the control, with three sprays with $\text{Ca}(\text{NO}_3)_2$ or CaCl_2 with a month interval. It is suggested that the more applications the better the control of bitter pit in apples. A spray program of four to seven calcium sprays is recommended for best results (122, 322, 325, 326). Soil applications of $\text{Ca}(\text{NO}_3)_2$ (1 kg/tree) was reported to have no effect on fruit Ca concentration and increased the incidence of bitter pit due to an increase in available nitrogen from the soil without increasing Ca (217, 220, 22, 227).

Calcium chloride may be more effective than $\text{Ca}(\text{NO}_3)_2$ in controlling bitter pit, but the CaCl_2 can cause leaf scorch under certain conditions. Spraying with CaCl_2 at high concentrations (10 lb/100 gal) early in the season may cause a slight leaf scorch (325, 326). Spraying during hot weather and under windy conditions may damage the leaves (325). Injury to the fruits may also result from high $\text{Ca}(\text{NO}_3)_2$ spray (322, 325, 326).

The use of additives and surfactants has a great effect on Ca uptake from sprays and increases the effectiveness of the treatments. Martin et al. (222, 224), studying the effect of Ca salts on the incidence of both bitter pit and internal breakdown of apples, concluded that dimethylsulphoxide (DMSO) was the most effective chemical added to $\text{Ca}(\text{NO}_3)_2$. 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 $\text{Ca}(\text{NO}_3)_2$ (0.05 M) alone.

Postharvest application of Ca has been used to reduce the occurrence of both disorders of apple and pear fruits, i.e., dipping (32, 146, 168, 197, 322, 393) and vacuum or pressure (32, 148, 168, 197, 315, 316, 322, 358, 393).

According to Fidler et al. (122), there are few instances where the relationship between bitter pit and low Ca concentration has not been established. They attribute this to inadequate sampling or analytical technique. Perring (267) found that low average Ca concentration in bulk samples of apples was associated with susceptibility to bitter pit but that the relationship between calcium and bitter pit in single apples stored individually was not established. Sharples (321) also makes reference to some exceptions of the relationship of Ca to bitter pit in apples.

Loss from bitter pit was negatively correlated with August fruit Ca analysis (324). In orchards where the fruit Ca exceeded 5 mg/100 g fresh weight, the risk of severe pit in air-stored fruits was low and where fruit Ca was lower the risk of pit was high (324). Wills

et al. (409) concluded that calcium concentration in fruit three weeks before and at harvest was a reliable guide for predicting bitter pit, but that between districts, varieties and seasons there were marked differences in fruit calcium concentrations above which bitter pit was unlikely to occur. Holland (156) found that fruit mineral analysis afforded a reasonably good tool for bitter pit predictions, and that the ratio K/Ca appeared superior to any single nutrient. He found no significant difference between K/Ca and bitter pit with regards to growth and cropping conditions of the orchards. A significant difference was found between regressions fitted in different years, but he points out that this difference was so small as to be of little consequence and that a single regression of K/Ca with bitter pit, generalizing over three years sufficed for predictive purposes.

Al-Ani (8), working on cork spot in D'Anjou pears, found that fruit Ca was negatively correlated with both cork spot at harvest ($r = -0.75$) and after storage ($r = -0.76$) for two consecutive seasons. Cork spot at harvest and after storage was positively correlated ($r = 0.74$ and 0.75) with N:Ca ratio. He pointed out that correlations between cork spot and both Ca and N:Ca ratio were highly significant at harvest and during the growing season as early as July. From his work it was concluded that the critical minimum calcium concentration in D'Anjou pear fruit for the development of corking disorder is about 7 mg/100 g of fresh weight, and that if the N:Ca ratio was

greater than 10:1, the critical minimum Ca was progressively greater than 7 mg/100 g fresh weight.

B. Internal breakdown. The relationship of low concentration of Ca in the fruit to breakdown in apple has been shown in Europe (267, 311, 319) and in North America (25, 247). Perring (267, 268) has attempted to establish limits of calcium concentration in Cox's Orange Pippin apples grown in England, and has suggested that if the level of Ca is not greater than 4.5 mg/100 g fresh weight, the fruit is liable to break down early in storage. The inverse relationship between Ca content of the fruit and susceptibility to breakdown has also been demonstrated in Denmark (293), Belgium (374), Canada (229, 230), Australia (314), and the USA (50, 381).

Some reduction in the incidence of the disorder has been achieved by the application of a calcium salt to the fruit as a preharvest spray (126, 292, 377), as a postharvest injection into the core (407, 408), and by dipping 'Jonathan' apples in CaCl_2 solution prior to storage reduced the development of internal breakdown from over 60% to less than 6% (25). Mason et al. and Scott (314) also reported substantial reduction of internal breakdown in apples dipped in CaCl_2 solutions.

Correlation coefficients of 0.9 were obtained using log Ca and linear breakdown, or log Ca and log breakdown in 'Spartan' apples. The incidence of breakdown was 100% at 70 ppm Ca (dry weight basis) from 46 to 100% at 100 ppm Ca, from 3 to 12% at 150 ppm Ca and less than 2% at 200 ppm Ca (230).

Whereas low calcium concentration in the fruit can be considered to be a contributory cause of senescent breakdown, this association does not seem to have been established for 'Jonathan' breakdown (342) and for 'Sturmer' apples.

Effect of Calcium on Senescence of Plant and Fruit Tissue.

Calcium plays an important role in maintenance of cell structure and function in plant tissues. Calcium is involved in the cell wall by forming linkages between the galacturonic acid chains, as well as between galacturonates and the carboxyl groups of other components such as protein (280). It is important also as calcium pectate, the cementing substances in the middle lamella between cells.

Low calcium content is associated with rapid senescence of fruit and vegetative tissue (24) and senescence may be deferred by addition of calcium to intact fruits, and this has been documented by several authors (47, 111, 411). Poovaiah and Leopold (277) also reported that Ca deferred senescence in leaf discs. In avocados and tomatoes, the delay and reduction in intensity of the climacteric in calcium-infiltrated fruit is associated with a similar deferral and reduced ethylene production (364, 411). Poovaiah and Leopold (278) have reported a similar delay in the ripening of bananas due to increased fruit calcium.

The rate of protein and chlorophyll degradation in corn leaf discs is retarded considerably by cytokinin (benzyladenine) and by 1 to 10 mM CaCl_2 in the solution on which they float (277, 279). The

studies of Thiemann and Satler (361, 362) implicate stomatal aperture as the principal cytokinin-sensitive factor in delaying senescence. It is not out of line with the known properties of Ca for it to act in maintaining transport and guard cell turgor (149).

In contrast to its effects on intact organs, calcium included in buffer systems has been reported to enhance ethylene production in excised mung bean hypocotyl (190), aged slices of postclimacteric apple (199), apple protoplasts (10) and potato discs (15). The effects of calcium in preserving the ethylene forming system in postclimacteric apple slices was attributed to the stabilization of membrane by calcium (191, 199).

Faust and Shear (111) suggest that calcium delays senescence due to preservation of the cellular organization (176), by preserving the cell membranes and also by maintaining nucleic acid and protein synthesis.

Effect of Calcium on Cell Membranes. One of the major functions of calcium 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 the nuclear envelope and the plasma and vacuolar membranes and "structureless" areas appear in the cells, followed by the disorganization of inner cristal of the mitochondria (209). Calcium deficiency caused a general degradation of the endoplasmic reticulum, mitochondria, and chloroplasts in green alga (254). Calcium

deficiency first leads to leaky membranes long before microscopically visible breakdown is evident (22).

The action of calcium is not only to prevent injury to cell membranes but also contributes actively to the formation of cytoplasmic organelles (62, 175, 201). Calcium deficiency decreased the synthesis of mitochondria and reduced their functional capability (62).

It has been reported (22, 62) that the addition of calcium can restore damaged membranes, which adds further evidence that Ca is essential to the maintenance of plasma membranes. It has been frequently reported that calcium participates in building up and maintaining cytoplasmic membranes in a functional condition (22, 62, 175, 250, 351, 379, 382).

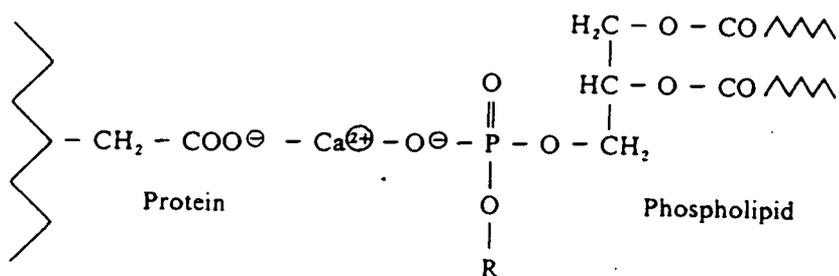
There is much evidence that calcium is of fundamental importance in regulating membrane permeability and for the maintenance of cell integrity (75). Electron microprobe studies by Roland and Bessoles (306) have revealed that calcium is located especially in the border zone between the cytoplasm and cell walls indicating high Ca content in the plasmalemma. Calcium can be removed from membranes by treatment with EDTA (382). This treatment increases membrane permeability to such an extent that inorganic and organic compounds can diffuse out of the cell and considerable damage may result (382). Impaired membrane permeability resulting from Ca deficiency, such as the effect of EDTA, influences the retention of diffusible cellular compounds (91). Membranes become leaky as deficiency progresses

(209). The disorder occurs first in meristematic tissues such as root tips, growing points of the upper plant parts and storage organs (243). Another aspect of the importance of Ca in membrane stability has been discussed by Marschner (210), where he suggests that low Ca content of storage organs induces a high membrane permeability and allows solute diffusion in these tissues.

Low Ca tomato fruits had a higher tissue permeability preceding the occurrence of blossom-end rot (379). Plasma membranes in pitted tissue of apple and in tomatoes with blossom-end rot are severely disturbed if not destroyed (23). Higher permeability of cell membranes may cause the acids as well as the phenols to permeate from the tonoplast into the cytoplasm and reduce or inactivate enzymes, mitochondria and other subcellular particles and thus damage the cell and tissue (8). This might happen in a Ca deficient fruit as in bitter pit and cork spotted tissues.

Calcium may affect membrane permeability and stabilization in two ways:

1. Probably the most important aspect of Ca^{++} interaction with membranes and how it affects permeability is that Ca interacts with adjacent phospholipid phosphate groups and pulls the membrane tighter together -- this reduces the average area occupied by each phospholipid molecule from 41-42 A^0 to 38-39 A^0 ; no other divalent cations exhibit this property. Calcium may bind to the phosphate groups of the phospholipid molecules in membrane. The phosphate group can be bridged by Ca^{++} to a carboxylic group of protein (243)



2. Calcium may bind to the protein molecule in the membrane. The phosphate group of phospholipids would bind to the NH_3^+ group of protein by electrostatic forces. This binding of Ca may alter the size of the pores in the membranes and reduce passive permeability (23).

Research within the last decade, first in animal tissues (192), then more recently in plants (241, 383), has revealed the presence of Ca binding proteins and, in particular, calmodulin. This protein interacts reversibly with Ca to form a calmodulin Ca complex, the activity of which is regulated by the cellular flux of Ca (192). Calcium binding proteins act as potential receptors of Ca mediating the effect of Ca in cellular reactions (243). Calmodulin has been shown to play a central role in cellular regulation in animals and the same seems likely to be the case for plants (73). Very recently, Fukumoto and Nagai (127) showed a possible relationship between calmodulin and the development of bitter pit in apple fruits. They infiltrated chlorpromazine, N-(6-aminohexyl)-5-chloro-1-naphthalene-

sulfonamide (known as W-7) and N-(6-aminohexyl)-1-naphthalenesulfonamide (known as W-5), all calmodulin antagonists, into the fruits for 20 minutes under reduced pressure (1×10^4 Pa). A few days later, numerous bitter pit-like spots were observed in both fruits treated with W-7 and chlorpromazine, while only a few spots were observed after the infiltration with W-5, a less potent calmodulin antagonist. They suggest that the development of bitter pit may be due to a failure of calmodulin activation.

Effect of Calcium on Respiration. The calcium ion is known to be involved in many fundamental physiological plant processes involving cell walls, membranes, chromosomes and enzyme activation, among others (175). In postharvest physiology, disorders such as bitter pit (109, 225, 255, 346) and internal breakdown (346) in apples and cork spot in pears (288, 289, 301, 384, 411) have all been directly linked to low Ca content of the fruits. It has been suggested that these disorders result from increased respiration rate (111) following membrane permeability changes (303) which lead to rapid cellular senescence and necrosis.

Respiration of apples was found to be inversely related to Ca content of flesh (111). Respiration was markedly increased if Ca concentration was below 110 ppm, and high Ca counteracted the increased respiration induced by ammonium-N (111). Wang and Mellenthin (390) found that pears affected with cork spot respired more rapidly than sound fruit. Bramlage et al. (47) reported a

highly significant negative correlation ($r = -0.82$) between peel Ca and respiration rate for 'Baldwin' apple fruits. A negative and highly significant correlation ($r = -0.83$) was reported by Richardson and Al-Ani (302) in D'Anjou pear. Higher calcium levels in the flesh depressed preclimacteric, climacteric and postclimacteric respiration of apples (46, 111), avocados (364) and pears (302, 385). The same inhibitory effect was observed for respiration of apple mitochondria (346). On the other hand, Bramlage et al. (47) found no influence of Ca content on the time of the climacteric occurrence in apples.

There are reports that addition of Ca^{++} to calcium deficient tissues reduces respiration (108, 279). Postharvest dips in CaCl_2 solution have reduced the rate of respiration of intact apple (25) and pear fruits (8).

One of the possible explanations for this high respiration in tissues with low Ca is that higher permeability of the membranes (277, 308) may increase substrate availability to respiratory enzymes in the cytoplasm and mitochondria (23).

Effect of Calcium on Ethylene Biosynthesis and Evolution.

Treating bean plants with CaCl_2 and then exposing them to ethylene gas or treating them with ethephon showed that CaCl_2 completely inhibited the leaf abscission-enhancing effect of ethephon or ethylene in intact bean leaves (279, 280, 281). Calcium also inhibited the abscission-enhancing effect of NAA in bean plants (279). It was found that Ca concentration ranging from 10^{-4} to 10^{-2} M CaCl_2 can reduce ethylene biosynthesis in bean plants (277).

D'Anjou pear fruits affected with cork spot produce more ethylene and produce it earlier than normal fruit (302, 390). Faust and Shear (110) reported that ethylene production by pitted apple tissues was two-fold higher than normal tissues. Calcium depressed the peak of ethylene production and delayed ripening of avocado fruit infiltrated with 0.1 M CaCl_2 (364). In avocados and tomatoes, the delay and reduction in intensity of the CO_2 climacteric in calcium-infiltrated fruits is also associated with deferred and reduced ethylene production (364, 411). There are reports that addition of calcium to calcium-deficient tissues suppresses ethylene production (108, 279), and calcium has been implicated in the regulation of C_2H_4 production (411). The close relationship between C_2H_4 production and IAA and the IAA oxidase system (123, 138) on the one hand, and Ca and IAA transport (255) and responses on the other, would suggest the possible link between Ca and C_2H_4 production. According to Dooley (94) his results on storage breakdown of 'Jonathan' apples confirms the antagonistic interaction between calcium and ethylene and suggests an accurate index of storage potential of apples could be based on both the calcium and ethylene levels.

In contrast to its effect on intact organs, calcium included in buffer systems was reported to enhance ethylene production in excised mung bean hypocotyls (190), aged slices of postclimacteric apple (199), apple protoplasts (10) and potato disks (15). The effect of calcium in preserving the ethylene forming system in postclimacteric

apple slices was attributed to the stabilization of membranes by calcium (191, 199).

Ethylene production in plants proceeds predominantly, if not exclusively, via the pathway methionine SAM ACC C₂H₄ (4). In most tissues, the rate-limiting step is SAM ACC, catalyzed by the enzyme ACC synthase (36, 64, 424). Changes that cause membrane disruption will impair this conversion since this step is membrane associated (12). Because loss of membrane integrity is one of the basic features of senescence, ACC conversion to C₂H₄ may be limited in senescent tissues. Most of the calcium-induced stimulation of ethylene production can be accounted for by its effect on ACC production, suggesting that the primary effect of calcium is on a step of ethylene biosynthesis preceding ACC production (103). However, calcium may also affect ACC conversion to ethylene, since a consistent increase in ACC-dependent ethylene production was observed in the presence of calcium (103).

Effect of Calcium on Fruit Firmness. Fruit firmness is a very important postharvest quality factor. This is especially important for long-term fruit storage. There have been many indications that fruit Ca content is related to the flesh firmness (25, 32, 90, 197, 228, 229, 232, 304, 315, 393). D'Anjou pear fruit low in Ca soften prematurely and cannot be stored for long periods of time (122, 233, 289, 300). Also, calcium dips maintained fruit firmness in Spartan (197, 229), McIntosh (32, 228, 393), Jonathan (25, 90), Golden Delicious (304), Newtown (386), Gravenstein, and Cox's Orange

Pippin (315) apples. D'Anjou pears dipped in CaCl_2 solution were also firmer than non-dipped pears after storage. Flesh firmness retention by postharvest Ca dips has varied from 3.7 N (229) up to over 9.8 N (228, 231, 304) depending on cultivar. Spray applications in the field during fruit growth also gave better fruit firmness retention. According to Riley and Kolatakudy (304), Golden Delicious apples individually sprayed with CaCl_2 solutions over a period of nine weeks immediately prior to harvest were 9.8 N firmer than untreated apples at harvest time, and that differences persisted during storage.

Contradictory reports on fruit dippings were made by Bramlage et al. (47) and Porritt (284) which have not found the Ca firming effect to be consistent. However, the initial concentration of flesh calcium and amount of calcium penetration from the postharvest dip may determine the magnitude of the effect and could account for these discrepancies.

Calcium chloride solution dips increase the calcium concentration of the flesh with detectable increases near the skin after two weeks and increased to 20 mm depth after eight weeks (232). The concentration of calcium in apple flesh resulting from a dip is increased considerably by addition of thickeners (e.g., Keltrol) and surfactant to the dip solution (171, 197, 232). Dewey (90) found no effect of a surfactant on the amount of Ca absorbed by Jonathan apples dipped in a CaCl_2 solution. Ca penetration was reduced by the inclusion of a surfactant, but was enhanced when a thickener was

added to the dipping solution (232). Lidster and Porrit (197) also reported higher flesh Ca with the use of thickener in dipping solution, with a substantial retention of flesh firmness. According to them, Spartan apples absorbed less Ca from a postharvest CaCl_2 dip than did McIntosh, which absorbed less than Golden Delicious or Red Delicious apples. The use of surfactant in CaCl_2 dipping solutions did not affect Ca penetration into McIntosh or Delicious apples but decreased penetration of Ca into Spartan and Golden Delicious, and this was reflected in differences in the gain of firmness among cultivars.

Relationship of Calcium with Organic Acids in Fruit.

Pitted tissues contain high concentrations of amino acids (aspartic and glutamic), more protein, and more organic acids than healthy tissues (20, 22, 120, 313, 340). Successive reduction of calcium solubility as well as citric acid accumulation in pit lesions may cause bitter pit in apples (95). The predominant organic acid in bitter pit is citric acid, while malic acid is the major constituent in the normal tissue (109). Citric acid also replaced malic as the principal acid in the pits of Cox's Orange Pippin apples (153), and was the only acid found in the pits of Golden Delicious and Ontario apples (122). Organic acids in the fruit can have antagonistic effects on Ca by providing H^+ (22). Organic acid can also remove calcium from its binding sites such as in the membrane by acting as calcium chelators (22). An increase in citric acid may increase the development of bitter pit (22).

The high acidity in the pitted tissue may cause the cations to move in the affected tissues to neutralize the excess of acidity (109).

Removal of calcium from the membrane by the action of organic acids can increase permeability, respiration, ethylene evolution and senescence (114).

Relationship of Calcium to Postharvest Diseases. All types of plant produce are susceptible to postharvest diseases (6). Post-harvest rot losses for fruits range from 12 to 23% (149). Prevention of postharvest decay often requires an integrated approach that includes protective treatments in the field or orchards and post-harvest treatments in the packing house (149). Modified environment techniques have improved the control of decay in fresh commodities (350). The initial quality of fruit is also very important for control of the decay (348).

The causes of rotting in apples and pears are mainly fungal infections, ranging from 20 to 30 species of fungi isolated from samples of fruit (9, 83, 181). However, the most common fungi found which cause the greatest losses of economic importance are Botrytis cinerea (30, 307, 403), Penicillium expansum (19, 139, 149), Gloeosporium album (6, 60, 149) and Mucor spp.

The rotting of apple and pear tissue is associated with the separation of cells and loss of pectic materials (80, 82). The loss of pectic substances in the middle lamellae and cell walls results in

loss of wall integrity (347). Solubilization of polyuronides from cell walls results in decreased fruit firmness (185), a key step in fruit ripening, and resistance to fungal attack is reduced. Analysis revealed protein loss during maturation until the fruit became susceptible to rotting and the amount of calcium in the susceptible fruit tissues was found to be lower than in resistant fruit (122). A pectin-protein-metal complex, resistant to fungal hydrolysis and therefore unable to serve as a source of carbon, was thought to be involved in the resistance of immature apples to the invasion of pathogens (122).

The incidence of rots in apples (47, 50, 88, 89) and pear (122, 384) fruits is believed to be at least partially related to calcium.

Wilkinson and Perring in 1964 (405) and Perring in 1968 (268) stressed the importance of calcium in relation to postharvest diseases of Cox's Orange Pippin apples.

The addition of CaCl_2 to benomyl increased the effectiveness of the fungicide in controlling decay following postharvest treatment (63). A more recent investigation showed that increased calcium content of fruit by postharvest applications may also reduce fruit decay (84). Conway and Sams (85) pressure infiltrated Delicious apples with an 8% CaCl_2 solution, stored for 3 months, then inoculated with a spore suspension of *P. expansum*. The infiltrated apples had 40% less decay than nontreated fruits. Wieneke (40) suggested that most calcium applied to the surface of apples penetrates through the lenticels and is located in the cell walls, possibly bridging

with the plasmalemma, and that the localization of native calcium and calcium originating from postharvest treatment is the same. Reductions in rotting have also been achieved by applications of calcium salts during the growing season (312, 326).

The mechanism by which calcium retards fungal decay may be similar to the effect that calcium has on the mechanisms that delay ripening or senescence and softening of the fruit. Calcium is known to help maintain cell wall integrity (23, 209, 297, 324). Since P. expansum produces enzymes that are related to cellular degradation leading to decay (81), Conway and Sams (85) suggested that Ca may maintain cell wall integrity by retarding fungal enzyme activity.

Nitrogen

Excessive nitrogen levels in the tree and fruit reduce fruit quality (48). Fruits high in nitrogen at harvest tend to be larger, softer and more likely to have cork spot and bitter pit (42, 48). Following storage, they develop greater amounts of bitter pit and internal breakdown (42). In the Pacific Northwest, probably 50 to 75% of apple orchards, and a smaller percentage of pear orchards, are excessively high in nitrogen (300). A high incidence of bitter pit has been associated with high fruit N (25, 107, 223, 226, 227, 313, 320, 322, 325, 326, 328, 329, 330, 331, 337, 338, 339). Pitted tissues have lower Ca and higher N (48, 120, 223, 226, 326). High N can have indirect effects of Ca concentration 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 (107, 109, 324, 330, 331, 332, 335, 336, 338, 339).

The form of N fertilization (NH_4^+ , NO_3^- , or urea) has also been reported to be related to the fruit susceptibility to bitter pit incidence (48, 59, 179, 274, 398, 414).

The form of N affects concentration of calcium in the fruits and its distribution within the plant. According to Shear (328) ammonium-N fertilizers can aggravate calcium deficiency in apples. Recently Ludders (204) demonstrated that the use of ammonium rather than nitrate nitrogen substantially increased K/Ca ratios in apples by reducing Ca accumulation, resulting in greater incidence of bitter pit.

Greater susceptibility of apple fruits to bitter pit relative to high levels of nitrogen fertilization depends on the apple cultivar. Link (202) reported that the incidence of bitter pit was increased significantly by higher rates of nitrogen in Gravenstein but not in Cox's Orange Pippin. Differences also exist between apples and pears. Richardson and Al-Ani (301) found that N:Ca ratio was positively related to cork spot in D'Anjou pear fruits at harvest ($r = .74$) and after storage ($r = .75$). However, they reported a weak correlation for N alone with the disorder. High N can affect the beneficial effect of normal concentration of calcium, so that a low N:Ca ratio and adequate Ca is required for good fruit quality (50, 301, 324, 331, 338). Cork spot in D'Anjou pear is expected to be more than 30%

if N:Ca ratio is higher than 10 (301) and in apples it has to be less than 10 to 14 to expect less than 10% bitter pit (331).

More recently, Ystass (423), working on nitrogen fertilization and quality of pears, reported no difference in fruit quality and size due to nitrogen supply, and it can be concluded that the pear fruit is insensitive to differential nitrogen fertilization.

Potassium

Potassium, although of less importance than Ca, seems relate to bitter pit in apples and cork spot in pear fruits. In fruits affected with the disorders, the concentration of potassium in the pit area is higher than in healthy tissues (56, 332, 378). High potassium fertilization has been reported to increase the incidence of bitter pit (57, 109, 221). Spray applications of potassium can increase the occurrence of the disorder (32, 57, 58, 221, 414).

It has been suggested that the K/Ca ratios in the fruit are more important than the absolute K levels (16, 17, 21, 38, 416, 417). A high K/Ca ratio favors the development of bitter pit in apples (17, 109, 135, 260, 404) and cork spot in pears (16, 416, 417). In a calcium deficiency situation, K was found to be greatly increased (58, 109, 324, 378, 404, 414) resulting in the development of bitter pit. The ratios (K + Mg)/Ca has been negatively correlated with bitter pit and cork spot (8, 16, 17). Schumacher (312) suggested the use of (K + Mg)/Ca ratios as the simplest index to predict bitter

pit. More recently, Holland (156) also pointed out that the ratio (K + Mg)/Ca appears superior to any single nutrient measure, but the simplest ratio, K/Ca, was just as good.

A high K/Ca ratio seems also to favor the development of internal breakdown in apples (104, 272). Very low levels of potassium reportedly cause a type of breakdown of apples during storage (322). Senescent breakdown and low-temperature breakdown of apples were reported to be reduced 50% when the fruits had adequate supply of K (402, 404).

Phosphorus

Reports conflict on the relationship of phosphorus to bitter pit (109). Phosphorus was reported to be significantly negatively correlated with bitter pit in apples (50, 217, 252); however, several authors have reported that the disorder was associated with high P content (54, 256, 318, 332). Faust and Shear (109) explained that in cases where P content was high, fruit calcium content was low (227) and so was K (252). Bitter pit only developed when the Ca was low and did not develop when K was low. Faust and Shear (109) suggest that Ca and K, and not P, played a role in pit development. In pitted areas, P content has been found in high levels (109), leading to the misconception that high P concentration was the cause of the disorder. However, it is known that mineral elements migrate to pit areas (17, 128); thus high P content in the pitted areas has no specific importance.

According to Bramlage et al. (48), in North America there is little evidence of effects of phosphorus concentration on postharvest fruit quality. It has however been reported to be a contributing factor to great deterioration of apples during storage (172, 370) in England.

Internal breakdown has also been suggested to be affected by low levels of phosphorus in the fruits (50, 267, 404). Letham (193) attributed the increase in susceptibility to the increased phospholipid level in the cell membrane. Johnson (170) sprayed triple superphosphate, calcium tetrahydrogen diphosphate, and mono-ammonium phosphate in Bramley's apples, noticing an increase of phosphorus in the fruit's flesh of 0.8, 1.2, and 1.1 mg/100 g, respectively. The severity of breakdown (index maximum 60%) in fruits was 16, 19 and 18% percent, respectively, compared with 31% in the untreated controls.

Critical levels of phosphorus in Bramley and Cox fruits was suggested to be 9 mg/100 g and 11 mg/100 g, respectively. Below these values internal breakdown was a risk (170).

Jakobsen (166) found that steady uptake of calcium may be improved by increasing the availability of phosphate early in the growing season, thus demonstrating an interaction between Ca and P on fruit nutrition.

Magnesium

High levels of Mg in fruit has been associated with increased

incidence of bitter pit (17, 376, 414). Pitting can be induced with Mg soil treatment (207, 282, 414), or by spraying with Mg salts (109, 221, 223, 227, 310, 332, 398, 415). Bramlage (48) however, points out there is little evidence for direct effects of either deficient or excess magnesium on fruit quality, and Oberly et al. (256) reported that apple fruit pitting decreased with increased leaf Mg content.

Magnesium, along with calcium and potassium, plays an important role in bitter pit development (310, 415), but due to antagonistic interactions at the membrane levels where they could occupy some of the non specific Ca attachment increasing membrane permeability.

In earlier discussion, $(K + Mg)/Ca$ was more closely related to bitter pit development than either K or Mg alone, or K/Mg. The effects of K or Mg on development of fruit disorders are a manifestation of a $(K + Mg)/Ca$ imbalance, and Mg was suggested to be less important than K in pit development (129).

Quinlan (287) explained the reason why high magnesium has often been correlated with fruit disorders. Magnesium and potassium tend to accumulate at a uniform rate in fruit. Therefore, any reduction in calcium accumulation in the fruit is reflected in a higher ratio of K or Mg, or both, to Ca. When calcium levels are below a certain threshold value, magnesium may substitute for Ca (327, 334, 369) and Hara et al. (148) showed that when calcium is low, magnesium may be partly incorporated into cell membranes, but when Ca is high, Mg remains in the cell sap. This replacement of Ca by Mg would bring

about membrane deterioration and predispose fruit to disorders (333).

The ratio Mg/Ca was suggested to be of importance in predicting bitter pit development (405). If the Mg/Ca ratio is less than 1.8, bitter pit is not expected, but if the ratio is above 2.0, more disorder is expected.

Boron

The relationship of Boron with cork-spot and bitter pit is unclear, and opinions among several authors are contradictory. There are some reports showing that boron treatments can reduce physiological disorders (40, 195, 250), while many others have shown them useless (35, 39, 399, 417). Unfortunately, low B is related to certain types of apple corking disorders, but their characteristics are distinct (usually) from Ca deficiencies.

Excessive levels of boron in fruits can cause earlier maturation and increased incidence of watercore and Jonathan spot at harvest, and increased incidence of internal breakdown and decay after storage (42, 49).

Boron deficiency may interact with calcium deficiency in the promotion of cork spot and bitter pit, since both reportedly could be reduced by boron application (48). According with Shear (332), this B effect is probably indirect since both soil and foliar application of boron can increase fruit calcium. The exact mechanism by which boron enhances calcium uptake is not understood but it has been shown to maintain more plant calcium in soluble forms (212). Cork spot of

'York Imperial' apples was reduced by increasing boron when calcium was in marginal supply (338). When calcium was deficient or merely adequate, increasing boron did not affect incidence of cork spot. Dixon et al (92) increased fruit calcium with foliar sprays of boron under orchard conditions. To be effective, B has to be applied at the time of greatest demand by the fruit calcium (333). Corkspot of 'Imperial' apples was reduced with boron sprays at full bloom and up to three weeks thereafter but later sprays were ineffective (151).

Increased movement of calcium went into leaves sprayed with boron (337). Boron may be required for the transport of Ca into fully developed tissues (109). The increase in Ca accumulation in leaves sprayed with B could be due to the stimulation of metabolic activities of the parenchyma cells of the leaf (336). The mobility of B from leaves to fruit is questionable (250, 257). One further possibility is that early B spray may increase fruit set and thus affect fruit Ca by having more, but smaller fruit.

According to Shear (328) there is variability between years and sites in responses to boron, he suggests that boron sprays are likely to be effective only when the availabilities of B or Ca or both, are restricted by soil content or moisture condition.

Other Nutrients

Jackson (162) found no effects of sprays containing vanadium, iron, strontium or tungsten on the incidence of bitter pit. Similarly, Martin et al (220) failed to obtain a response to sprays of

barium or strontium nitrate. 'Cox' apples treated with 36 minor elements to control the incidence of bitter pit had no beneficial influence (27). Spray application of sodium (251) and zinc (266) was reported to increase the development of bitter pit in apples. By contrast, Shear reported (332) that Zn spray, or soil applications may be able to reduce bitter pit (Magness et al, (207)).

Effect of Fruit Size, Crop Load and the Development of Bitter Pit, Internal Breakdown and Rots

Large fruit size has generally been associated with poor keeping quality (122). A number of studies have been made of the effect of size and in general, all of them agree in finding that large fruits are more susceptible to physiological disorders and rotting (109, 116, 216, 259, 320).

Bitter pit is particularly prevalent in large sized apples which are generally produced by lightly cropping trees, and this relationship has been demonstrated for many cultivars (109, 375). Large apples have also been found to be more susceptible to core breakdown than smaller ones (67, 137, 219).

According to Perring (269), analysis of individual apples and of bulk samples picked at different stages of development and from different orchards and trees receiving different orchard treatments, showed that the calcium concentration in the fruit was mainly dependent on fruit size, the larger apples having lower concentrations of calcium (404, 405). Ratkowsky and Martin (94) reported a positive association between bitter pit in apples and mean fruit

weight, and a negative relationship with calcium content. They emphasize the importance of including mean fruit weight as one of the variables when studying the relationships between disorders and mineral content. Generally, those conditions which lead to calcium deficiency tend to lead to increased fruit size per tree and greater susceptibility to bitter pit. Mean fruit size is dependent on cropping level, and hence, often relate to environmental factors (270). Frost before or during pollination can result in fewer, larger apples with subsequent lower calcium concentration, and cold weather during flowering can have a similar effect by inhibiting pollination (270). Conversely, heavier crops of small apples usually have high calcium concentration and less Ca deficiency disorders.

Rootstocks can influence fruit Ca concentration, and thus susceptibility to develop bitter pit. This effect appears to result mainly from their influence on mean mass per apple (270) and is related, in part, to fruit set.

The low viability of pollen from certain pollenizing cultivars was also suggested to lead to low fruit calcium and high incidence of pitting (265). Heavy rainfall or irrigation can result in large fruit size of low calcium concentration and increased occurrence of bitter pit and internal breakdown (270). Recently, Perring (271) reported different degrees of bitter pit susceptibility among different cultivars of apple based on their fruit calcium concentration and weight.

Some increase in fruit calcium content has resulted from treatments that reduced mean mass per apple, for example, the application of lime (270), late boron sprays (167), and hormone sprays (323) have had these effects.

Lidster et al (198) reported that large fruit require greater flesh calcium concentration than smaller fruit to prevent breakdown. Also large fruits would require greater calcium uptake from postharvest dips to give adequate control of breakdown. This was both confirmed by Lidster et al (197) where they demonstrated an inverse relationship between fruit weight and increase in calcium content in Spartan apples resulting from postharvest CaCl_2 dips. The greater increase which occurred in small fruit might be partly explained by geometry: small fruit has a greater surface to volume ratio than large fruit and hence the dip provides relatively more calcium for absorption into the tissue. Small fruit might also have thinner, less well developed cuticular wax than larger fruit.

Martin et al (209) showed that large fruits from light-cropping trees have larger cells and a higher rate of respiration per unit protein than smaller cells of fruits from heavy-cropping trees. They suggested that the more rapid senescence and susceptibility to storage disorder of the larger fruited, light-crop apples might be due to this higher rate of respiration.

Postharvest Quality Aspects of Apples and Pears

Introduction

Fruit quality is closely related to maturational processes, and is a crucial characteristic which determines acceptance by consumers. Many factors interact to determine fruit quality. A schematic for fruit quality could be outlined as follows:

Quality factors for fruits and vegetables:

- a - Appearance - Size; shape and defects
 - Color - Pigments
 - Phenolic compounds
- b - Texture - Firmness - Polysaccharides, cell turgidity
 - Toughness -Lignin formation
- c - Flavor - Taste - Sweetness - sugar, starch
 - Sourness - organic acids, minerals
 - Astringency - Tannins
 - Aroma - Volatile compounds
- d - Nutrition - Vitamins
 - Minerals
 - Proteins
 - Carbohydrates
 - Fat

These various factors interact and must be at proper balance to develop optimum dessert quality for the species or cultivar considered. Dessert quality of a ripe fruit may be related to

organic substances such as acids, sugars and cell wall materials maintained in the fruit during storage as well as to enzyme activities during ripening (286, 349). Knowledge of the composition of apple and pears is necessary to understand their role in fruit quality.

The approximate composition of apples and pears (on fresh weight basis) is shown in the following table.

As the fruits on the trees develop they undergo a series of biochemical changes (373). Wang et al (391) reported that maturation of pears results in a decline in organic acids and firmness and an increase in weight, soluble solids and soluble pectins.

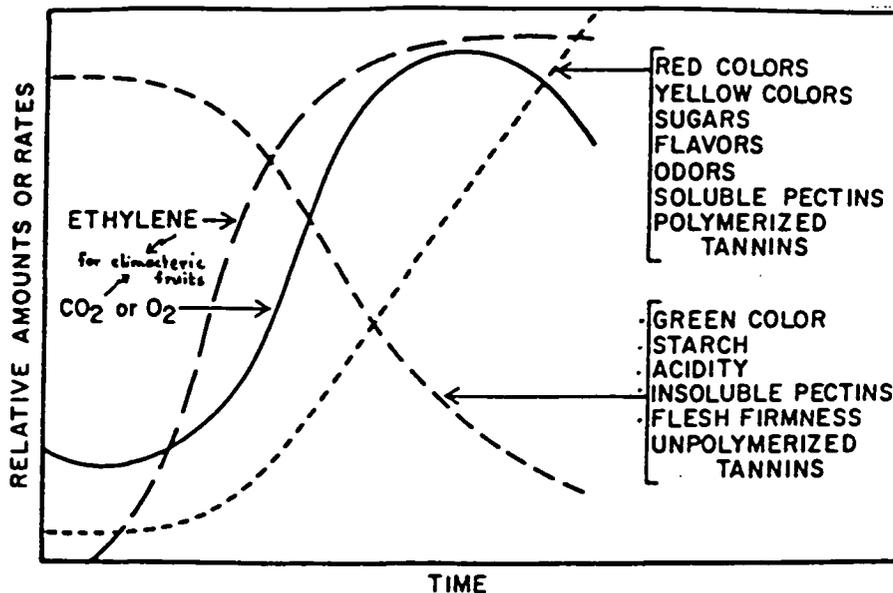
During ripening, a fruit passes through a series of overt changes in color, texture and flavor, indicating that compositional changes are taking place. Attainment of optimal eating quality of a fruit requires coordinating completion of such chemical changes. However, this can only be accomplished if the fruits are picked at the proper maturity; otherwise, immature or overmature fruits will have unsatisfactory quality, even after desirable ripening changes are completed (264).

A schematic for compositional changes associated with ripening of fruits is indicated on the following page.

According to Kupferman (189), fruit quality at harvest, harvest timing, storage regime, length of storage, and method of shipment will determine that fruit quality in the market. An error at any of these steps can adversely affect the entire program.

Approximate Composition of Apples and Pears
(on fresh weight basis)

Constituent	Apples	Pears
Water (%)	81-85	82-84
Total carbohydrates (%)	14.1-18.8	12.5-15.3
Fiber (%)	1.0	1.4
Total proteins (%)	0.2	0.7
Total fats (%)	0.6	0.4
Total sugars (%)	11.5-13.0	9.7-13.0
Reducing sugars (%)	6.0-8.5	7.0-10.5
Sucrose (%)	2.4-4.4	1.0-2.5
Fructose (%)	5.0-6.5	6.0-8.0
Glucose (%)	0.7-1.8	0.8-2.4
Titratable acidity (%)	0.40-0.50	0.25-0.35
Main organic acids	<u>Malic</u>	<u>Malic</u> or <u>Citric</u>
Pigments		
Anthocyanins (mg/100g)	0.1-21.6 (pell)	0-? (red skin cultivars)
Carotenoids (mg/100g)	5.5-12.6	0-0.6
-carotene (mg/100g)	0.2-7.6	0-0.1
Vitamin A value (IU)	90	20
Ascorbic acid (mg/100g)	2-10	2-6
Total phenolics (%)	0.1-1.0	0.2-0.5
Volatiles of organoleptic significance	<u>Hexanol</u> , <u>2-Hexenal</u> (green)	<u>Methyl-trans-2-cis-4-decadienoate</u> (pear flavor)
	Ethyl-2-methylbutyrate (ripe)	



By A. A. Kader, U.C. Davis, 1982.

Fruit Firmness

According to Bramlage (45) apple firmness is used worldwide as a measure of ripeness and condition of the fruit. It is used for 'Imperatore' and 'Stark Delicious' apples as one quality index (366). However, several authors disagree about its validity in apples (93, 290), at least for use as a dominant quality index.

Flesh firmness is a very useful index for Anjou pears and they should be harvested at optimum maturity, 63 N flesh firmness, to provide good dessert quality after long term storage (244). Hansen and Mellenthin (143) suggested a flesh firmness between 67 and 57 N as an optimum maturity index for D'anjou pears. D'anjou pears are

considered to be at end of commercial storage life when they soften to a firmness of 45 N (283). Conference pears are at optimum maturity with firmness values of 49 to 65 N (121), and according to Williams (412), Bosc at 58 N, Bartlet at 76 N, Kieffer at 62 N and Seckel at 71 N.

The softening of fruits is caused largely by the breakdown of insoluble protopectin into soluble pectin and there may be some contribution by hydrolysis of starch or fats. Lignin synthesis in some vegetable fruits may also adversely affect texture (264).

Color

The ground color of the skin of fruits is determined by the concentrations of chlorophyll and carotenoids (133). As the fruit matures, chlorophyll begins to disappear and carotenoids contribute more to the color (122, 133). Therefore color changes may be due to either degradative or synthetic processes, or both.

In most varieties of pears and for some apples, i.e. Newtown apples, skin color varies from deep green to yellow. For certain cultivars of apples, red coloration is an important criterion of quality (324). Skin color has been suggested as an index of optimum maturity at harvest associated with dessert quality; as it is used for Gravenstein (134) and Golden Delicious (366) apples.

The green color retention of certain cultivars of apples and pears during and after storage has been suggested to be an indication of the fruit's quality condition (47, 125, 315, 384).

The orchard's mineral nutrition is known to affect fruit color (104, 105). High nitrogen has been associated with poor red color in certain cultivars (28, 41, 206). Increased potassium fertilizer was found to offset the adverse effect of high nitrogen on red fruit color (394). Growing temperature also influences color. Warm night temperatures before harvest was associated with deep green color of Golden Delicious (358).

Soluble Solids

Soluble solids measurements, usually determined by a hand held refractometer, largely represent free sugars as the major solutes. Other constituents, such as dissolved salts, organic acids, etc., also contribute to the soluble solids value, but generally sugars are the major contributors. The optimum eating quality which is characteristic of a fruit variety is largely determined by its sugar-acid balance.

Soluble solids increase as fruits develop on the tree. Chen (68) studied harvest date on ripening capacity and post-harvest life of Anjou pears, and reported that fruits picked at the time of commercial harvest (September 4) had 12.1% SS content which increased to about 13.1% if left on the tree for an additional 21 days. He also reported that usually there is little change of SS during storage of pear fruits. In apples it is known that soluble solids vary greatly and are dependent upon moisture supply and climatic conditions during development (140). The time of bloom and the

subsequent pollination is suggested to affect the soluble solids content, the earlier the bloom the higher will be the SS (357). A highly positive correlation exists between apple fruit soluble solids: acid ratio and days from full boom (145).

Generally SS increase as fruit ripening proceeds and can be influenced by the storage regime. The average amount of SS in unripened fruit was reported to be 11.5% irrespective of the storage condition. However SS of 1% O₂-stored fruits increased significantly to 12.5% upon ripening while SS of air-stored fruits increased only slightly to 11.7% (70). The increase in SS of ripened fruit could be due to conversion of starch to soluble sugars and/or a release of cell-wall-bound neutral sugars (7, 70, 136, 360). Mellenthin and Wang (245) reported that soluble solids in Anjou pear fruits vary with season, location and preharvest temperatures. It is suggested not to be a reliable measurement of fruit maturity (68). However, soluble solids have been used as common indicators of fruit quality and storage life (63, 68, 125, 245, 286) of certain types of fruit, especially citrus.

Titrateable Acidity

Ulrich (373) suggested that most of the fruit acids are localized in the vacuole of the pulp cells. Usually, fruit acids decrease from the time of fruit set to harvest time (145). According to Chen and Mellenthin (68) TA shows little relationship with different harvest dates, and that it varies in the fruit with season,

location and preharvest temperature (245). They discourage the use of TA as a reliable measurement of pear fruit maturity.

Titrateable acids are used up more rapidly than the sugars during storage and if acid losses are too great, the apple fruit can taste overly sweet (269). Mellenthin and Wang (269) stated that Anjou pears with high acid and sugar content at harvest have better post-harvest quality than those with low acid and sugar content.

Measurement of 7 identified organic acids in Anjou pear fruits, revealed that malic acid is the predominant one (72), and that the flavor differences of ripened fruit are suggested to be at least partially due to quantitative differences among those 7 organic acids.

Retention of acids in fruits is nearly always associated with prolonged storage life (186, 196). Titrateable acidity is known to decrease during fruit storage (68, 71, 105, 269).

Different regimes of postharvest storage can influence the final amount of TA in fruits after storage. Mellenthin et al (244) stored Anjou pears for 3 months at different O₂ concentrations. The changes in titrateable acids were as follows. Fruit stored at 0.5% O₂ maintained their TA with little change. Fruit stored at 1.0 - 5.0% O₂ and those stored at 2.5% O₂ plus 1.0% CO₂, gradually decreased in TA. Fruits stored in air underwent a rapid decrease in TA from 270 mg to 170 mg/100 ml of juice. The rate of loss in total and some individual organic acids has also been reported to be retarded in apples (186) and pears (196) during controlled atmosphere storage.

Postharvest Physiology, Cold Requirement and Ethylene Biosynthesis
in D'Anjou Pears

Some cultivars of pear fruits, particularly the winter types (68, 69, 392) have a special cold or chilling requirement before ethylene production begins and normal ripening can take place. Ripening resistance in immature Bartlett pears can be abolished by storage at -4.4°C (203). Fully mature fruits need a short period of cold treatment before appreciable amounts of ethylene production occurs, which consequently leads to ripening (69, 203, 392). The cultivar Passe Crassane requires a period of storage at 4°C to initiate ripening once transferred to room temperature (141). According to Sfakiostakis and Dilley (317), preclimacteric 'Bosc' pears held continuously at 20°C produced ethylene at a very low rate and resisted ripening for 12 days. However when held at 5 or 10°C for 7 days, ethylene production at 20°C began to increase almost immediately resulting in rapid and uniform ripening. The longer the pears were held at 5°C , up to 6 days, the greater was their ethylene production capacity at 23°C .

The ripening capacity of pears after varying the period of cold storage can be determined by the production of external ethylene and CO_2 at 20°C . If the external ethylene production is below measurable levels, there will be no climacteric rise in respiration and the pears fail to ripen (391). The length of time in low temperature storage that Anjou pears require to develop ripening capacity is different from season to season. Ripening of Anjou fruits has been

reported to occur right at normal picking time, without any chilling treatment. After 16 days at 20°C those fruits produced about 11 ml C₂H₄/Kg-hr (391). However this cultivar of pear has been cited to require at least 50 days at -1.1°C to develop ripening capacity (68, 72), although sometimes only 30 days (69). In another study, ethylene production on transfer to 20°C was not detectable until after 60 days of storage at -1.1°C (71). The number of days at -1.1°C necessary for Anjou pears to produce detectable amounts of internal ethylene is different for different growing locations (365).

The temperature during fruit development might also have an effect on chilling requirements of pears. Bartlett pears undergo premature ripening, while still attached on the tree when exposed to cool night temperatures (203, 392). Exposure to 18°C during the day and 8°C during nights induced premature ethylene production and climacteric rise in respiration in Bartlett pears, whereas 24°C day and 16°C night did produce not effect (392).

Exposure to low temperature storage thus activates the fruit's ability to produce ethylene. Cool temperatures induce the fruits to produce ethylene by temperature dependent metabolic reactions (317).

Ethylene, considered to be a plant hormone, is involved and is produced in association with ripening of fruits and senescence of plant tissues (2, 61, 236, 286, 298).

Initiation of ripening activities of climacteric fruits is controlled by the threshold level of internal ethylene concentration (68, 285, 299, 392). When the internal ethylene reaches 1.5-2.0 ppm,

Anjou fruits are capable of ripening normally at 20°C (68). The threshold values are different for different pear cultivars, e.g. for Bosc it is about 0.2-0.3 ppm which occurs after 10 days of storage at -1.1°C (69). The biosynthesis of ethylene in both cultivars was induced by low storage temperature (-1.1). Different pear cultivars have different chilling requirements before they ripen (68, 69, 71, 283, 317, 392). Bosc pears have less chilling requirement need than Anjou (69).

Maturity stage greatly affects the chilling requirement of Anjou pears. Premature pears ripen slower than fully mature fruits when treated with exogenous ethylene (391). Late harvested (i.e. late maturity) Anjou pears develop the capacity to ripen much earlier than fruit harvested at optimum maturity (68). However, winter pears harvested at an advanced stage of maturity tend to develop coarser texture and have shorter storage life (68, 143).

The chilling requirement of pears can be overcome by treating the fruits with ethylene (203, 389). Anjou pears, which had not had their chilling requirement satisfied, when treated with exogenous ethylene attained full ripeness. However, this occurred without a concomitant change in respiratory activity (142).

The concentration of applied ethylene as well as the time required to initiate a response is different for respiration and ethylene production (391). Anjou pears of different maturities were treated with different concentrations of ethylene. The first response found was the development of sharp peaks in respiration on

the first day at 20°C when treated with ethylene concentrations above 0.5 ppm (391). Peak heights were in proportion to ethylene and decreased with increase in maturity. This early increase in respiratory activity in response to applied ethylene was observed previously in Anjou pears (142, 389), in apples (285) and in cantaloupes (239). Respiration of the fruit at harvest is responsive to ethylene concentrations considered below the minimum required for later stimulation of the climacteric rise (142, 391).

Therefore, Anjou pears must be stored at low temperature or be treated with ethylene, before they ripen. However, the mechanism of low-temperature induction of ripening is still unknown in pears. One approach to understand this low temperature-induction of ripening in winter pears is via the biosynthesis of ethylene.

1-Aminocyclopropane-1-carboxylic acid (ACC) is an intermediate in ethylene biosynthesis (5, 205). The pathway, Methionine \rightarrow SAM \rightarrow ACC \rightarrow ethylene has been proposed and generally accepted to occur in plant tissues (422). The regulation of ethylene biosynthesis has been studied (421). The key enzyme in this biosynthetic pathway has been indicated to be ACC synthase (36, 421, 424). In tissues producing ethylene at a rapid rate, ACC formation precedes or parallels ethylene evolution (13, 44, 155, 425). However during cold storage of Bosc and Anjou pears, the increase in ACC content was roughly parallel to the increase in internal ethylene (71). ACC synthase is rapidly induced in response to various factors (37, 159, 177, 187).

Wang and Adams (387) subjected cucumber fruit to chilling and noticed an increased synthesis of ACC. However, the next step in the conversion of ACC to C_2H_4 was inhibited while the chilled cucumbers were kept at cold temperature (388), which later increased when there was an increase in the temperature. Temperatures below $12^{\circ}C$ were shown to inhibit this conversion in eggplant fruits (1). In tomatoes and apples this sensitivity of ACC conversion to ethylene by low temperature was also demonstrated (234).

The length of time fruits remain in cold storage is suggested to affect either the ACC synthase activity and, consequently, formation of ACC or the conversion of ACC to ethylene. Pear flesh discs were unable to convert exogenous ACC to ethylene after 21 days of cold storage, but were able to after 41 days (240). Endogenous ACC in Anjou pears kept at $-1.1^{\circ}C$ was not detected when measured weekly from the time of harvest to eight weeks storage (365). For Bartlett, Bosc and Comice pears, the ACC concentration decreased continuously upon transfer to $20^{\circ}C$, and did not parallel ethylene evolution (365).

In apple fruit tissues, ACC conversion to ethylene has also been demonstrated to be temperature sensitive, and the site is believed to be cell wall, or membrane bound (124, 199). Any change induced in the cell wall or membranes is suggested to affect such conversion. Cold, osmotic shock and surface active agents inhibit ACC \rightarrow C_2H_4 conversion, as do temperatures above $29^{\circ}C$ or below $12^{\circ}C$ (12). In many fruits and vegetables, their conversion seems also to be affected by short chain fatty acids, which also affect membrane

functions (158). When Morning Glory flower rib segments were treated with exogenous ethylene, an increase in membrane permeability was verified (178), which adds support for the hypothesis that breakdown of cellular compartmentalization allows mixing of ethylene producing components. This likely is the basis for ethylene-induced ethylene synthesis (178). Perhaps there is a release or activation of part of the ethylene producing system on the cell wall of membrane (240). In tobacco leaf discs, a fungal cell wall degrading mixture promotes formation of ACC and synthesis of ethylene (11).

CHAPTER III

DEVELOPMENT OF PREDICTIVE MODELS FOR THE PHYSIOLOGICAL DISORDERS
BITTER PIT AND INTERNAL BREAKDOWN ON NEWTOWN APPLES
BY PREHARVEST FRUIT MINERAL ANALYSIS

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Abstract

In 1982 and 1983, 20 and 10 orchards respectively in Hood River, Oregon, were sampled and analyzed for fruit Ca, N, K, Mg, P, B, Mn, Fe, S, Zn, Na, and Se. The orchards were sampled approximately 60, 40, and 20 days before harvest as well as at harvest. Fruits were evaluated for bitter pit at harvest and 10 months in storage, and for internal breakdown after 10 months in storage.

We have been attempting to predict the occurrence of bitter pit and internal breakdown far ahead of harvest by developing regression equations, associating the mineral nutrient status of the fruits and the occurrence of the physiological disorders. Among the variables tested, Ca, fruit Wt, and Wt/Ca ratio accounted for the most consistent predictive factors. Calcium concentration and fruit Wt varied from season to season but the shape

of the curve was about the same. Bitter pit occurrence in the area has varied from 1% up to 23% every year, and internal breakdown from 0.5 up to 12%. Most of the bitter pit incidence occurred during fruit storage. Bitter pit could be predicted 20 days before commercial harvest with about 70% probability of certainty. It appeared that internal breakdown could be predicted from fruit sampled 60 days before harvest.

Introduction

Bitter pit (BP) is a physiological disorder of apples which disfigures the fruit surface destroying its market value. The quality conscious market demands that apples have a BP incidence well below 5% (9). Losses from 1 up to 40% have been reported due to this disorder (8,15,47). It is not a new disorder, and was first identified in 1869 (16). It has become of increasing concern because several modern orchard management practices tend to favor its occurrence.

Internal breakdown (IB) is also a physiological disorder responsible for considerable postharvest losses (38,47). Both disorders have been associated with nutritional levels of P, K, Ca, and Mg (5,11,14,35,46). Ca occupies a prominent position in fruit nutrition, and the effects of other elements in these disorders is often through their interaction with Ca (4). It has been established that the occurrence of BP (1,13,14,44) and IB (3,5,30,38,39,47) in apples is associated with a localized Ca deficiency. Because of the well-known nutrition relationships to BP and IB, fruit analysis constitutes an integral part of BP and IB research (5,11,24,30,35,46). The purpose of fruit analysis is to find concentration norms for ensuring BP and IB free apples. Leaf and fruit samples (5,7,9,10,41) have been used in attempts to find the best correlations of mineral elements to these disorders. Fruit analysis has been much more useful than leaf analysis in evaluating storage disorders (5,9,40,47). IB has been negatively correlated with Ca (20,30,39,47) and positively correlated to K

and Mg (39). BP has been negatively correlated with Ca (5,7,15,41,47).

Bitter pit can be detected at harvest but greater incidence becomes evident during storage. Based on the correlations between IB, BP and nutritional elements, attempts have been made to develop a predictive model for these disorders (5,9,14,20,47) during storage. There are different approaches to BP predictions. These include leaf and fruit Ca analyses and rapid induction of BP in fruit after harvest by the method of Ginsburg and Bangerth as used by Eksteen (9). However, limitations exist and IB cannot be detected by the latter method. Some predictive models have dealt with a very small sample from each orchard and concentrated mainly in the last 15 days prior to harvest time, which does not leave sufficient time to cope with the problem other than to help identify susceptible lots of fruit. Incidences of these disorders vary with the fruit cultivar, growing location and growing season, and thus may require varying models. So a relatively simple method is needed for estimating seasonal BP and IB potential, enabling producers and packers to plan their market strategies. Knowledge of which orchards are likely to develop IB and BP and to what extent can help packing houses program short, medium, or long term storage of fruit, as well as estimate the fruit's quality at the end of a given storage period. If the relative incidence of disorders could be predicted well before harvest, it could reduce the rush in fruit sampling and analysis and would permit time for

corrective treatments such as orchard sprays or postharvest dips to be applied.

Therefore, the main objective of the work is to provide a way of predicting IB and BP based on preharvest fruit mineral analysis for Newtown apples grown in the Hood River area of Oregon.

Materials and Methods

Twenty commercial Newtown apple orchards in the Hood River area of Oregon were sampled in 1982 and 10 in 1983. Ten typical fruits per tree were randomly sampled from the periphery of each of three trees per orchard on July 19, August 10, 29, and September 19, the commercial maturity in 1982 and July 20, August 10, August 29, and September 19, in the 1983 season.

On the final harvest date 1 box of fruits (Ca 100 fruits) were picked from each tree. Fruits were treated with Benlate and diphenylamine and stored in 20 Kg cardboard boxes with perforated polyliners at 1°C and 96% RH for 8 to 10 months.

Mineral analysis

All harvested samples during fruit development were sealed in plastic bags (31,49) and placed in cold storage until analyzed. All fruits were weighed, washed with distilled water and allowed to air dry on paper towels (28,34) just before cutting sections for analysis. Pairs of opposite quarters were cut longitudinally (27,28,33,34) from 6 fruits per sample and composited. Seeds and stems were removed to prevent errors in analytical results (28,33,52). Samples of 18 fruit slices were weighed and blended with an equal amount of deionized water in a blender for 2 min. The mixture was further homogenized for 1 min to make a fine suspension (26,27,31,32,34) which is referred to as the fruit water suspension.

In 1982 calcium was extracted by the method suggested by Perring (32) with minor modifications: ten ml of fruit water

suspension was transferred into a 50 ml Erlenmeyer flask and 10 ml concentrated 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 cooled digested contents were then filtered through Whatman 41 paper. The filtrate was diluted to 100 ml with deionized water and strontium chloride was added to reduce interferences to a final concentration of 3% SrCl_2 to all extracts. Calcium was determined by Atomic Absorption spectrophotometry (Perkin Elmer, Model 303) at 212 nm.

1983 samples were prepared as in 1982 up to the final digestion with HCl. Thereafter some modifications were introduced. The digested contents were filtered and 4.5 ml of the solution taken for analysis, Ca, P, K, Mg, Mn, B, Zn, Fe, Na, S, and Se were determined by an Inductively Coupled Argon Plasma Spectrometer (Jarrell Ash ICAP 9000).

Five ml of the fruit water suspension were dried at 70°C. Digestion and automated N determinations were made with a Technicon-autoanalyzer.

Bitter pit and internal breakdown rating

The incidence of BP was evaluated at harvest and after 10 and 8 months in 1982 and 1983 respectively. After storage all the fruits were peeled and the internal BP determined. For total incidence of BP, harvest BP was added to storage BP.

The incidence of IB was determined at the end of storage on peeled fruits.

Simple linear correlations between the incidence of BP and IB and mineral concentrations of fruits were calculated on the Cyber computer, using the SIPS program. Multiple correlations and the simple linear correlations were used to select the variables which were most closely correlated with the incidence of harvest, storage, and total BP as well as the incidence of IB.

Results

Scatter diagrams (not shown) relating mineral elements with the physiological disorders BP and IB in apple fruits were essentially linear and therefore only linear correlations were calculated.

Bitter pit

There was a marked difference in fruit Ca concentration from samples of 1982 and 1983 (Fig. 3.1). The higher the fruit Ca the lower the incidence of bitter pit. Fruits of 1982 season were higher in Ca and had lower incidence of the disorder. The average fruit Ca concentration at the time of fruit harvesting was from 1.9 to 3.6 and from 0.15 to 2.6 mg/100 g of fresh weight in 1982 and 1983 respectively. The incidence of total bitter pit in both years ranged from 2.0 to 12.9% and from 2.0 to 22.5% respectively. However the distribution of the data points follow the same pattern in both years.

Simple linear correlation coefficients (r-values) of mineral elements in relation to bitter pit of Newtown apple fruits are shown in Tables 3.1, 3.2A, and 3.2B. These tables are the results of seasonal fruit mineral analysis of all orchards sampled in 1982 and 1983. BP incidence was negatively correlated with Ca at harvest, after 8 months in storage and with total BP in both years. These correlations were highly significant in both years for most of the sampling dates. The correlations between Ca and the incidence of BP increases and becomes more significant, as the sampling date approaches final harvest date for 1983. The highest

correlation ($r = -.83$) was found for the September 6 sampling date for 1982 (Table 3.1). The incidence of BP during storage was higher than at harvest. The best correlations were obtained when plotting total bitter pit with fruit Ca levels in 1983 (Table 3.2A).

The incidence of BP after storage was negatively correlated with N in 1982 but positively correlated in 1983. This correlation was not significant at harvest in 1982 nor for the sampling dates in relation to after storage BP in 1983. Generally the correlations with N decrease as sampling approaches final harvest in both years.

Phosphorus was negatively correlated with BP and highly significant except in July 20, 1983 for harvest BP and in August 10, 1983 for storage BP when there was a nonsignificant correlation. The correlation coefficients increased as sampling approached August 29, and decreased thereafter. The highest correlation values are obtained with the disorders after storage.

Potassium was positively and highly significantly correlated to BP for the July 20, August 29, and September 19 fruit samplings. These correlations were slightly stronger when sampled at 60 days before harvest, decreasing thereafter toward the 3rd sampling date but again then increased even more at final harvest time. The strongest K relationship occurred with total BP.

Several other elements; Mg, Mn, Fe, Se, S, Zn, and Na; showed some correlation to BP, but typically only on the late August

and/or early September sampling date and these did not exhibit any consistency.

BP was positively correlated with N/Ca ratios in 1982 and 1983 (Tables 3.1 and 3.2B). In both years the strongest correlations were obtained approximately 20 days before harvest, where it was highly significantly related to the disorder except with storage BP. The correlation values increased as sampling approached the 3rd sampling date.

The P/Ca ratios (Table 3.2B) correlated positively and highly significantly with BP mainly at the August 29, 1983 and September 19, 1983 sampling dates, and was strongest at 20 days before harvest, August 29. The ratio showed increased r-values as compared with P alone, but lower values as compared to Ca, thus Ca was still the dominant factor.

The ratio K/Ca was also positively correlated to BP. For some sampling dates the ratio improved the r-values as compared to K or Ca individually, whereas for other dates there was no advantage of the K/Ca ratio. The same was true for Mg/Ca ratios. To conjugate some of the beneficial effects of K/Ca and Mg/Ca ratios, a $(K + Mg)/Ca$ ratio was calculated. There was a positive correlation expressed sometimes by a highly significant relationship with BP for the 4 sampling dates. BP correlated negatively with $Ca/(K + Mg)$. The use of these ratios raised the significant relation of K and Mg. The strongest correlation was for the September 19 sampling date.

Fruits sampled in 1983 were larger than those of 1982 (Fig. 3.2), ranging from 143 to 235 g in 1982 and from 160 to 278 g in 1983. The greatest incidence of total bitter pit was in the larger fruits.

Fruit weight always correlated positively and highly significantly with BP in both years. The correlation was the strongest for the fruit sampling approximately 20 days before harvest in 1982 and 1983. Since fruit weight was demonstrated to be strongly correlated to BP, as was Ca, the ratio Wt/Ca was calculated. A positive relationship existed between Wt/Ca with BP in both years. These correlations were always highly significant. The strongest values were obtained from fruit sampling 20 days before harvest in 1982 and 1983.

Multiple correlation between bitter pit and mineral elements

Results of multiple linear correlations of mineral elements in relation to BP of Newtown apple fruits are expressed in coefficient of determinations (r^2 values), as shown in tables 3.3 and 3.4.

Multiple linear correlations between mineral elements and the incidence of BP at harvest, BP after 8 or 10 months in storage and total BP show (Tables 3 and 4) that Ca and fruit weight are the most closely related variables to the incidence of this physiological disorder. The equation that best fits the model for prediction of harvest BP:

$$\text{BP at harvest} = 2.1253 - 1.4273 \text{ Ca} + 0.0218 \text{ Wt}$$

can be used for predictions of BP at the time of commercial harvest by knowing the fruit Ca levels and fruit weight 20 days before harvest which together explains 77% of the variation in the incidence of BP.

The fitted equation to predict storage BP was obtained with fruit samplings at either 20 or 40 days before commercial fruit harvest. However for the total incidence of BP, sampling fruits 20 days before harvest provided the best fitted equation:

$$\text{Total BP} = 1.2084 - 3.1682 \text{ Ca} + 0.0822 \text{ Wt}$$

Where Ca and fruit weight accounted for 71% of the variation in total BP incidence.

Multiple linear regressions between BP and mineral elements in 1983, shows that Ca and fruit weight are again the most closely correlated variables to the incidence of BP.

Based on the coefficients of determination, harvest BP could be predicted by knowing fruit levels of K, N, and Ca and the ratio fruit Wt/Ca as early as 60 days before harvest (mid-July). These parameters accounted for 66% of the variation in BP incidence.

The coefficient of determination ($R^2 = .74$) indicated that by knowing fruit wt and fruit Ca concentration 20 days before harvest the incidence of BP after 8 months in storage can be predicted fairly well.

Most of the BP develops during fruit storage, the incidence at the end of storage is far greater than at harvest time. Equal coefficients of determination on August 29 and July 20, indicates

the possible use of either one of these equations to predict total BP if their corresponding parameters are known.

Internal breakdown

In 1982 the incidence of IB in Newtown apples ranged from 0.2 to 6.5% while in 1983 the fruits had a considerably larger incidence, ranging from 3.0 to 11.0% (Fig. 3.3). The higher the fruit Ca content, the lower the incidence of IB.

Simple linear correlations between mineral elements and IB are shown in Tables 3.5A and 3.5B. Ca always correlated negatively and significantly with IB. For modelling purposes, the 1st sampling date represented by the highly significant correlation in 1982 demonstrated considerable potential. In 1983 highly significant correlations were also found. The correlation coefficient value ($r = -.82$) for the August 29 sampling expresses the highly significant relationship between IB disorder and Ca and the potential for the development of a predictive model. In 1983 as the sampling dates approached final harvest, there was an increase in the correlation values.

Nitrogen did not always correlate significantly with IB disorder, since only for the last sampling in 1983 was there a positive and highly significant relationship.

Phosphorus correlated negatively and highly significantly with IB with increased r-values up to the 3rd sampling but decreased thereafter. K related positively and highly significantly with IB, especially 20 days before harvest. Mg only correlated

with this disorder 40 and 20 days before harvest. These correlations were highly significant.

Several other elements, Mn, Fe, S, B, Zn, Na, and Se showed some correlations to IB, but typically only for the late August and early September sampling date and were not consistent.

Fruit weight always correlated positively and highly significantly with IB in both years. With the exception of the first sampling date in 1982 and 1983, the correlation coefficient increases as sampling approached final sampling date. The ratio Wt/Ca expressed also a positive and highly significant correlation with the incidence of IB in Newtown apple fruits for both seasons.

The use of N/Ca ratio improved the statistical significance of the correlation between IB and N alone, although in 1982 the positive significance of N/Ca was not consistent throughout all sampling periods. However, for the 1983 season, N/Ca for all sampling dates exhibited positive correlations with the disorder. P/Ca and Mg/Ca ratios related positively and highly significantly with IB for the last two sampling dates. The ratio K/Ca improved the inconsistent significance of the correlation of K alone in relation to IB. The positive and highly significant correlation values increased up to August 29, but decreased thereafter. Utilizing a $\text{Ca}/(\text{K} + \text{Mg})$ ratio, a negative and highly significant correlation existed, whose r-values increased throughout the sampling dates towards September 19.

Multiple correlation between IB and mineral elements

Multiple linear regressions between IB and mineral elements in 1982 and 1983, show that Ca and fruit weight were also the most closely related variables to the incidence of this storage disorder (Table 3.6). The high coefficients of determination $r = .82$ and $r = .85$ indicates the possibility of using fruit samples from 9/06 and 7/19 to predict the occurrence of IB by using the corresponding formulas for the season of 1983. In 1982 despite the fact that the r^2 values were lower, still IB could be predicted by fruit sampling as early as July 19.

Testing the model

To test the model, fruit samples taken 20 days before harvest in 1982 were entered in the model and the predicted BP at harvest as well as the total BP was compared with the actual harvest BP and total BP for the same season (Table 3.7). The correlation coefficient between actual and predicted BP was $r = .84$ and $r = .85$, respectively.

Harvest and total BP were also checked (Table 3.8) against the actual values by entering the data of 1983. However, fruit sampled from 60 days before harvest was used. The correlation coefficient between actual and predicted BP was $r = .81$ and $r = .85$, respectively. However a more accurate comparative test was carried out, by comparing the model of 1982 (Tables 3.3 and 3.9):

$$\text{Total BP} = 1.2084 - 3.1682 \text{ Ca} + 0.0822 \text{ Wt}$$

with the model of 1983 (Tables 4 and 9):

$$\text{Total BP} = -7.3179 + 0.0756 \text{ Wt} + 0.0177 \text{ Wt/Ca}$$

For both years fruit sampled August 29 and September 6 (third sampling) were used. Both equations had comparable coefficients of determination $r^2 = .71$ and $r^2 = .73$, respectively.

The best equation of 1983 was that generated with data 60 days before harvest. However since this equation also involved K and Mg values, which were not analyzed in 1982, it could thus not be compared for both seasons.

Therefore fruit Ca and fruit wt data of 1982 were entered in the equation of 1983 and the predicted total BP in 1982 compared with the actual total BP in the same season and vice-versa for the data of 1983. The values $r^2 = 0.62$ and $r_2 = 0.65$ for 1982 and 1983 respectively were obtained. The results from Table 3.9 and Figs. 3.4 and 3.5, clearly demonstrate that using either the model of 1982 or 1983 an accuracy over 70% is obtained in the prediction values of the incidence of BP.

The models for prediction of IB based on 1982 and 1983 could not be compared since the 1983 equation involved parameters which were not analyzed in 1982 (Table 3.6).

Discussion

Simple linear regressions of BP and IB in relation to fruit Ca concentration at all four sampling dates in both years were all significant and negatively correlated. The significance and consistency of the Ca relationship with BP and IB as compared with other mineral elements implies that Ca deficiency in the fruit is the major factor in the incidence of BP at harvest and after storage as well as IB in Newtown apples. The higher the fruit Ca the lower the incidence of BP and IB, as reported before (7,10,12,20,30,39,40). Seasonal fruit Ca analysis suggests also that the relation between Ca concentration during growth and harvest BP incidence and BP and IB after storage can be used as a component to develop a predictive model. These results agree with previous reports (1,13,14,30,39,44,47). Our results showed that N may also be related to the occurrence of these disorders in apple fruits, but the results are not consistent between years and sampling dates. However, when the ratio N/Ca was computed the fluctuations were reduced and more significant correlations were obtained than for N alone, thus reflecting a degree of balance between Ca and N. The effect of N on these disorders is mainly indirectly through the interaction of fruit size and Ca and may also relate to the vegetative competition component. Our results agree with previous reports on apples (13,18,43) and pears (36,37). A high incidence of BP has been associated with high fruit N (13,18,25,36) and low fruit Ca (7,10,22,40,56).

Samples with decreasing levels of fruit P developed more BP and IB, with significant correlations at $p < .05$ and $p < .01$.

Our findings agree with other results (4,17,23), which showed highly significant negative correlations between BP in apples and P, but contrast with some reports showing a positive correlation between BP and P (5,42). The possible link between low fruit P and predisposition to IB during storage has also been reported (5,29,48). Since P is negatively correlated with BP and IB its concentration should be kept at high levels in the fruits. The ratio P/Ca is less correlated with BP and IB than Ca or P alone, suggesting non-interaction between these 2 elements.

The positive and significant correlation between K and BP and IB suggests that high fruit K may develop higher incidence of these fruit disorders. The K effect may be through an antagonistic interaction for Ca uptake by the roots, as suggested previously (5,11,23,46). A high K/Ca ratio in the fruit seems to favor the development of BP and IB (4,6,12). Depending on the sampling date, this K/Ca ratio correlates better with disorders than K or Ca alone. This also reflects the antagonism hypothesis. Although Mg was less significantly related with BP and IB for the August 29 and September 19 sampling dates than some other nutrients, it does positively correlate to these disorders. Fruit with higher Mg concentration is more likely to develop BP and IB than those with medium to low Mg concentration. Evidence for (11,19,55) and against (53,54) involvement of Mg in BP and IB of apples has been reported. The ratio Mg/Ca improved the correlation with the

disorders, demonstrating a possible interaction of the antagonistic kind between Mg and Ca in the fruit as well as in the whole tree.

IB and BP were also correlated with $(K + Mg)/Ca$ ratios.

Apple fruits with higher levels of Mg and K and lower levels of Ca are most likely to develop disorders.

It has been suggested (2) 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. It was also suggested that K and Mg may occupy some nonspecific Ca attachment site and thus antagonize the function of Ca in the membrane. The most serious effect on Ca is caused by Mg. Large portions of Ca in the membrane are exchanged by Mg, K, or H^+ ; and this increases the permeability and fragility of the membranes (1,2). Calcium deficiency is relative and conditioned by the concentration of Mg, K, and N. The relative Ca deficiency may precondition the replacement of Ca in the membrane by Mg, K, and H^+ (1).

The always positive and highly significant correlations between fruit size and IB and BP demonstrate that large size fruits are most likely to develop BP and IB than smaller fruits. Related factors include a dilution effect and/or preferential Ca movement to new meristematic tissues which could deprive the fruits of Ca, or alter the balance of this nutrient with others. Since Ca concentration in the fruit is mainly dependent on fruit size, larger apples having lower concentration of Ca agrees with other

reports (10,50,51). Wilkinson and Perring (51) suggested that the final level of Ca in the fruit is probably determined early in fruit development, this is basically substantiated by the present models.

Since the introduced ratio Ca/Wt (or Wt/Ca) was always highly significant and strongly correlated with IB and BP and was demonstrated to be more significantly correlated than either Wt or Ca alone for most sampling dates, this indicates the interdependence of fruit Ca and fruit weight.

The present data shows that a correct balance of macro and micronutrients in the fruit is very important to ensure freedom from BP at harvest and IB and BP during storage of apples. To develop a predictive model, the main emphasis was to find variables most closely correlated with disorders substantially before harvest. Multiple regression analyses of fruit Ca and fresh wt were revealed to be the most directly correlated parameters in 1982, with the best association at the September 6 sampling date, 20 days before final harvest, for harvest BP. The same variables and sample times were also found to be the most highly correlated with total BP in 1983.

One can develop prediction of harvest, storage and total BP by using any equation from any of the sampling dates. If total BP is considered, a result of adding both harvest + storage BP together, better accuracy is obtained with the prediction. Therefore the best equation for 1982 was:

$$\text{Total BP} = 1.2084 - 3.1682 \text{ Ca} + 0.0822 \text{ Wt.}$$

and for 1983 season:

$$\text{Total BP} = 7.3179 + 0.0756 \text{ Wt.} + 0.0177 \text{ Wt./Ca}$$

Based on these two equations for 1982 and 1983, the total incidence of BP in each year could be predicted with more than 70% accuracy 20 days before the commercial harvest. However, even at 60 days before harvest (July 19) it was possible to predict total BP with the same accuracy, once fruit wt., and the ratio Ca/(K + Mg) and Ca/Wt. are also used.

Internal breakdown can also be predicted from fruit samplings 60 days before harvest, with good accuracy (Table 3.6) with the formula:

$$\text{IB} = -5.9475 + 0.1371 \text{ Wt.} + 1.0211 \text{ Mg/Ca} - 0.0582 \text{ Wt./Ca}$$

To know the potential for IB and BP incidence far before harvest is of considerable importance, because it allows enough time for corrective preharvest treatments, i.e., CaCl_2 spray applications (3,9); summer pruning (45) or other cultural manipulations. To be able to sample in July would also eliminate the rush of sampling and analyses if fruit samples for analysis are taken late as in September, just 20 days prior to commencement of commercial harvesting.

Results for the 1982 and 1983 seasons indicate that relatively reliable predictions can be made for expected bitter pit and internal breakdown potential for Newtown apples either 20 or 60 days prior to the commencement of harvesting, at least for the conditions of the Hood River area of Oregon. Further work is still required to study the correlation between the predicted

values and BP and IB potential during different seasons and locations so that a more generalized model can be generated.

Figure 3.1

Relationship fruit Ca and total bitter pit in Yellow Newtown apples at combined sampling date September 27, 1982 and September 19, 1983.

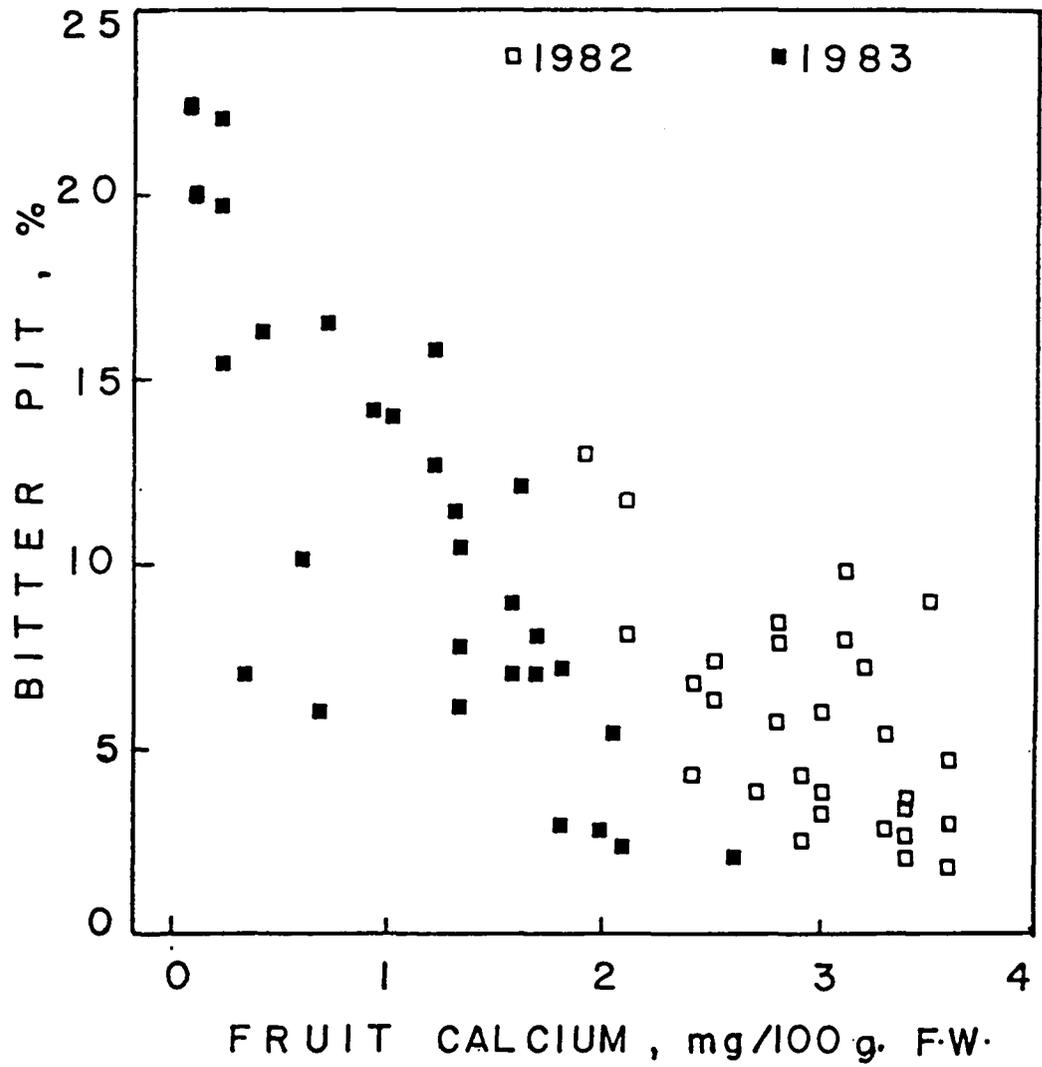


Figure 3.1

Figure 3.2

Relationship fruit wt and total bitter pit in Newtown apples at combined sampling date September 27, 1982 and September 19, 1983.

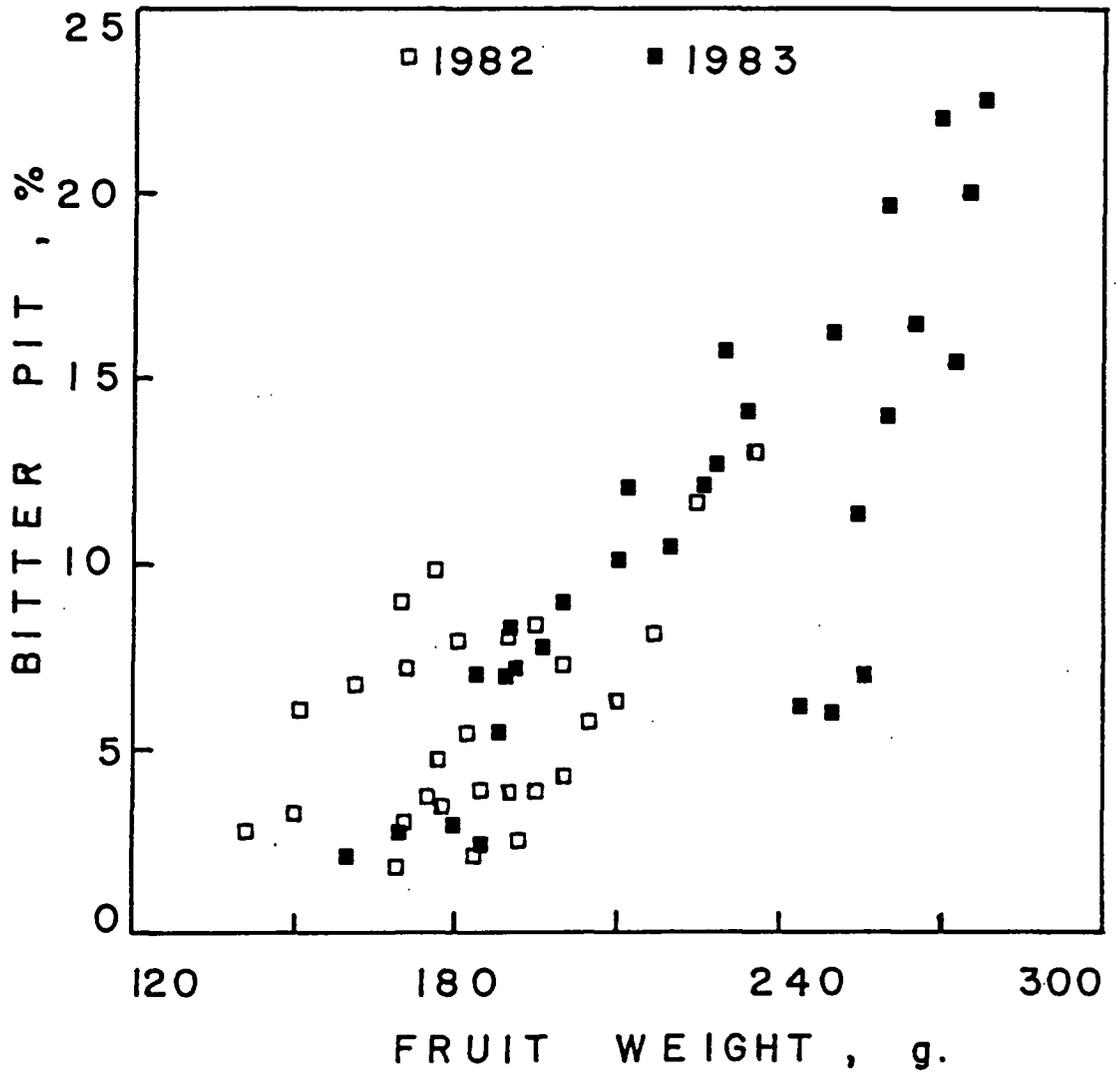


Figure 3.2

Figure 3.3

Relationship fruit Ca and internal breakdown in Yellow Newtown apples after 10 mo storage. Combined data of 1982 and 1983.

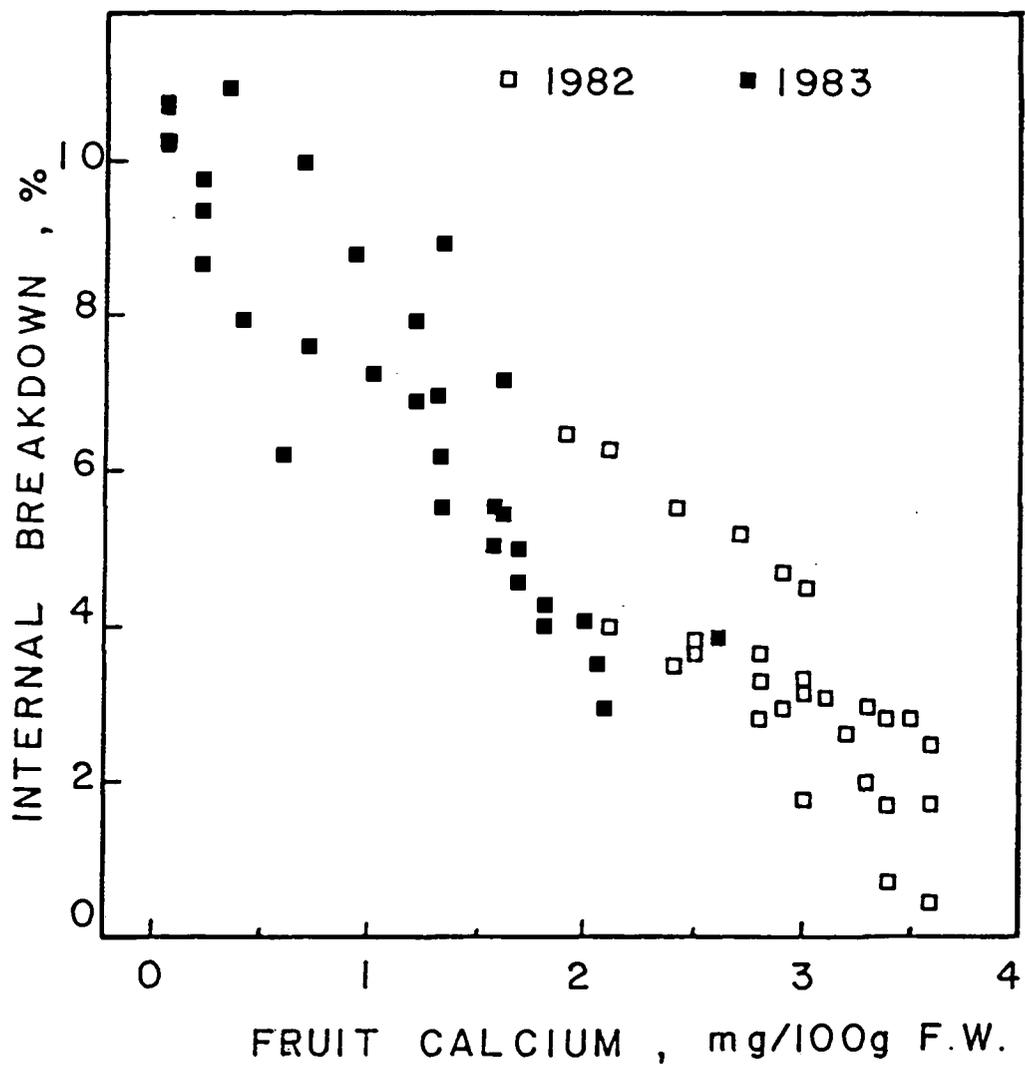


Figure 3.3

Table 3.1. Linear correlations (r-values) between the incidence of harvest, storage, and total bitter pit on Newtown apple and fruit mineral analysis, fruit weight, and their ratios in 1982.

Sampling Dates Survey, 1982	Ca	N	Fruit Weight	N/Ca	Ca/Wt	Wt/Ca
Harvest Bitter Pit						
07/19	-.54**	-.20NS	.48**	.47**	-.54**	.54**
08/13	-.38*	-.16NS	.43**	.25NS	-.52**	.52**
09/06	-.83**	-.25NS	.74**	.60**	-.85**	.85**
09/27	-.68**	-.19NS	.59**	.49**	-.67**	.67**
Storage Bitter Pit						
07/19	-.59**	-.48**	.53**	.31NS	-.59**	.63**
08/13	-.37*	-.42**	.57**	.10NS	-.59**	.63**
09/06	-.66**	-.41*	.70**	.28NS	-.74**	.74**
09/27	-.65**	-.36*	.58**	.31NS	-.64**	.67**
Total Bitter Pit						
07/19	-.61**	-.41*	.55**	.38*	-.61**	.64**
08/13	-.40*	-.36*	.56**	.10NS	-.61**	.65**
09/06	-.76**	-.38*	.76**	.40*	-.82**	.83**
09/27	-.70**	-.36*	.62**	.40*	-.69**	.71**

** = significant at 1% level; * = significant at 5% level; NS = non-significant;
df = 34.

Table 3.2A. Linear correlations (r-values) between the incidence of harvest, storage, and total Newtown apple bitter pit and fruit mineral elements in 1983.

Sampling Dates	Ca	N	P	K	Mg	Mn	Fe	S	B	Zn	Na	Se
Harvest Bitter Pit												
07/20	-.38**	.53**	-.25NS	.58**	.10NS	-.29NS	-.29NS	.10NS	-.16NS	-.29NS	.16NS	-.10NS
08/10	-.49**	.54**	-.46**	.12NS	.09NS	-.51**	.36NS	-.16NS	.09NS	-.58**	.25NS	-.08NS
08/29	-.72**	.35NS	-.49**	.42**	.53**	-.50**	-.16NS	-.15NS	-.42**	.16NS	.30NS	-.35NS
09/19	-.71**	.40*	-.58**	.77**	.68**	-.49**	-.53**	.42*	-.22NS	-.09NS	.42*	.27NS
Storage Bitter Pit												
07/20	-.41*	.25NS	-.42*	.61**	.14NS	-.20NS	-.42*	-.10NS	-.21NS	-.13NS	.14NS	-.09NS
08/10	-.50**	.26NS	-.33NS	.21NS	.10NS	-.44*	.62**	-.20NS	.26NS	-.76**	.12NS	-.10NS
08/29	-.74**	.28NS	-.73**	.60**	.40*	-.54**	-.10NS	-.26NS	-.53**	.19NS	.21NS	-.21NS
09/19	-.78**	.46**	-.59**	.89**	.80**	-.38*	-.60**	.25NS	-.43*	.22NS	.53**	.42*
Total Bitter Pit												
07/20	-.43*	.32NS	-.41*	.63**	.11NS	-.23NS	-.41*	-.10NS	-.22NS	-.17NS	.16NS	-.10NS
08/10	-.53**	.34NS	-.37*	.18NS	.09NS	-.48**	-.60**	-.20NS	.24NS	-.76**	.16NS	-.08NS
08/29	-.77**	.30NS	-.72**	.60**	.44*	-.56**	-.12NS	-.26NS	-.54**	.18NS	.22NS	-.24NS
09/19	-.80**	.46**	-.60**	.91**	.81**	-.41*	-.63**	.30NS	-.41*	.16NS	.54**	.40*

** = significant at 1% level; * = significant at 5% level; NS = non-significant; df = 28.

Table 3.2B. Linear correlations (r-values) between the incidence of harvest, storage, and total Newtown apple bitter pit and fruit mineral elements, fruit weight, and various ratios in 1983.

Sampling Dates	N/Ca	P/Ca	K/Ca	Mg/Ca	$\frac{(K + Mg)}{Ca}$	$\frac{Ca}{(K + Mg)}$	Wt	Wt/Ca	Ca/Wt
Harvest Bitter Pit									
07/20	.46**	.27NS	.58**	.40*	.58**	-.57**	.60**	.45*	-.53**
08/10	.49**	.22NS	.31NS	.20NS	.27NS	-.37*	.58**	.46**	-.56**
08/29	.70**	.69**	.67**	.70**	.68**	-.69**	.64**	.69**	-.71**
09/19	.63**	.63**	.67**	.64**	.67**	-.75**	.66**	.64**	-.71**
Storage Bitter Pit									
07/20	.39*	.08NS	.58**	.46**	.58**	-.62**	.76**	.53**	-.61**
08/10	.46**	.42*	.39*	.10NS	.37*	-.48**	.75**	.56**	-.62**
08/29	.80**	.70**	.79**	.78**	.79**	-.73**	.82**	.83**	-.76**
09/19	.62**	.62**	.68**	.63**	.68**	-.82**	.80**	.63**	-.79**
Total Bitter Pit									
07/20	.43*	.12NS	.61**	.47**	.61*	-.65**	.76**	.54**	-.63**
08/10	.50**	.41*	.40*	.13NS	.38*	-.48**	.75**	.57**	-.64**
08/29	.80**	.72**	.79**	.79**	.79**	-.76**	.82**	.83**	-.79**
09/19	.65**	.65**	.71**	.66**	.71**	-.84**	.81**	.66**	-.80**

** = significant at 1% level; * = significant at 5% level; NS = non-significant; df = 28.

Table 3.3. Multiple linear correlation (r^2 -values) between the incidence of harvest, storage, and total bitter pit and fruit weight and mineral levels of Newtown apple fruit during development and at harvest.

Sampling Date Survey, 1982	Dependent Variable	Independent Variables	r^2 value
07/19	Bitter Pit	= 0.2979 + 0.1532 Wt/Ca	.31**
08/13	at	= 0.4991 - 0.6997 Ca + 0.0351 Wt	.29**
09/06	Harvest	= 2.1253 - 1.4273 Ca + 0.0218 Wt	.77**
09/27	"	= 5.016 - 1.3172 Ca	.47**
07/19	Bitter Pit	= 13.489 - 0.8638 Ca - 0.0907 N	.43**
08/13	After 10	= 0.3915 - 0.1495 N + 0.1146 Wt	.56**
09/06	Months in	= -0.7367 - 1.7685 Ca + 0.0596 Wt	.57**
09/27	Storage	= 17.059 - 2.478 Ca - 0.1316 N	.55**
07/19	Bitter Pit	= 5.3524 - 0.1210 N + 0.1391 Wt	.38**
08/13	Total	= -0.9980 - 2.0376 Ca + 0.1353 Wt	.42**
09/06	Incidence	= 1.2084 - 3.1682 Ca + 0.0822 Wt	.71**
09/27	"	= 31.111 - 6.161 Ca - 0.471 N/Ca	.58**

** = Significant at the 1% level.

Table 3.4. Best multiple linear correlation (r^2 -values) between the incidence of harvest, storage and total bitter pit and fruit weight and mineral contents of Newtown apple fruit during development and at harvest.

Sampling Date Survey, 1983	Dependent Variable	Independent Variables	r^2 value
07/20	Bitter Pit	= -9.3804 + 0.039 K + 0.1082 N + 0.0325 Wt/Ca	.66**
08/10	at	= -7.4437 + 0.1798 N - 70.611 Ca/Wt	.60**
08/29	Harvest	= 4.1483 - 1.730 Ca	.52**
08/19	"	= -0.2001 + 0.0459 K - 39.168 Ca/(K + Mg)	.65**
07/20	Bitter Pit	= -15.0366 + 0.2416 Wt + 0.2239 K/Ca - 0.1775 Wt/Ca	.65**
08/10	After 8	= 2.5925 + 7.0706 Ca + 7.8691 Fe - 719.113 Ca/Wt	.69**
08/29	Months in	= -6.709 + 0.0638 Wt + 0.01546 Wt/Ca	.74**
08/19	Storage	= -6.6376 + 0.2726 K - 2.4224 Ca	.86**
07/20	Bitter Pit	= -18.652 + .3658 Wt - 818.88 Ca/(K + Mg) + 559.892 Ca/Wt	.73**
08/10	Total	= 4.730 + 7.5866 Ca + 8.5875 Fe - 811.578 Ca/Wt	.67**
08/29	Incidence	= -7.3179 + 0.0756 Wt + 0.0177 Wt/Ca	.73**
08/19	"	= -23.884 + .3795 K - 5.2654 Ca + 0.0459 Wt + 290.646 Ca/(K + Mg)	.92**

** = Significant at the 1% level.

Table 3.5A. Linear correlations (r-values) between incidence of internal breakdown on Newtown apples and fruit mineral content during development and at harvest.

Sampling Date	Ca	N	P	K	Mg	Mn	Fe	S	B	Zn	Na	Se
Survey 1982												
07/19	-.70**	-.27NS										
08/13	-.34*	-.12NS										
09/06	-.52**	-.13NS										
09/27	-.85**	-.12NS										
Survey 1983												
07/20	-.52**	.30NS	-.47**	.44*	.06NS	-.26NS	-.46*	.37*	-.42*	-.34*	.19NS	-.18NS
08/10	-.65**	.23NS	-.64**	.19NS	.12NS	-.46*	.23NS	-.07NS	-.20NS	-.22NS	.01NS	-.23NS
08/29	-.82**	.31NS	-.75**	.76**	.55**	-.24NS	-.11NS	.19NS	-.72**	-.19NS	.40*	-.43*
09/19	-.88**	.53**	-.46*	.54**	.83**	-.57**	-.17NS	.41*	-.58**	.25NS	.76**	.38*

** = significant at 1% level; * = significant at 5% level; and NS = non-significant; (1982 df = 34, 1983 df = 28).

Table 3.5B. Linear correlations (r-values) between incidence of internal breakdown on Newtown apples and fruit weight and ratios of various fruit mineral contents during development and at harvest.

Sampling Date	Wt	Wt/Ca	N/Ca	P/Ca	K/Ca	Mg/Ca	Ca/ (K + Mg)
Survey 1982							
07/19	.77**	.79**	.53**				
08/13	.66**	.67**	.25NS				
09/06	.71**	.65**	.36*				
09/27	.72**	.82**	.65**				
Survey 1983							
07/20	.87**	.59**	.45*	.14NS	.56**	.52**	-.65**
08/10	.84**	.80**	.74**	.37*	.62**	-0.1NS	-.56**
08/29	.85**	.79**	.78**	.70**	.80**	.78**	-.82**
09/19	.91**	.68**	.66**	.68**	.62**	.67**	-.85**

** = significant at 1% level; * = significant at 5% level; and NS = non-significant; (1982 df = 34, 1983 df = 28).

Table 3.6. Best multiple linear correlation (r^2 -values) between the incidence of internal breakdown after 8 and 10 months in storage in 1982 and 1983 respectively, and the fruit weight and ratios of mineral contents of Newtown apple fruit during development and at harvest.

Sampling Date	Dependent Variable	Independent Variables	r^2
Survey, 1983			
07/19	Internal	= $-5.9475 + 0.1371 \text{ Wt} + 1.0211 \text{ Mg/Ca} - 0.0582 \text{ Wt/Ca}$.82**
08/13	Breakdown	= $2.1083 - 0.1711 \text{ P} + 0.0511 \text{ Wt}$.76**
09/06	"	= $-7.2492 + 0.072 \text{ Wt} + 0.0721 \text{ K/Ca} - 0.0282 \text{ Wt/Ca}$.85**
09/27	"	= $1.3263 + 0.03379 \text{ Wt} + 0.0058 \text{ K/Ca} - 98.0875 \text{ Ca/(K + Mg)}$.75**
Survey, 1982			
07/19	Internal	= $0.1605 + 0.3349 \text{ Wt/Ca}$.63**
08/13	Breakdown	= $-4.3161 + 0.0494 \text{ Wt} + 0.0889 \text{ Wt/Ca}$.52**
09/06	"	= $-7.2149 + 0.0657 \text{ Wt}$.50**
09/27	"	= $10.7708 - 2.5520 \text{ Ca}$.73**

** = Significant at 1% level.

Table 3.7. Percent of the incidence of harvest and total bitter pit actual and predicted in Newtown apples from sampling in September 06 (20 days before harvest) 1982, to measure the goodness of fitting with the corresponding linear equation. All values in table are percents. Thirteen orchards were compared.

BP at Harvest				BP at Harvest + BP After 8 Mo.			
Actual	Predicted	Variation From Actual BP	Predicted Accuracy	Actual	Predicted	Variation From Actual BP	Predicted Accuracy
3.5	3.3	-5.7	94.2	12.9	10.4	-19.4	80.6
2.8	3.0	+6.7	93.3	8.1	9.9	+18.2	81.8
1.9	1.6	-15.8	84.2	4.2	5.8	+27.6	72.4
1.7	1.6	-5.9	94.1	5.8	6.5	+10.8	89.2
2.6	2.4	-7.7	92.3	6.8	6.4	-5.9	94.1
2.6	2.3	-11.5	88.5	8.4	8.8	+4.6	95.4
1.4	1.5	+6.7	93.3	3.2	3.8	+15.8	84.2
1.8	1.7	-5.6	94.4	2.0	2.6	+23.1	76.9
1.9	1.1	-42.1	57.9	3.7	4.1	+9.8	90.2
0.8	0.89	+10.1	89.9	5.5	4.6	-16.4	83.6
1.0	1.2	+16.7	83.3	4.0	4.9	+18.4	81.6
1.2	1.5	+20.0	80.0	.7	0.3	-57.2	42.8
0.8	0.93	+14.0	86.0	4.2	5.1	+17.7	82.3
			87.0				81.1

Table 3.8. Percent of the incidence of harvest of total bitter pit actual and predicted in Newtown apples from sampling in July 20 (60 days before harvest) 1983 to increase the goodness of fitting with the corresponding linear equation. All values in table are percents. Thirteen orchards were compared.

BP at Harvest				BP at Harvest + BP After 8 Mo.			
Actual	Predicted	Variation From Actual BP	Predicted Accuracy	Actual	Predicted	Variation From Actual BP	Predicted Accuracy
3.4	3.9	+12.8	87.2	22.5	20.6	-8.4	91.5
3.2	2.9	-9.4	90.6	20.0	19.2	-4.0	96.0
3.4	3.4	0	100.0	22.0	19.0	-13.7	86.3
4.5	3.2	-28.9	71.1	16.5	17.9	+7.8	92.2
2.0	2.7	+25.9	74.1	14.0	14.0	0	100
1.2	1.5	+20.0	80.0	14.1	11.1	-21.3	78.7
3.6	2.1	-19.3	80.7	10.2	9.7	-4.9	95.1
3.0	2.2	-26.7	73.3	8.1	5.5	-32.1	67.9
2.2	2.6	+15.4	84.6	12.6	13.1	+3.8	96.2
1.3	1.8	+27.3	72.2	7.0	5.4	22.9	77.1
.50	.87	+42.5	57.5	5.4	4.6	14.8	85.2
0	.83	+83.0	17.0	2.4	3.5	31.4	68.6
3.9	2.8	-28.1	71.9	2.8	3.3	15.2	84.8

Table 3.9. Incidence (percent) of total bitter pit predicted in Newtown apples from 11 orchards with fruit sampled 20 days before commercial harvest, calculated from 1982 and 1983 equations. All values in table are percents.

August 29, 1983				September 6, 1982			
Actual Total BP in 1983	Predicted Total BP in 1983 Model 1982	Variation From Actual BP	Predicted Accuracy	Actual Total BP in 1982	Predicted Total BP in 1983 Model 1983	Variation From Actual BP	Predicted Accuracy
20.0	20.0	0	100	12.9	8.6	- 33.3	66.7
19.7	18.3	- 7.2	92.8	8.1	7.9	- 2.5	97.5
12.1	10.5	- 13.2	86.8	4.2	5.8	+ 27.6	72.4
14.0	16.1	+ 13.0	87.0	6.3	7.6	+ 17.1	82.9
15.8	11.5	- 27.2	72.8	5.8	6.8	+ 14.7	85.3
9.0	12.0	+ 25.0	75.0	6.1	3.9	- 36.1	63.9
10.4	12.5	+ 16.8	83.2	7.2	5.8	- 19.5	80.5
7.8	11.8	+ 33.9	66.1	3.4	5.3	+ 35.8	64.2
5.4	7.8	+ 30.8	69.2	4.7	4.8	+ 2.0	98.0
2.8	7.5	+ 62.7	37.3	2.1	3.2	+ 3.4	65.6
2.0	6.6	+ 69.7	<u>30.3</u>	1.1	2.8	+ 60.7	<u>39.3</u>
			<u>72.7</u>				<u>74.2</u>

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CHAPTER IV

DEVELOPMENT OF A PREDICTIVE MODEL FOR THE PHYSIOLOGICAL DISORDER
CORK SPOT ON D'ANJOU PEARS BY PREHARVEST FRUIT MINERAL ANALYSIS

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Abstract

In 1982 and 1983, 15 and 10 orchards respectively in Hood River, Oregon, were sampled and analyzed for 11 mineral nutrients in the fruits. We have been attempting to predict the occurrence of cork spot in d'Anjou pear before harvest, so that alternative methods could be applied to overcome the losses generated by such physiological disorders. The orchards were sampled approximately 40 and 20 days before harvest as well as at harvest, when the fruits were evaluated for cork spot, and again at the end of 8 months storage.

A model has been developed using regression equations, which associated the mineral nutrient status of the fruits, the occurrence of cork spot and best sampling time. Among the elements tested, Ca, wt/Ca, and N/Ca ratio accounted for the most consistent predictive parameters. Cork spot could be predicted from

fruit samples 40 days before harvest with about 70% probability of certainty.

Introduction

Cork spot (CS) is a physiological disorder of pear fruit similar to bitter pit in apples. It is characterized by sub-epidermal darkened lesions which become increasingly apparent and enlarge in storage. D'Anjou pear is quite susceptible to this disorder, and postharvest losses have ranged from 1 up to 60% (1,30,31,35,48). Nutritional imbalance between Ca and other elements are major causes of many physiological disorders of fruits and vegetables. Cork spot in pears and bitter pit in apples have been associated with nutritional levels of P, K, Ca, N, and Mg (1,3,9,21,32,44,46,54). Calcium seems to occupy a central position in fruit nutrition, and the effect of the other elements in relation to these disorders is often through their interactions with Ca (6). The relationship between fruit Ca content and the physiological disorders known as bitter pit in apple (3,9,44,46) and cork spot in pear (1,21,30,31,35,48,54) has been well documented. High levels of N have been reported to aggravate apple (9,20,43) and pear (1,32,35,36) fruit Ca deficiency symptoms. High Mg has also been associated with increased bitter pit in apples (2,5,17) and cork spot in d'Anjou pear (21,54). High K has been related to greater incidence of bitter pit in apples (9,44,47) and cork spot in pears (21,54). Phosphorus has been correlated both positively (9) and negatively (19,32) with bitter pit in apples and cork spot in pears. It has been reported that the occurrence of bitter pit in apple is related to the fruit

size, the larger fruit being affected more than smaller fruit (10,16).

Several workers (1,10,28,35,46,53) have suggested threshold levels of Ca above which little or no pit can be expected to develop, and below which the fruit is likely to be increasingly susceptible to the disorders.

The use of fruit Ca and N/Ca ratios have been suggested as a predictive assay for the occurrence of cork spot in d'Anjou pears (35).

A need exists for producers and packers to be able to estimate their seasonal cork spot potential, enabling them to plan their market pattern. Knowledge regarding which orchards are likely to develop CS and to what extent, can help the packing houses to program the length of fruit storage, as well as to anticipate the respective fruit's quality at the end of storage. If the relative incidence of these disorders could be predicted far ahead of harvest, it could allow for corrective treatments such as orchard sprays to be used, and would avoid late season rush to analyze fruit.

Therefore the objective of this work is to examine the use of preharvest fruit mineral analysis relative to future prediction of the incidence of cork spot in d'Anjou pears at harvest and after storage. Also potential interactions with P, K, Mg, Mn, B, Zn, S, Na, and Fe were examined.

Materials and Methods

Fifteen commercial orchards in the Hood River area of Oregon were sampled in 1982 and 10 in 1983. Fruits were sampled from three uniform trees per orchard and analyzed individually. Ten typical fruits per tree were randomly sampled from the periphery of the trees on July 18, August 9, and September 1, the time of commercial harvest.

At harvest, September 1, one box of fruits (100 fruits) were picked from each tree. Fruits were stored in 20 Kg cardboard boxes with perforated polyliners at -1°C and 96% RH for 8 months in 1982 and 1983.

Mineral analysis

All fruit samples were held in plastic bags (25,52) in cold storage until analyzed. Fruits were weighed, washed with distilled water and allowed to dry in the air on paper towels (31,42) just before cutting sections for analysis. Pairs of opposite quarters were cut longitudinally (23,24,27,28) from 6 fruits per sample and bulked. Seeds and stems were removed to prevent errors in analytical results (24,27,51). Samples of 18 fruit slices were weighed and homogenized with an equal amount of deionized water for 2 min. The mixture was further homogenized in a 'Virtis 45' for 1 min to make a finer suspension (22,23,25,26,28) which is referred to as the fruit water suspension.

Calcium was extracted in 1982 by the method suggested by Perring (26) with some modifications: ten ml of fruit water suspension was transferred into a 50 ml Erlenmyer flask and 10 ml

concentrated 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 then filtered through Whatman 41 or 42 paper. The filtrate was diluted to 100 ml with deionized water. Strontium chloride was added to reduce interferences at a final concentration of 3% SrCl_2 to all extracts. Calcium was determined by Atomic Absorption spectrophotometry (Perkin Elmer, Model 303).

The samples in 1983 were prepared as in 1982 up to the final digestion with HCl. From there on some modifications were introduced. The digested contents were filtered and 4.5 ml of the solution taken for analysis of Ca, P, K, Mg, Mn, B, Zn, Fe, Na, and S determined in an Inductively Coupled Argon Plasma Spectrometer (Jarrell Ash ICAP - 9000).

Five ml of the fruit water suspension were dried at 70°C for digestion and micro-kjeldahl N determinations with a Tecnicon-autoanalyzer.

Cork Spot Assessment

Fruit samples at harvest in 1982 and 1983 were evaluated for superficial cork spot and designated harvest CS.

The incidence of CS was also evaluated after 8 months in 1982 and 1983. All the fruits were peeled and the internal CS determined and designated storage CS. Total incidence of CS was the sum of harvest CS and storage CS.

Simple linear correlations between the incidence of cork spot and mineral content of the fruits were calculated on the Cyber

computer, using the SIPS Regression program. Multiple correlation coefficients were obtained with the SIPS regression system by adding and dropping the best and the worst variables respectively. Multiple correlations and the simple linear correlations were used to select the variables which were most closely correlated with the incidence of harvest CS and total cork spot.

Results

Average 'Anjou' fruit weights for July 18, August 9, and September 1, 1982 sampling dates were 48, 100, and 198 gr respectively. Comparable average weights for about the same dates in 1983 were 85, 140, and 235 gr, substantially larger than 1982 data (Figs. 4.1 and 4.4). As will be shown, this size difference is an important parameter in fruit Ca and cork spot associations.

Fruit Ca changes between sampling dates and season

Fruits sampled 40 days before harvest presented the highest Ca concentration in 1982 and 1983 (Fig. 4.3). As sampling dates approached final harvest in 1982 there was a substantial decrease in fruit Ca concentration. Fruits initially with 12 mg Ca/100 f.w. decreased to 8 and 5 mg Ca/100 g f.w. for the July, August, and September sampling periods respectively, or an equivalent decrease in fruit Ca of 32% and 33% between the 1st, 2nd and 3rd fruit sampling periods.

Average fruit Ca concentrations in 1983 were 8, 5, and 3 mg/100 g f.w. for the July, August, and September sampling periods respectively. The decrease in fruit Ca concentration from the 1st to the 2nd sampling period was 41% and from the 2nd to the 3rd sampling, a decrease of 30% in fruit Ca content.

There was a substantial difference in fruit Ca concentration for the 2 seasons. Thus, average fruits sampled in 1983 were 77, 40, and 18% lower in Ca concentration than fruit samples of 1982 for the 1st, 2nd, and 3rd sampling dates respectively.

Incidence of cork spot

In the 1982 season, the incidence of total cork spot was low, ranging from 0.8 to 13% (Figs. 4.1 and 4.2). Fruits from the 1983 season showed higher incidence of total cork spot in d'Anjou fruits, ranging from 2.6 to 17.8% (Figs. 4.1 and 4.2). On average, twice as much CS developed in storage as was present at harvest in 1982 and 1983 and this is comparable to previous reports (1,35,36).

Larger fruits and low fruit Ca were associated with higher incidence of cork spot in both years. This relationship was clearly evident especially in the lower and higher extremes of the ranges (Figs. 4.1 and 4.2) which had very low and very high occurrence of the disorder respectively.

The 1983 fruits were larger and had lower Ca than 1982 fruits and therefore higher incidence of the disorder.

Simple linear correlations between cork spot and mineral elements

Inspection of the scatter diagram (not shown) demonstrated that the relationship of mineral elements with the physiological disorder cork spot in d'Anjou fruits is mostly linear and therefore only linear correlations were calculated.

Simple linear correlation coefficients (r-values) of mineral elements in relation to cork spot fruits are shown in Tables 4.1, 4.2A, and 4.2B, and are the results of seasonal fruit mineral analyses of all orchards sampled in 1982 and 1983.

Fruits sampled in 1982 (Table 4.1) had highly significant negative correlations between harvest cork spot and total inci-

dence of cork spot, with fruit Ca content and Ca/fruit wt ratios. Higher correlation values were obtained with the ratio Ca/wt. Nitrogen did not correlate significantly with the disorder at either stage of evaluation. However, when the ratio N/Ca was used, a significant positive correlation was detected even at the July sampling date, and increased in value and significance as sampling approached the final harvest date. Weight of the fruits was found to correlate positively and was highly significant with the CS disorder with the values increasing toward the last sampling date as well.

Several other fruit mineral elements besides Calcium and Nitrogen were measured in 1983 relative to CS disorder (Tables 4.1, 4.2A, and 4.2B). Negative correlations were found for both harvest CS and total CS with N, P, Ca, Mg, Mn, Zn, S, Na, and Ca/K + Mg and Ca/wt ratios. Positive correlations were found for K, Fe (July and August only), B, N/Ca, P/Ca, K/Ca, Mg/Ca, (K + Mg)/Ca, wt/Ca, and fruit wt, and CS disorder incidences.

Most of the mineral elements correlated at 5 or 1% level of significance with fruit cork spot. There was a general increase in r-values from the time of the 1st sampling date, July 18 toward the final sampling, September 1st. There was a greater incidence of cork spot during storage than at the time of commercial harvest, and this was reflected by generally higher values of the coefficient of determination for the various mineral elements.

Since there was a consistently higher significant correlation between fruit Ca and cork spot at all sampling dates, and since

the ratio of this element with P, K, and Mg has been cited to be closely related to bitter pit in apples (2,12,14,35,43) ratios of those elements (with Ca) most likely to be related to the disorder in d'Anjou pear were calculated. Generally there was an additional gain in significance with the Ca adapted ratios.

Multiple correlation between cork spot and mineral elements

Results of multiple linear correlations of mineral elements in relation to harvest and total incidence of cork spot of d'Anjou fruits are expressed in coefficient of determinations (r^2 -values), as shown in Tables 4.3 and 4.4.

Multiple linear correlations between mineral concentrations, fruit weight, and the incidence of harvest CS and after 8 months in storage for 1982 and 1983 showed that Ca and fruit weight were the most closely related variables. However, N, K, P, and Mg also influenced the occurrence of the disorder, but mainly for harvest CS and for the 2nd and 3rd sampling dates.

Simple linear correlations between cork spot and Ca, fruit weight, N, and fruit wt/Ca ratio

Since fruit Ca; wt; N and the ratio wt/Ca were found to be closely related with CS in both seasons and for most of the sampling dates, simple linear correlations were calculated between these parameters and the incidence of harvest and total CS. However, data of 1982 were combined with those of 1983 and calculated as a whole. The results in Table 5 show that among N, Ca and fruit weight, fruit Ca was the best correlated with the disorder at the early sampling dates July 18 ($r = -.66$ and $r = -.71$), and

August 9 ($r = -.74$ and $r = -.76$). However, when the ratio fruit wt/Ca was considered, an even higher value of the correlation coefficient was detected.

Nitrogen alone did not relate significantly with CS, but the ratio N/Ca was found to be significantly positive with both harvest and total CS in the fruits.

There was always an increase in the correlation coefficient as sampling date approached harvest, September 1 for these limited parameters. Also, correlation values for total incidence of CS were always higher than for harvest CS for the same parameters referred above.

Multiple correlation between total cork spot and fruit Ca, N, wt, N/Ca, and fruit wt/Ca

The results of the multiple linear regression are shown in Table 4.6. Based on these figures the ratios N/Ca and fruit wt/Ca were the most consistent variables relating to total CS for all sampling dates.

For July 18 sampling, Ca, N/Ca, and wt/Ca accounted for 65% of the variation in CS. However for August 9 and September 1, only N/Ca and wt/Ca were related directly with CS which accounted for 68% and 85% of the variation respectively.

The coefficient of determination increased to $r^2 = .85$ as the fruit sampling approached the commercial harvest, September 1.

Testing the model

To test the efficacy of the model, fruit samples from July 18, 40 days before harvest in 1982 and in 1983 were entered in the

model and the predicted total incidence of CS compared with the actual total CS in each year respectively, using the equation generated by both years, data combined.

The results presented in Table 4.7 clearly demonstrate the predictive accuracy over 70% and 80% in 1982 and 1983 respectively of the proposed model based upon July fruit sampling. The obtained R^2 between the predicted and actual CS were $R^2 = .69$ and $R^2 = .84$ for 1982 and 1983 respectively, which demonstrate the effectiveness of the model.

Discussion

Simple linear regression of cork spot in relation to Ca concentration of the fruit for all three sampling dates in both years were all significant and negatively correlated. The significance and consistency of the Ca relationship with CS as compared with other mineral elements confirmed that Ca deficiency in the fruit is the major factor in the incidence of cork spot at harvest and after storage. Fruit size was also a very closely related variable in the occurrence of the disorder. The larger the fruit the lower was the fruit Ca concentration and the higher the incidence of cork spot in d'Anjou fruits. Since large fruits are more likely to develop CS than smaller fruit it might be considered to be due to a dilution effect (50). According to Wilkinson and Perring (50) and Shear and Faust (41) the final level of Ca in the fruit is probably determined early during fruit development and this basically was substantiated by the present data. In 1982 fruit started with higher Ca concentration which persisted to harvest in contrast to 1983 when the fruits showed lower Ca concentrations already on July 18.

The results of multivariate analysis, together with previous experience, indicated the importance of including mean fruit weight as one of the variables when trying to study the relationship between disorder incidence and mineral content. Generally, those conditions which lead to increased mean fruit size per tree also lead to Ca deficiency and greater susceptibility to cork spot

(16,29,34). The best correlations were attained when considering the ratios fruit weight/Ca for all three sampling dates.

The seasonal analysis for Ca suggested that the relation between Ca concentration during growth and the incidence of harvest and total cork spot could be used as a component of a predictive model. The results presented here agree with some previous reports for Ca (7,39,40) and fruit weight (8,45,49).

Our results show that N may also be related to the occurrence of this disorder in d'Anjou pears, although the results are largely inconsistent between years and sampling dates. However, when the ratio N/Ca was computed some of these fluctuations in significance were corrected and more significant correlations were obtained, reflecting a degree of balance between Ca and N. The effect of N on cork spot was mainly indirect, possibly by increasing vegetative vigor competition or through the interaction of fruit size and Ca. These results agree with other reports on apples (11,18,42) and pears (31,35).

D'Anjou pear fruits with higher levels of Mg and K and lower levels of Ca are most likely to develop the disorder cork spot. It has been suggested (4) that Mg and K are the main ions that antagonize the function of Ca in cork spot development, perhaps because their concentration is high enough to compete with Ca in the fruit. It is also suggested that K and Mg may have occupied some nonspecific Ca attachment sites and antagonized the function of Ca in the cell membrane. The most serious effect on Ca may be that caused by Mg. Large portions of Ca in the membrane are

exchanged by Mg, K, or H^+ ; this exchange increases the permeability and fragility of the membranes (3,4). Calcium deficiency is relative and conditioned by the concentration of Mg, K, and N. Larger fruits may disproportionately accumulate more mineral elements especially K and Mg, resulting in an imbalance with Ca, therefore predisposing the occurrence of the disorder. Under drought stress conditions, Ca may move out of the fruits and accumulate in the leaves (13) where it becomes quite immobile (15,37). Therefore in a condition where fruits are large, the amount of some micro- and macronutrients become high and Ca low since it relates inversely with fruit size. Any adverse growing condition may further aggravate a marginal ionic and physical balance in the fruit, thus inducing the occurrence of the physiological disorder.

The results showed that the Ca, fruit weight and cork spot relationship was consistent for different orchards and seasons. Of course, some variation existed. But considering all the environmental effects and different cultural methods used for different orchards, still quite significant correlations existed for each isolated year or as a whole group, suggesting the potential adoption of these variables as a predictive tool. One way of reducing some of the variations involved would be to sample a larger number of fruits per orchard and from a larger number of orchards in a growing district, and this is currently under study.

The main objective of this work has been to find a practical way for assessing the likely risk of cork spot in individual

orchards so that managerial decisions could be made prior to harvest and the more lead time, the better. Based on two years of data, by adopting the equation:

$$\text{total cork spot} = 18.0249 - 0.8585 \text{ Ca} - 1.8086 \text{ N/Ca} + 1.2918 \text{ wt/Ca}$$

and sampling fruits on July 18, 40 days before the beginning of commercial harvest, we obtained accuracy over 70% in 1982 and 80% in 1983. Thus we are encouraged by the potential for cork spot prediction ahead of time. Naturally, individual histories of particular orchards must be taken into account and as cultural factors are more intensively studied, the predictive model may become modified.

Once one can predict the orchard's risk, an efficient program of spray application of Ca (30), cultural practices (45) and pre-storage dipping or drenching of the fruits (34), could be applied, thus raising the Ca concentration in the fruits and reducing the incidence of the disorder. Also, the possibility of pinpointing potentially high risk orchards, managerial decisions concerning length of storage time, and fruit quality after storage can be made even if no other treatments have been made to reduce the incidence of cork spot.

In conclusion it appears that fruit weight, together with Ca and N analysis can reliably be used to predict cork spot incidence in seasons aimed at better prediction and reducing variation between years, by developing a model averaged over a five-year period.

Figure 4.1. Relationship between fruit weight and total cork spot in D'Anjou pears at the sampling date September 11, 1982 and 1983.

Figure 4.2. Relationship between fruit Ca and total cork spot in D'Anjou pear at the sampling date September 1, 1982 and 1983.

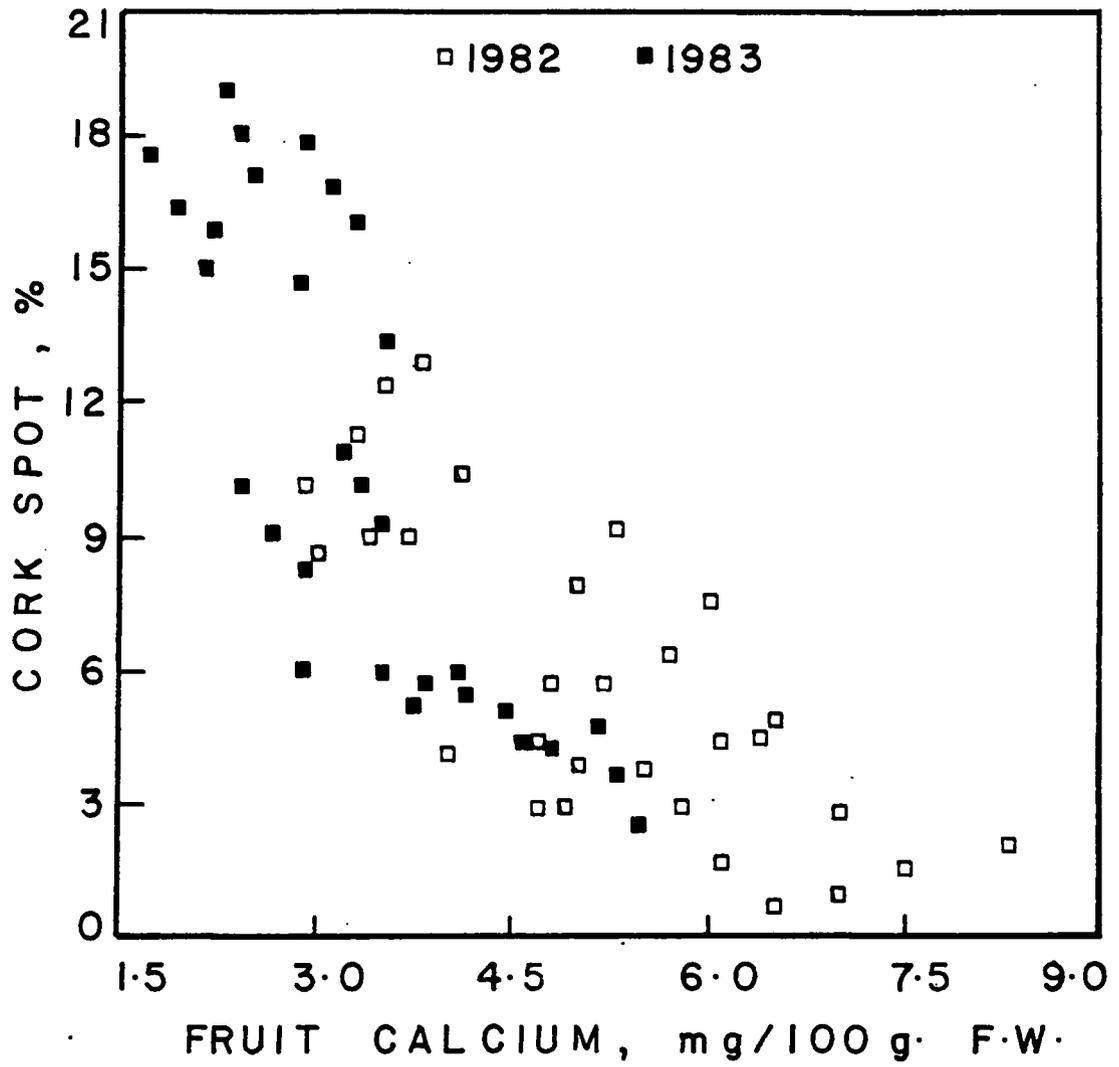


Figure 4.2

Figure 4.3

Influence of sampling date on the fruit Ca concentration of D'Anjou pears, in 1982 and 1983 respectively.

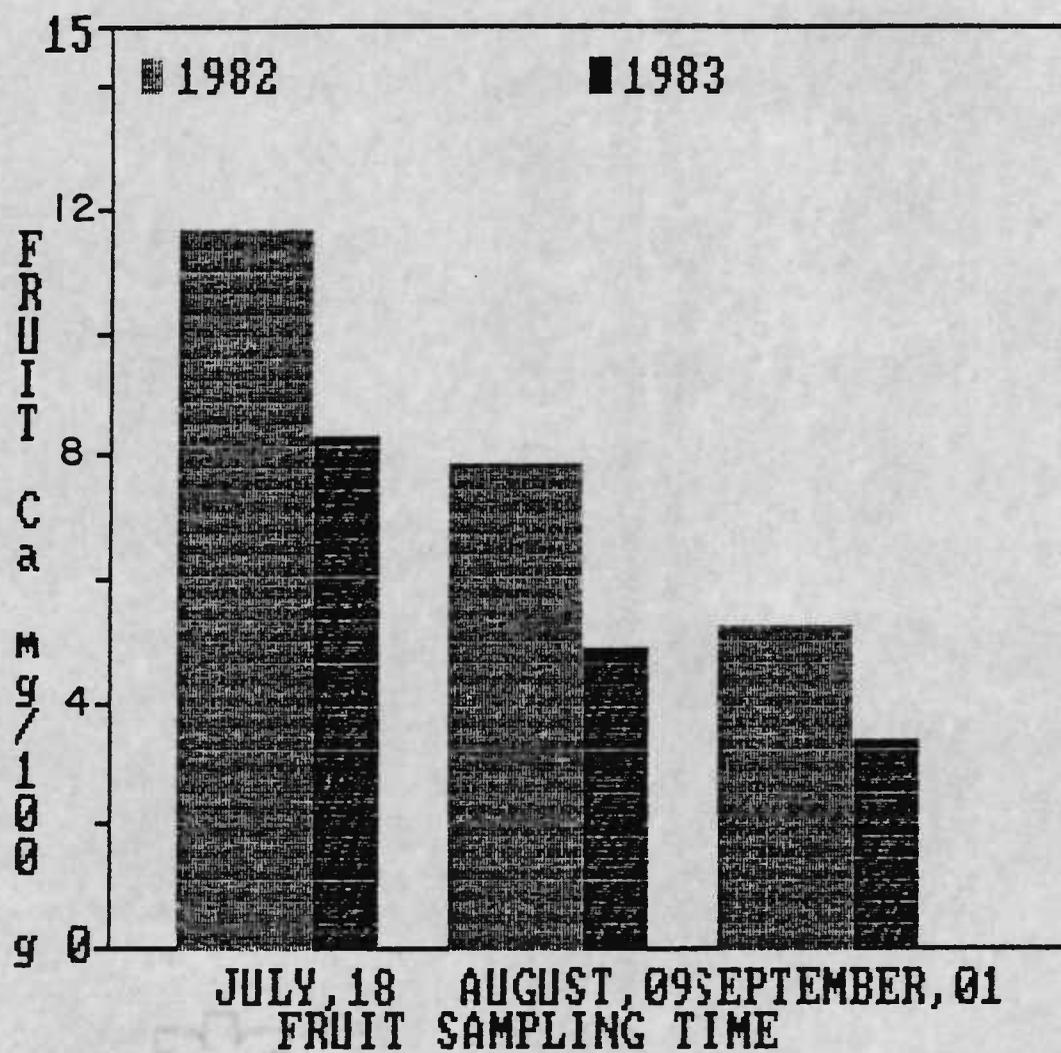


Figure 4.3

Figure 4.4. Influence of sampling date on the fruit Ca concentration of D'Anjou pears, in 1982 and 1983 respectively.

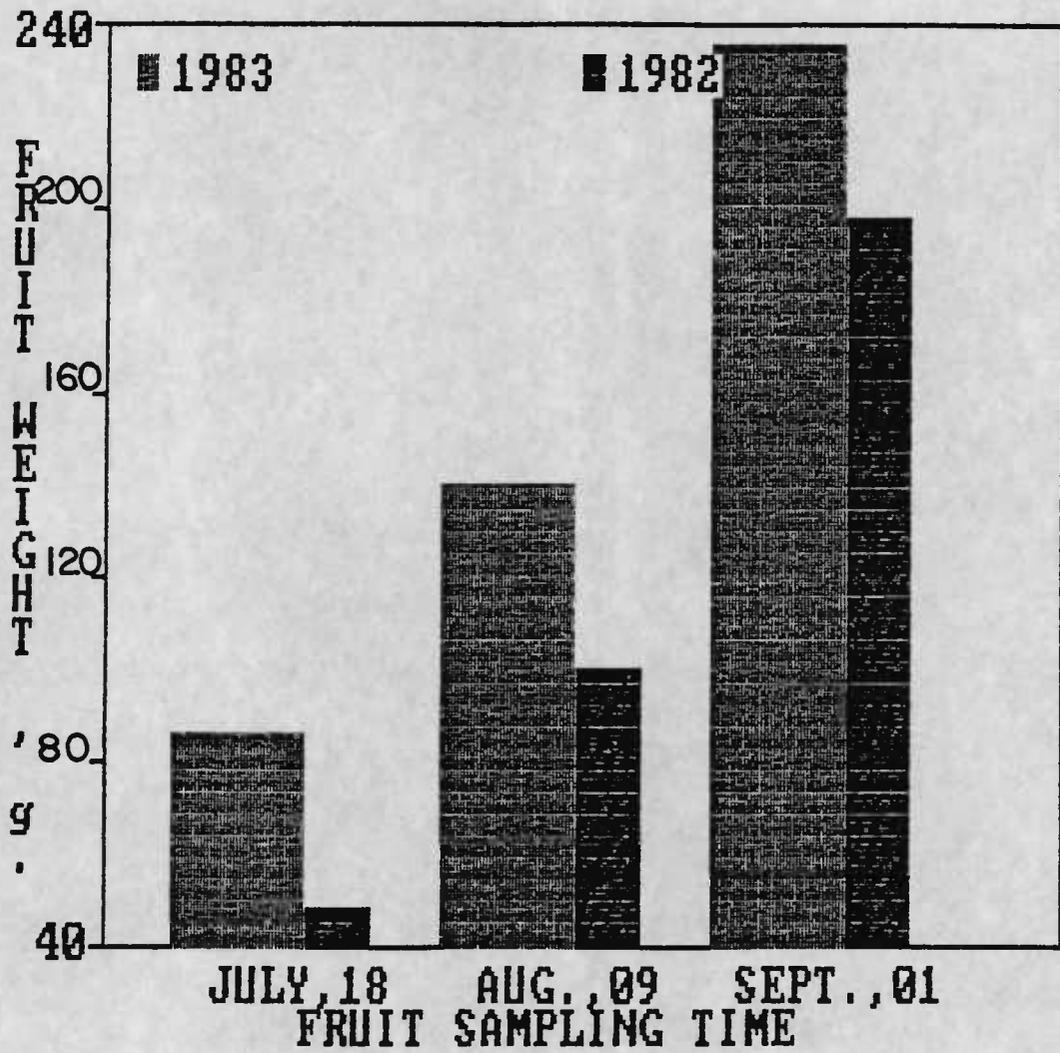


Figure 4.4

Table 4.1. Linear correlations (r-values) between the incidence of harvest and total D'Anjou cork spot and fruit weight and fruit mineral elements in 1982.

Sampling Date of 1982	Ca	N	N/Ca	Fruit Weight	Fr Wt/ Ca	Ca/Fr Wt
<u>Harvest Cork Spot</u>						
July 18	-.59**	-.18NS	.33*	.52**	.70**	-.68**
August 9	-.73**	-.15NS	.55**	.75**	.81**	-.78**
September 1	-.70**	-.14NS	.59**	.87**	.78**	-.78**
<u>Total Cork Spot</u>						
July 18	-.67**	-.22NS	.56**	.52**	.72**	-.70**
August 9	-.80**	-.33*	.58**	.78**	.84**	-.81**
September 1	-.81**	-.45*	.61**	.93**	.86**	-.83**

** = Significant at 1% level, * = significant at 5% level, NS = non-significant, df = 28.

Table 4.2A. Linear correlations (r-values) between the incidence of harvest and total D'Anjou cork spot and fruit mineral elements in 1983.

Sampling Date of 1983	P	K	Ca	Mg	Mn	Fe	B	Zn	S	Na
Harvest Cork Spot										
July 18	-.37*	.20NS	-.54**	-.38*	-.05NS	.41*	.40*	-.33*	-.05NS	.05NS
August 9	-.53**	.63**	-.75**	-.73**	-.56**	.39*	.52**	-.37*	-.45*	-.14NS
September 1	-.70**	.46**	-.74**	-.34*	-.39*	-.68**	.26NS	-.63**	--	-.34*
Total Cork Spot										
July 18	-.37*	.33*	-.67**	-.41*	.14NS	.45*	.48**	-.40*	.01NS	.10NS
August 9	-.61**	.77**	-.80**	-.68**	-.59**	.31NS	.65**	-.36*	-.36*	-.16NS
September 1	-.68**	.63**	-.81**	-.26NS	-.57**	-.71**	.26NS	-.61**	-.52**	--

** = Significant at 1% level, * = significant at 5% level, NS = non-significant, df = 28.

Table 4.2B. Linear correlations (r-values) between the incidence of harvest and total D'Anjou cork spot and fruit mineral concentration, weight, and various ratios in 1983.

Sampling Date of 1983	N	N/Ca	P/Ca	K/Ca	Mg/Ca	$\frac{(K + Mg)}{Ca}$	$\frac{Ca}{(K + Mg)}$	Wt/Ca	Wt	Ca/Wt
Harvest Cork Spot										
July 18	-.31NS	.39*	.34*	.44*	-.06NS	.20NS	-.38*	.65*	.82**	-.67**
August 9	-.40*	.49**	.60**	.68**	-.16NS	.63**	-.74**	.68**	.65**	-.78**
September 1	-.52**	.47**	.53**	.55**	-.39*	.46**	-.36*	.68**	.73**	-.77**
Total Cork Spot										
July 18	-.22NS	.56**	.52**	.61**	-.01NS	.33*	-.42*	.78**	.89**	-.78**
August 9	-.33*	.58**	.61**	.79**	-.26NS	.77**	-.68**	.75**	.74**	-.82**
September 1	-.45*	.61**	.67**	.71**	-.54**	.63**	-.28NS	.81**	.84**	-.83**

** = Significant at 1% level, * = significant at 5% level, NS = non-significant, df = 28.

Table 4.3. Best multiple linear correlations (r^2 -values) between the incidence of harvest cork spot in D'Anjou pear and fruit mineral elements in 1982 and 1983.

Sampling Dates	Dependent Variable	Independent Variables	r^2
<u>Survey, 1982</u>			
July 18	Cork Spot	= 3.0079 - 0.4528 Ca + 0.1056 Wt	0.47**
August 9	at	= 2.1854 - 0.4792 Ca + 0.0431 Wt	0.67**
September 1	Harvest	= -5.9316 + 2.3059 Ca - 269.427 Ca/Wt + 0.0985 Wt/Ca	0.65**
<u>Survey, 1983</u>			
July 18	Cork Spot	= 1.180 + 3.9975 Ca - 293.349 Ca/Wt	0.78**
August 9	at	= 44.742 + 8.778 K - 0.1315 Wt - 8.7692 (K + Mg)/Ca - 399.738 Ca/Wt	0.76**
September 1	Harvest	= 27.826 - 0.707 P - 0.1515 N - 302.041 Ca/Wt	0.83**

** = Significant at 1% level.

Table 4.4. Best multiple linear correlations (r^2 -values) between the incidence of total cork spot in D'Anjou pear and fruit mineral elements in 1982 and 1983.

Sampling Dates	Dependent Variable	Independent Variables	r^2
<u>Survey, 1982</u>			
July 18	Harvest &	= -4.5545 + 2.4701 Wt/Ca	0.52**
August 9	Storage	= -1.6947 + 0.5510 Wt/Ca	0.71**
September 1	Cork Spot	= -11.0225 + 0.0851 Wt - 0.3595 N/Ca + 0.0897 Wt/Ca	0.89**
<u>Survey, 1983</u>			
July 18	Harvest &	= -33.4959 + 0.5165 Wt	0.80**
August 9	Storage	= 26.9724 - 8.2755 Ca + 0.367 Wt - 0.8979 Wt/Ca	0.76**
September 1	Cork Spot	= -36.6877 + 0.1373 Wt - 0.6112 K/Ca + 0.4024 Wt/Ca	0.85**

** = Significant at 1% level.

Table 4.5. Linear correlations (r-values) between the D'Anjou pear cork spot at harvest (H) and after 8 months storage (S) and the fruit mineral content during development of 1982 and 1983 data combined.

Sampling Date	Ca		N		Fruit Wt		N/Ca		Fruit Wt/Ca		Ca/Fruit Wt	
	H	S	H	S	H	S	H	S	H	S	H	S
July 18	-.66**	-.71**	.05NS	.09NS	.66**	.69**	.54**	.64**	.68**	.76**	-.66**	-.68**
August 9	-.74**	-.76**	-.05NS	.03NS	.72**	.74**	.62**	.68**	.75**	.80**	-.71**	-.71**
September 1	-.76**	-.80**	-.08NS	-.05NS	.78**	.81**	.63**	.72**	.78**	.86**	-.76**	-.77**

** = Significant at 1% level, NS = non-significant, df = 56.

Table 4.6. Best multiple linear correlations (r^2 -values) and equations between the incidence of total 'Anjou' cork spot (harvest plus storage) and fruit mineral content during development. Combined data of 1982 and 1983.

Sampling Dates	Dependent Variable	Independent Variables	r^2
July 18	Total CS	= 18.0249 - 0.8585 Ca - 1.8086 N/Ca + 1.2918 Wt/Ca	0.65**
August 9	Total CS	= 2.6335 - 0.8584 N/Ca + 0.6304 Wt/Ca	0.68**
September 1	Total CS	= 1.2115 - 0.8341 N/Ca + 0.3171 Wt/Ca	0.85**

** = Significant at 1% level.

Table 4.7. Actual and predicted total Anjou pear cork spot incidence in 1982 and 1983 based upon the combined predictive equation from July 1982 and 1983 fruit sample dates. All values in table are in percents. The same 10 orchards were compared both years.

Actual Total Cork Spot 1982	Predicted Total CS 1982	Predictive Accuracy	Actual Total CS 1983	Predicted Total CS 1983	Predictive Accuracy
9.3	6.6	71	16.5	15.1	91
10.5	5.6	53	5.2	9.0	58
2.8	2.5	89	10.2	12.4	82
7.7	4.1	53	6.2	7.3	85
5.0	2.6	52	9.3	8.4	90
10.3	9.7	94	18.3	16.1	88
2.1	3.6	58	17.1	13.7	80
11.4	9.4	82	5.3	6.9	77
9.2	6.3	68	5.8	8.0	73
5.8	6.3	<u>92</u>	14.8	14.1	<u>95</u>
		Ave. 71			Ave. 82

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CHAPTER V

EFFECT OF CALCIUM ON RESPIRATION RATE, ETHYLENE PRODUCTION AND OCCURRENCE OF CORK SPOT IN D'ANJOU PEARS (PYRUS COMMUNIS L.)

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Abstract

D'Anjou pear fruits were sampled from 10 commercial orchards near Hood River, Oregon, and analyzed for calcium concentrations by plasma emission spectrophotometer (ICAP). Fruits with high, medium, and low Ca^{++} were evaluated at harvest and after storage 3 and 7 months at -1.1°C and 96% RH, for CO_2 and C_2H_4 production and incidence of cork spot.

Calcium concentrations in the fruits were closely correlated to cork spot; the symptoms increasing with decreasing fruit Ca concentration and increasing storage duration. Respiration, C_2H_4 evolution and internal C_2H_4 of fruits varied with different Ca levels at harvest, and after 3 and 7 months in storage. Higher Ca levels were related to lower respiratory activities of the fruits. The higher the Ca in the fruits, the lower the ethylene evolution. Initial C_2H_4 production was high only in low Ca fruits. Fruit with high Ca had delayed onset of C_2H_4 evolution. The higher the

fruit Ca, the longer was the chilling requirement for proper fruit ripening.

Therefore, calcium had a marked effect on delaying ripening and senescence of D'Anjou pear tissues. Fruits with high Ca concentrations can be stored successfully for longer time periods.

Introduction

Cork spot, a physiological disorder of D'Anjou pear (18, 30), is characterized by a bumpy, uneven appearance of the surface with brown, dry, corky tissue in the flesh when peeled. The lesions are usually most common near the calyx end.

A number of factors which contribute to the disorder have been identified, but the most significant is low tissue calcium content (18, 21, 22, 26, 30). The disorder is similar to bitter pit in apples (19), which is also characterized by low calcium level. The mechanisms by which Ca influences growth and retards senescence are partially understood and there are reports that adding Ca to Ca deficient tissues reduces respiration and suppresses C_2H_4 production (12, 16, 20).

Bramlage et. al. (4) found that calcium levels markedly influence respiration rate of apples, but that differences in Ca level did not influence time of fruit ripening. However, calcium not only depressed avocado fruit respiration and ethylene production, but also delayed the onset of the climacteric rise when the fruits were treated with 0.1 M $CaCl_2$ (25).

Nitrogen is related to bitter pit in apples, and respiration was higher with high N concentration (11). Apple fruits affected with bitter pit showed low calcium as well as high nitrogen content in the fruit flesh.

Calcium and nitrogen have been shown to be related to cork spot and respiratory activities of D'Anjou pears (21, 23, 24). However, additional studies are required to demonstrate the effect

of existing natural levels of these mineral elements on respiration, ethylene production and internal C_2H_4 , as well as on the climacteric behavior of D'Anjou fruits, which is still not well understood. The generated information could help determine the relative importance of Ca and N in the development of disorders and could lead to a predictive storage potential model if they are shown to be key factors in fruit physiology, especially if relative indices of these nutrient levels can be characterized.

Materials and Methods

Three uniform and mature trees of D'Anjou pear trees were selected from 10 commercial orchards near Hood River, Oregon, in 1983. At the initial date of commercial harvest, when fruits were 100% mature, based on a 147 day post-bloom growth period, about 100 fruits per tree (300 fruits per orchard) were harvested, treated with benomyl (600 ppm), diphenylamine (1000 ppm), and Ketrol (3000 ppm). Fruits were stored in 20 kg. cardboard boxes with perforated polyliners at -1°C and 96 % R.H. for 3 and 7 months.

Six fruits from each box (3 boxes/orchards) were washed, then rinsed with distilled water. Axial sectors were cut in 4 pieces. The pieces were replicated in 3 sub-samples, each containing 6 pear slices. Cores were removed prior to maceration. Minerals were extracted with hydrochloric acid, filtered, and analyzed on a fresh-weight basis using an inductively coupled plasma emission spectrometer. After storage, fruits of each treatment were again analyzed for minerals. Calcium is the most important element reported here, although P, K, Mg, Mn, Ca, Fe, Zn, B, and Cu were also analyzed but found to be unrelated to the parameters in this study. Automated N determinations were made with a Technicon autoanalyzer.

Once fruit samples from all orchards were analyzed and their Ca levels known, fruits were grouped into 3 lots: high, medium and low, averaging 5,3: 3,3: and 1,9 mg of Ca/100 g of fresh weight, respectively.

Samples of 6 fruits (about 1 kg), 3 replicates, were placed into 4-liter glass jars, and sealed with large rubber stoppers containing inlet and outlet tubes. Respiration was measured and air flow rate was maintained at 150 ml per minute per container to insure that CO_2 accumulation did not exceed 0.4%. CO_2 was measured by an infrared gas analyzer.

One ml gas samples were drawn by syringe from the outlet tubes of the jars and evolved ethylene was measured with a Carle 311 flame ionization gas chromatograph.

CO_2 and C_2H_4 evolution was monitored only the first and eighth days at 20°C ripening, following harvest and after 3 and 7 months in storage. Simple linear correlations were calculated.

Internal ethylene (10 fruits per treatment) was extracted by a modification of the method of Bussel and Maxie (5) and described by Vaz (27).

Fruits from the same 10 orchards containing different Ca levels were analyzed as above and used to study the relationship of Ca, N and N/Ca with incidences of cork spot, respiration and ethylene production. Respiration, ethylene production and internal ethylene for the ripening study were measured every 2 days at 20°C for 14 days after harvest, and after 3 and 7 months in storage.

Fruits were evaluated for cork spot incidence at harvest and after 7 months in storage. The fruits with superficial cork and those with deeper spots were rated as cork spot fruits.

Results

The incidence of cork spot of D'Anjou pears closely correlated with decreasing fruit calcium concentrations (Tables 1, 2, and 3) at harvest ($r = -0.68$) and was even stronger after 3 and 7 months storage ($r = -0.83$ and -0.80 , respectively). Nitrogen did not correlate significantly with cork spot incidence. The N:Ca ratio, however, correlated poorly and positive ($r = 0.52$) with cork spot at harvest but was much stronger after 3 and 7 months storage ($r = 0.72$ and 0.77 , respectively).

Fruit Ca was always negatively correlated with respiratory CO_2 evolution (Tables 1, 2 and 3) the first day placed in 20°C ripening at harvest, after -1°C storage 3 or 7 months (r -values = -0.78 to -0.84). CO_2 evolution rates at the climacteric peak, typically on day 8 of ripening, also correlated negatively with fruit Ca concentration. Ethylene evolution on days 1 and 8 of ripening after 3 and 7 months storage (Tables 2 and 3) was parallel to the CO_2 relation with fruit calcium and the correlations were even stronger (r -values = 0.80 to 0.91). Thus, the more calcium, the lower the incidence of cork spot disorder, and the lower the respiration rate and ethylene evolution. Nitrogen alone had little, if any, influence. The high correlation with N:Ca ratio is probably due to the Ca alone.

Respiration. Fruit Ca affects D'Anjou pear fruit respiration in three ways: a) it alters the time needed in cold storage to initiate the climacteric response; b) it affects the maximal

rates of CO_2 produced; and c) it shifts the time to reach the climacteric peak.

D'Anjou pears with a normal amount of Ca (more than 5 mg Ca/100 g) usually required 50 to 60 days of -1°C storage to be capable of ripening with good quality (7). Pears with low Ca (1.9 mg Ca/100 g fresh wt) were already able to ripen at harvest and went through a climacteric rise in respiration (Figure 1). Pears with 3.3 or 5.3 mg Ca/100 g fresh wt were not capable of ripening at harvest even after 13 days at 20°C . CO_2 rates were 6 to 8 mg CO_2 /kg-hr for the 3 to 5 mg Ca fruits, but CO_2 peaked at 19 mg CO_2 /kg-hr for the low Ca pears on day 11.

After 3 months at -1°C storage (Figure 2), the low Ca pears reached 40 mg CO_2 /kg-hr on day 7, whereas the intermediate 3.3 Ca pears reached 22 mg CO_2 on day 9 and the high 5.3 mg Ca pears reached 14 mg CO_2 on day 13.

After 7 months storage (Figure 3), fruit ripening at 20°C still had low Ca pears respiring most rapidly, but by day 5 rots and fruit breakdown were so severe that measurements were stopped. Medium Ca pears peaked at 21 mg CO_2 on day 5, whereas high Ca pears peaked at 18 mg CO_2 on day 9. The low Ca fruits had already gone through the climacteric in storage and were quite senescent. Chlorophyll loss was nearly complete in the low Ca fruit, while the medium Ca fruit was showing some color loss, and the high Ca pears were still green and very firm prior to ripening. No rots or breakdown were present in the medium and high Ca pears.

Ethylene. Even with no chilling treatment, low Ca D'Anjou fruit produced ethylene at harvest (Figure 4), maximizing to 10 ul C_2H_4 /kg-hr on day 11 at 20°C. Pears with greater than 3 mg Ca produced virtually no ethylene at harvest. Storage for 3 months at -1°C (Figure 5) satisfied the chilling necessary for C_2H_4 synthesis in all fruit regardless of Ca concentration. However, low Ca pears produced the most C_2H_4 : 45 ul/kg-hr on day 7. Medium Ca fruit peaked at 21 ul/kg-hr on day 9. High Ca pears scarcely produced 1 ul C_2H_4 /kg-hr until day 7, rising to 14 ul C_2H_4 /kg-hr on day 13. After 7 months cold storage (Figure 6), the low Ca pears were senescent and producing 26 ul C_2H_4 /kg-hr on day 5. Medium and high Ca pears peaked at 14 ul C_2H_4 on day 5 and 20 ul C_2H_4 on day 9, respectively. At this late stage of storage, high Ca fruit still were able to make C_2H_4 and ripen to good quality. Fruit with lower Ca had already spent much of their ripening capacity.

Internal ethylene concentrations paralleled the ethylene evolution patterns for various fruit Ca levels at harvest (Figure 7) and after 3 months (Figure 8) and 7 (Figure 9) months storage. Low Ca pears at harvest showed a rise in internal ethylene to about 30 ul/l at 11 days of ripening. D'Anjou pears above 3 mg Ca/100 g fresh wt had no increase in internal C_2H_4 at harvest. After 3 months storage, low, medium and high Ca pears had peak internal ethylene after 5, 9 and 13 days respectively (Figure 8). After 7 months internal C_2H_4 had decreased in the low and medium

Ca pears, both of which had weak peaks at 3 and 5 days, respectively. High Ca pears still had high internal C_2H_4 which peaked at day 9.

Discussion

Fruit calcium concentration clearly has dramatic effects on the incidence of cork spot disorder, ethylene synthesis and respiration rate. Symptoms of cork spot increase as fruit Ca concentrations decrease. This has been shown previously for bitter pit in apples (8, 9, 19) and for D'Anjou pears (1, 23, 24). Although some symptoms appear at harvest, much more appear as storage duration increases. Thus, a model for predicting cork spot disorders based on prestorage fruit Ca analysis appears feasible. Although high N in fruits has been associated with bitter pit of apples (13), there was no significant relationship between fruit N and cork spot of pears in this study.

Respiration rates and C_2H_4 increased considerably and the onset of the climacteric rise was earlier as fruit calcium concentration was less in this study. This is similar to previous reports in apple (10) and in D'Anjou pear (29). This study was the first to demonstrate the effects of low Ca on decreasing the time of the chilling requirement, and helped to explain some of the season-to-season variations in storage time to undergo ripening that has been observed. Clearly there would be advantages to segregate D'Anjou pears by Ca concentration in order to market the low Ca fruit early as it will not keep as well as high Ca fruit. In other words, Ca plays a major role in retarding fruit ripening and senescence.

Physiologically, Ca has been implicated in maintenance of cellular integrity via membrane stabilization (17) and effects on

permeability (3). Apple fruits with bitter pit showed some disintegration of the plasmalemma and tonoplast membranes (2). Low Ca fruit membrane instability may be an important factor in the accelerated senescence observed in such fruit which seldom survives cold storage of 3 months. Such fruits were rapidly subject to physiologic breakdown, susceptible to fungal attack (26) and ripen with poor quality even without obvious cork spot symptoms.

Calcium was strongly associated with C_2H_4 production. When Ca was deficient, C_2H_4 was greatly enhanced. Ethylene production by cork spot D'Anjou pears was 4 times that of normal pears (15). Ethylene of bitter pit-affected apples was also higher than normal fruit (10).

D'Anjou and other winter pear varieties such as Bosc and Comice are known to require varied periods of cold storage to ripen satisfactorily (6, 7). This study showed that fruit Ca concentration in some way regulated the chilling requirement for ripening of pears. Previously, Wang et. al. (28) reported detectable C_2H_4 in mature D'Anjou pears at harvest which increased to 13 $\mu\text{l}/\text{kg}\text{-hr}^2$ after 14 days at room temperature, 20°C. It would have been interesting to have measured the fruit Ca concentration of those fruits, as it is highly likely that they were Ca deficient. High Ca in pear fruits not only suppressed C_2H_4 production, but also delayed the onset of the climacteric rise in respiration. Since ripening is initiated by a threshold value of internal C_2H_4 that accumulates in the fruit (14), our data relating fruit Ca to internal ethylene and respiration rates agreed with that concept.

The rate of internal C_2H_4 increase of D'Anjou pears was faster than evolved C_2H_4 . The shape of both internal and evolved C_2H_4 curves (over time) was similar. Although other reports (1) indicated an effect of apple fruit Ca on internal C_2H_4 , our data indicated that there are also differences, but they are not large. There was a dramatic effect of pear Ca concentration on the time required both in storage and ripening out of storage on maximal C_2H_4 production. Low Ca pears made C_2H_4 right after harvest, with no chilling requirement. High Ca pears did not make C_2H_4 at harvest, but developed and retained C_2H_4 synthesis quite late in storage. Medium Ca pears were intermediate in their pattern of C_2H_4 . A study by Chen et. al (7) measured internal C_2H_4 in stored D'Anjou pears in two seasons. In one season, peak internal C_2H_4 (5 ppm) occurred after 60 days in storage, whereas in another season, peak production to the same 5 ppm C_2H_4 was only reached after 90 days in storage. Although fruit Ca was not measured in that study, other fruits measured those seasons gave indications that the 90-day peak internal C_2H_4 pears may well have had higher fruit Ca than the pears exhibiting a C_2H_4 peak at 60 days in storage.

Although we expected some interaction between fruit N and respiration and ethylene as reported previously (11, 24), the effects of fruit N on cork spot, respiration and C_2H_4 were rather insignificant in our study.

Conclusion

Pear fruit Ca concentration was a major factor in determining susceptibility to cork spot at harvest and after storage. Calcium had great effect on the magnitude of respiration, internal ethylene and ethylene production in D'Anjou pear fruits, as well as on the time to reach the climacteric. The chilling requirement of D'Anjou fruits appeared to be related to the Ca content in the fruit tissues. Ripening and senescence of the fruit was delayed when Ca was present in increasing numbers. Nitrogen did not affect the incidence of the disorder, respiration or C_2H_4 evolution of the fruits.

This data emphasizes the importance of establishing adequate Ca concentration in D'Anjou pears if they are to be stored successfully for long periods of time.

Table 5.1 Simple linear correlations between respiration incidence of cork spot and Ca, N, and N/Ca ratio of D'Anjou pears at harvest.

	Initial CO ₂	CO ₂ After 8 Days	Cork Spot
Ca	-.78**	-.75*	-.68*
N	-.52 NS	-.60 NS	-.24 NS
N/Ca	+.70*	+.68*	+.52 NS

Significant at ** = 1% level; * = 5% level; and NS = not significant.

Table 5.2 Simple linear correlations between respiration, ethylene production, and Ca, N, and N/Ca ratio after 3 months in storage of D'Anjou pears.

	Initial CO ₂	CO ₂ After 8 Days	Initial C ₂ H ₄	C ₂ H ₄ Peak After 8 Days
Ca	-.81**	-.79**	-.80**	-.83**
N	-.31 NS	-.50 NS	-.39 NS	-.45 NS
N/Ca	+.76*	+.73*	+.77**	+.72*

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Table 5.3 Simple linear correlations between respiration, ethylene production, incidence of cork spot, and Ca, N, and N/Ca of D'Anjou pears after 7 months in storage.

	Initial CO ₂	CO ₂ After 8 Days	Initial C ₂ H ₄	C ₂ H ₄ After 8 Days	Cork Spot
Ca	-.84**	-.83**	-.87**	-.91**	-.80**
N	-.38 NS	-.13 NS	-.29 NS	-.40 NS	-.12 NS
N/Ca	+.75*	+.78**	+.83**	+.87**	+.77**

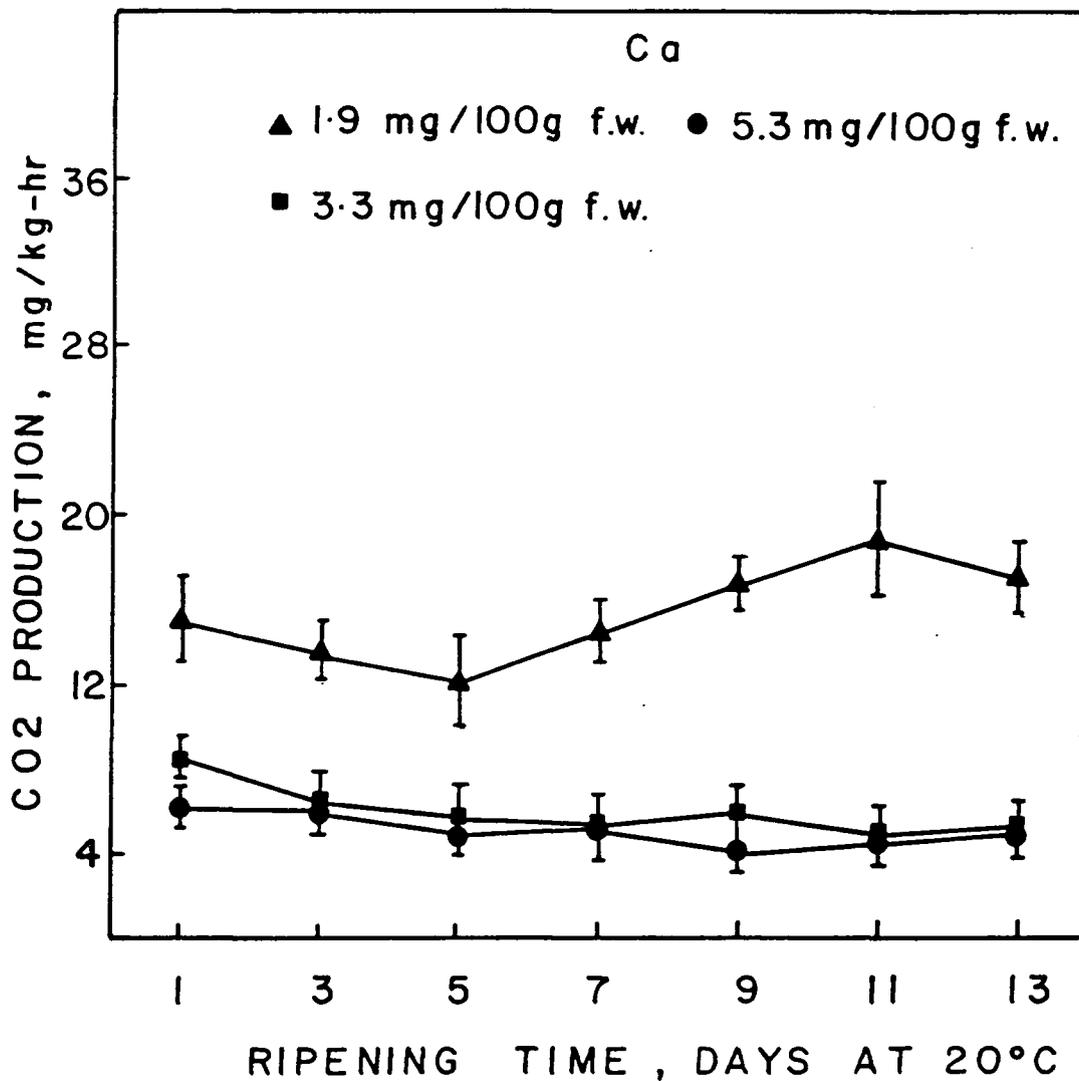


Figure 5.1 Respiratory climacteric of D'Anjou pear fruits in relation to Ca⁺⁺ concentration in the fruit at harvest.

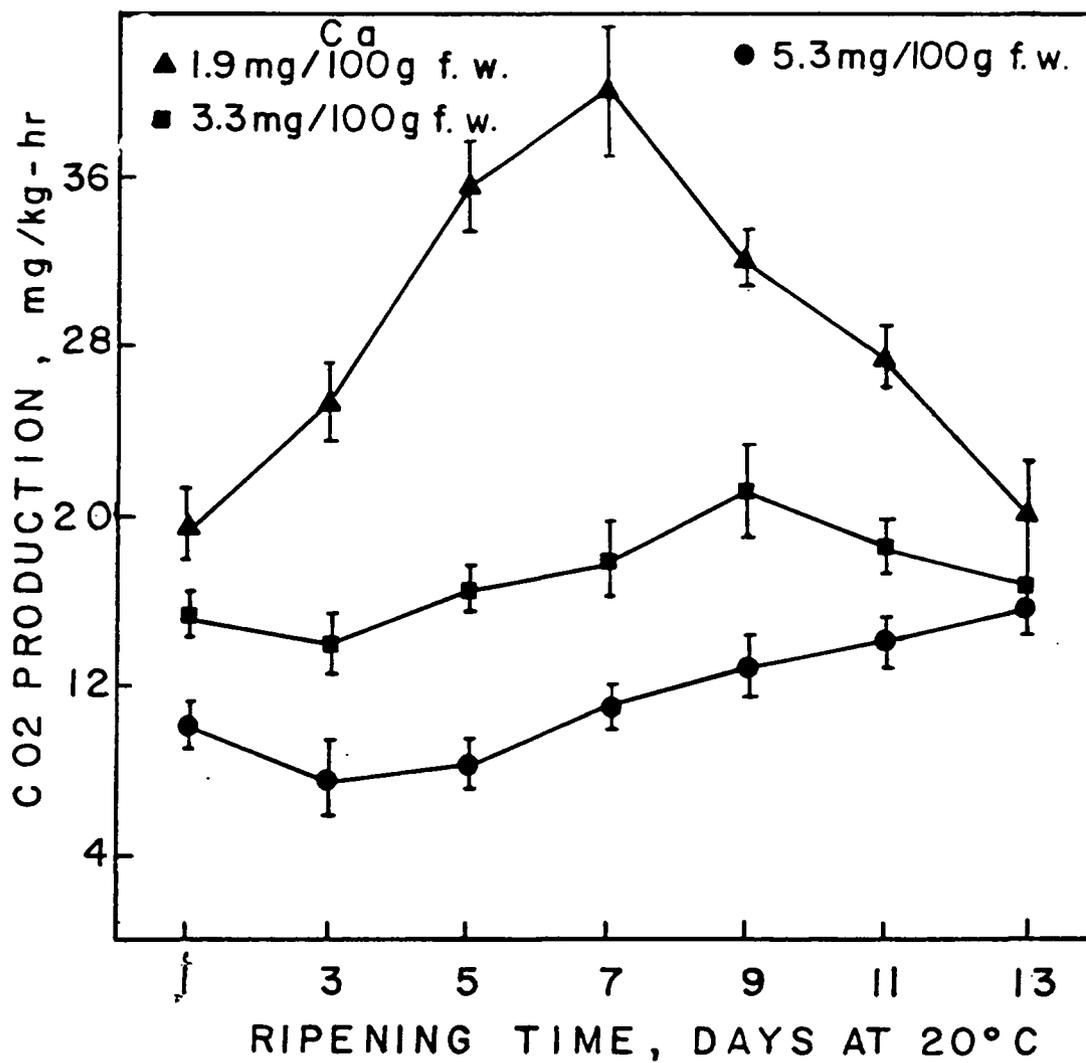


Figure 5.2 Respiratory climacteric of D'Anjou pear fruits in relation to Ca⁺⁺ concentration in the fruit after 3 months at -1°C.

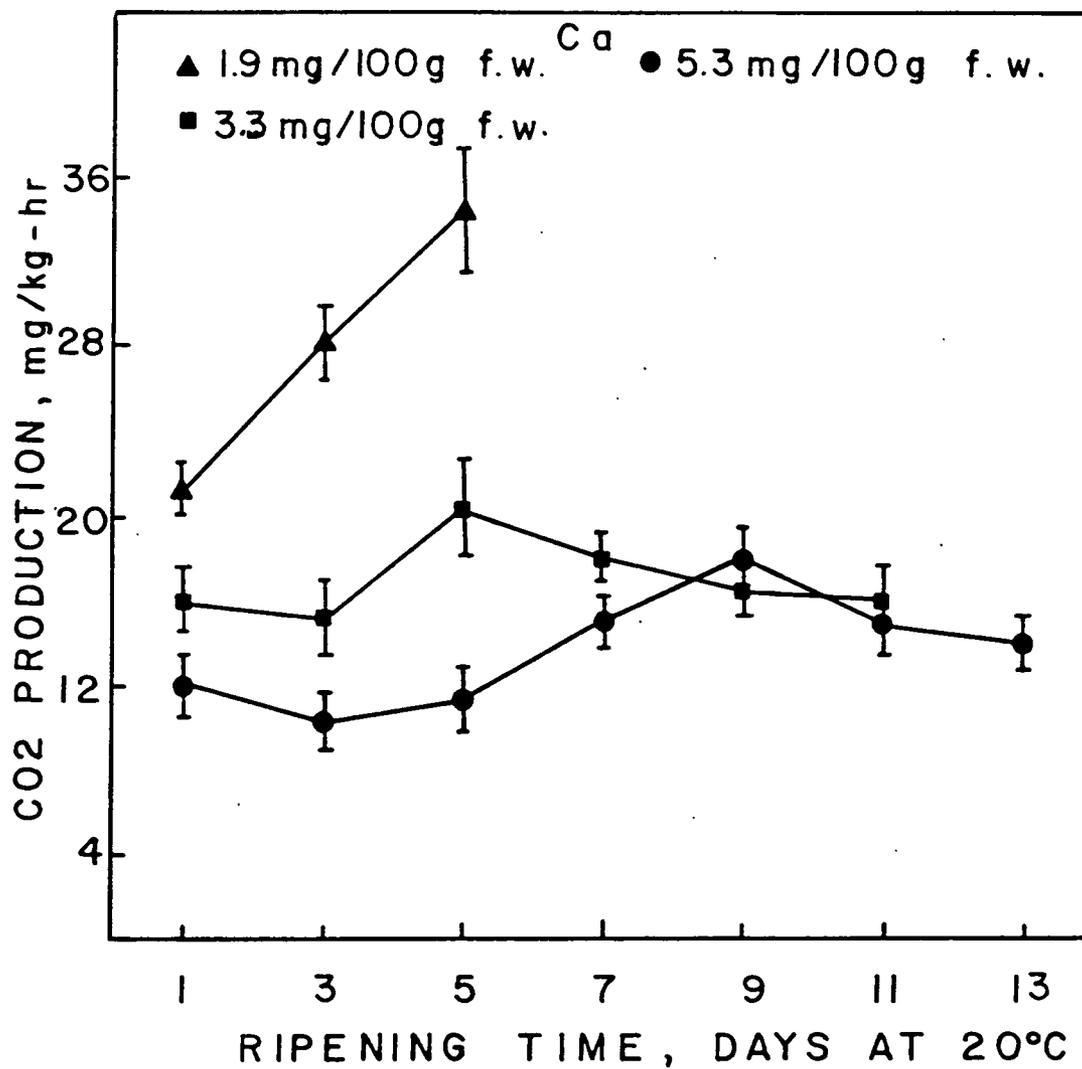


Figure 5.3 Respiratory climacteric of D'Anjou pear fruits in relation to Ca⁺⁺ concentration in the fruit after 7 months at -1°C.

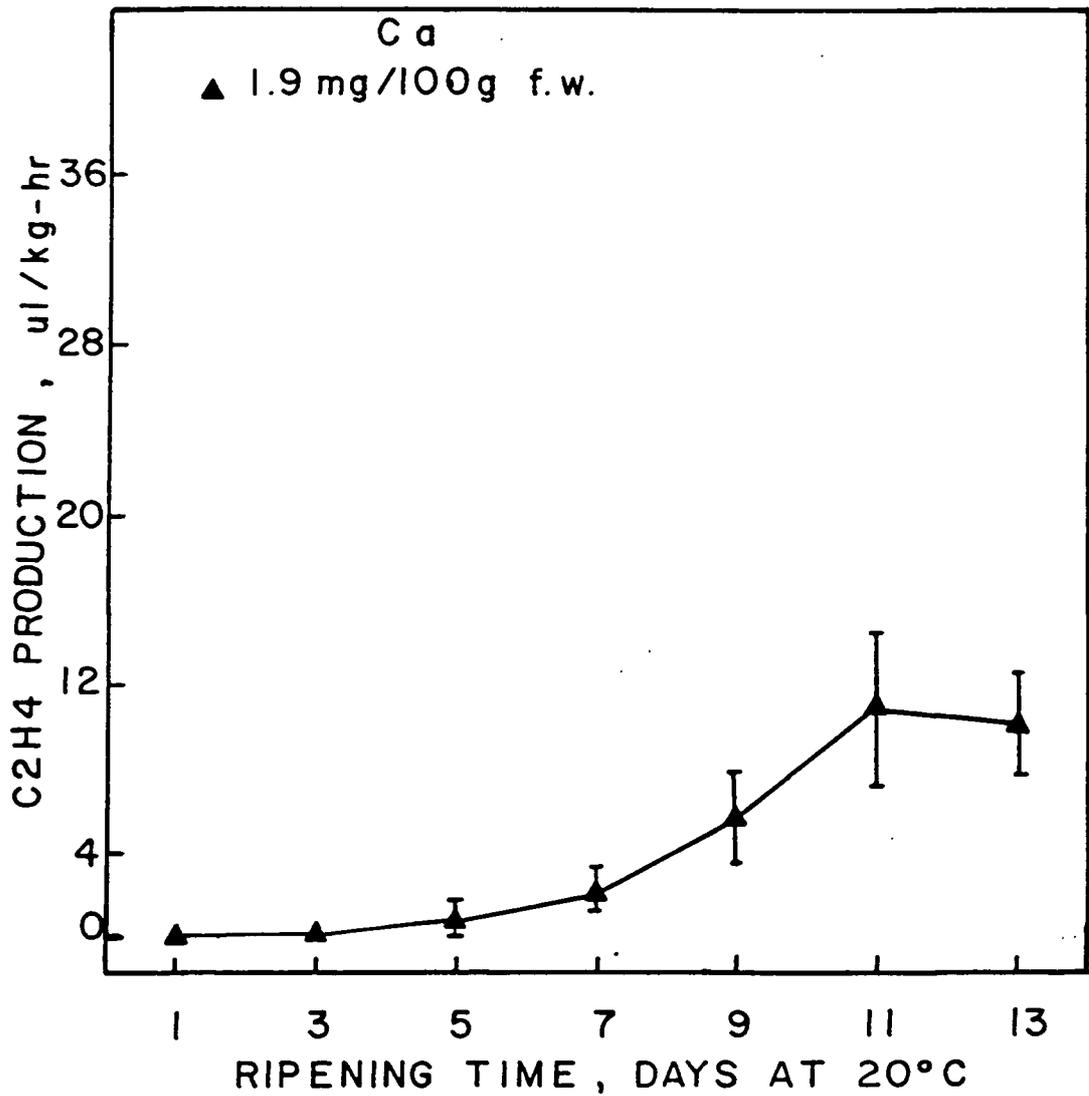


Figure 5.4 Ethylene evolution of D'Anjou pear fruit in relation to Ca^{++} concentration in the fruit at harvest.

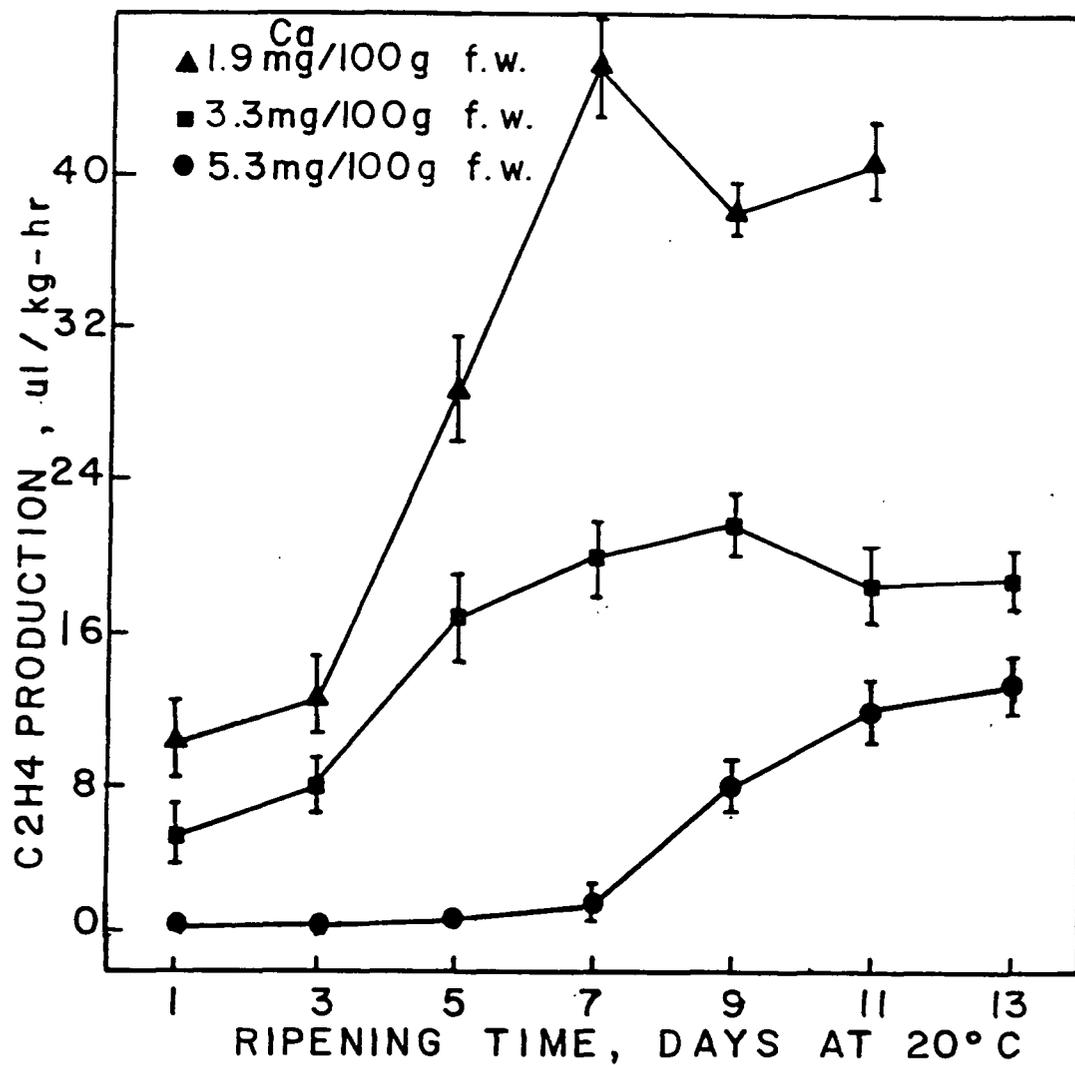


Figure 5.5 Ethylene evolution of D'Anjou pear fruit in relation to Ca⁺⁺ concentration in the fruit after 3 months at -1°C.

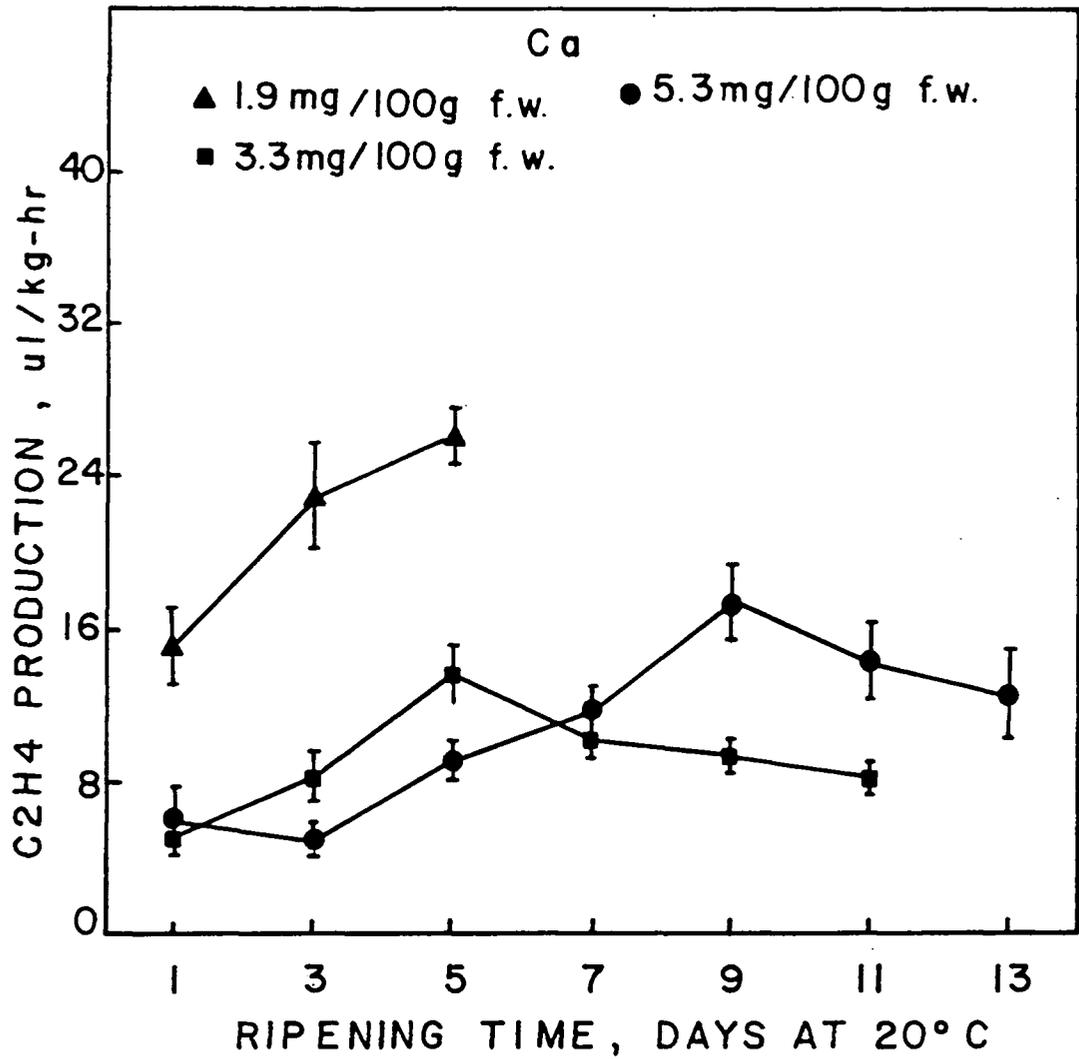


Figure 5.6 Ethylene evolution of D'Anjou pear fruit in relation to Ca^{++} concentration in the fruit after 7 months at -1°C .

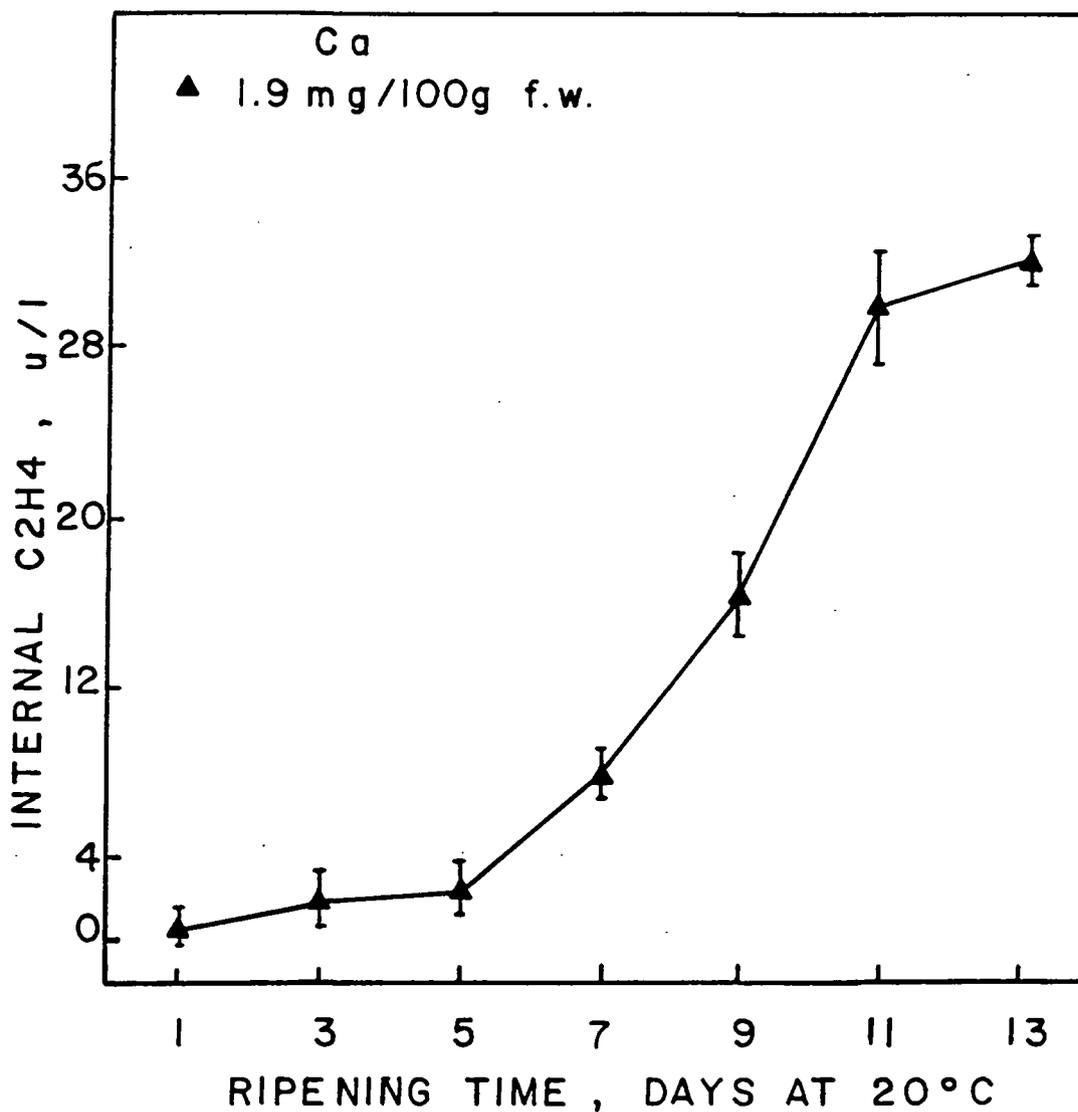


Figure 5.7 Relationship between ethylene concentration in the internal atmosphere of D'Anjou pear fruit during ripening and the concentration of Ca⁺⁺ in the fruit at harvest.

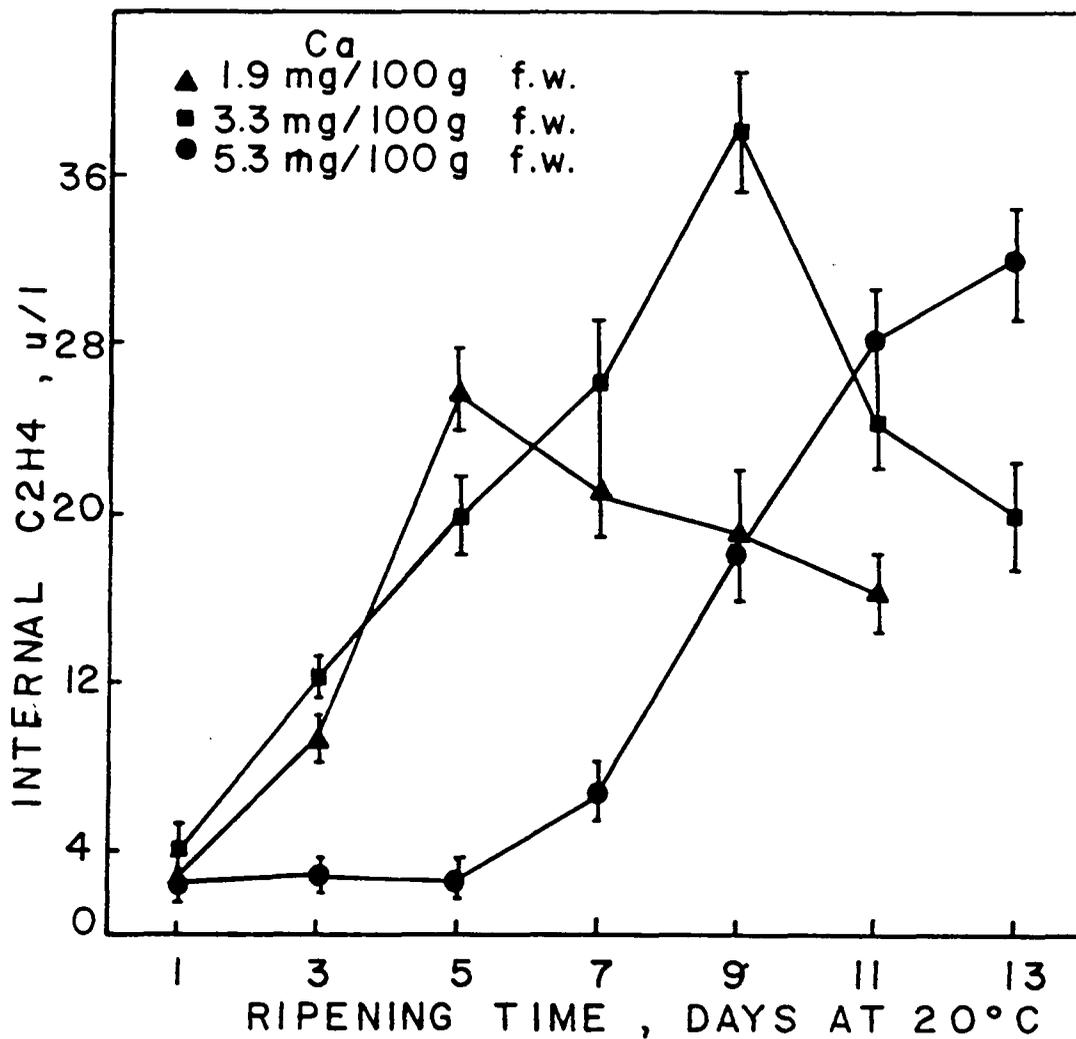


Figure 5.8 Relationship between ethylene concentration in the internal atmosphere of D'Anjou pear fruit during ripening and the concentration of Ca⁺⁺ in the fruit after 3 months at -1°C.

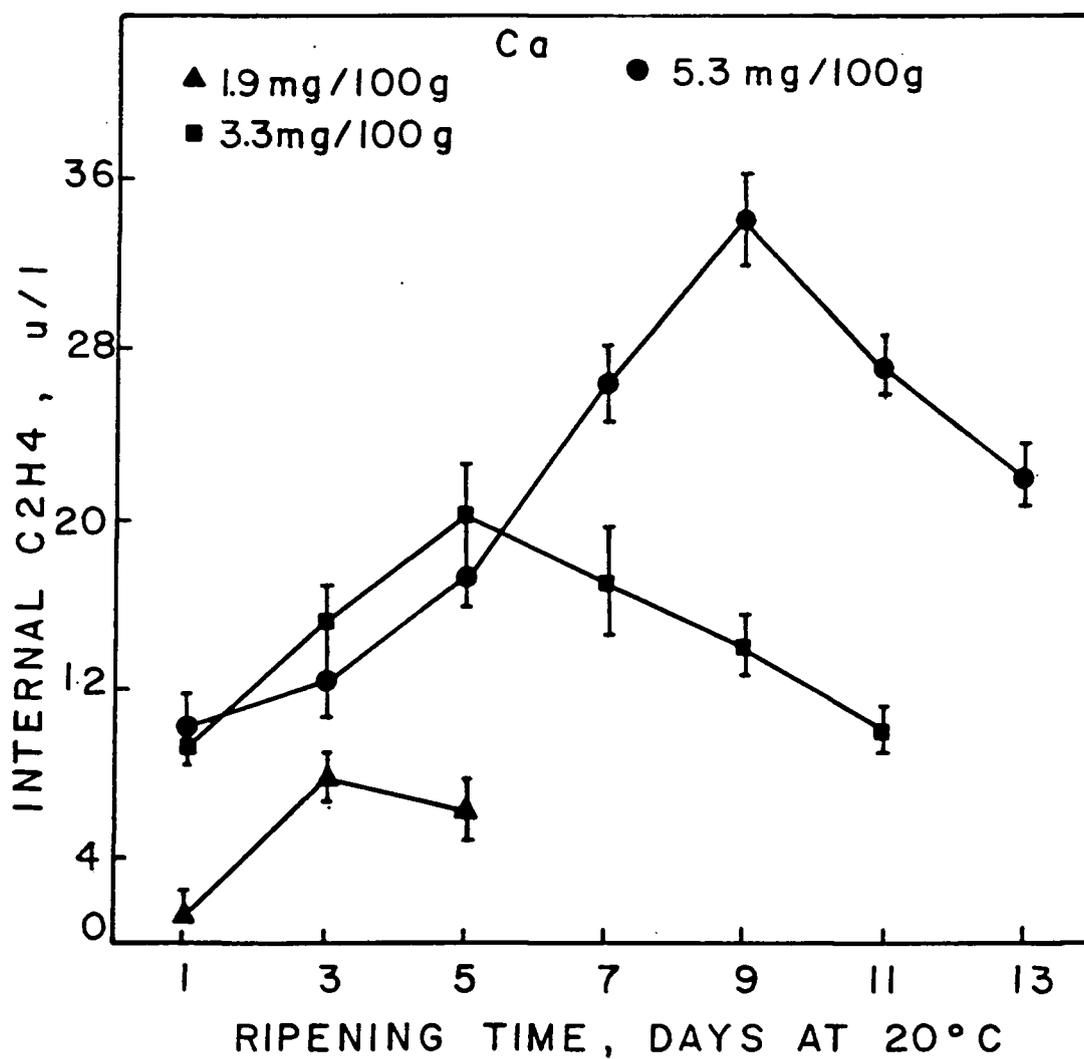


Figure 5.9 Relationship between ethylene concentration in the internal atmosphere of D'Anjou pear fruit during ripening and the concentration of Ca⁺⁺ in the fruit after 7 months at -1°C.

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CHAPTER VI

INTERACTION BETWEEN CHILLING REQUIREMENT FOR D'ANJOU PEAR
FRUIT RIPENING, ETHYLENE SYNTHESIS, AND RESPIRATION
WITH FRUIT CALCIUM CONCENTRATION

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Additional index words. Pyrus communis, postharvest physiology,
storage, fruit nutrition, quality, ACC.

Abstract

D'Anjou pears normally require 60-70 days at -1°C after harvest to initiate C_2H_4 production and consequently the ripening process. We have found that fruit Ca concentration affects this process. Fruit containing low (1.9), medium (3.3), and high (5.3 mg Ca/100 gr fresh weight) Ca were stored for 30, 40, 50, 60, and 70 days at -1°C and 96% RH. Respiration rate and C_2H_4 evolution were measured every two days for 13 days at 20°C following the respective storage periods. ACC was also measured. Following harvest, low Ca fruits initiated C_2H_4 production after 5 days at 20°C , and after 11 days, a production of $10.5 \mu\text{l}/\text{kg}\text{-hr}$ was measured. After 30 days in storage, still only the low Ca fruit produced C_2H_4 . Medium Ca fruits started to produce C_2H_4 after 50 days storage, although it was only about $0.06 \mu\text{l}/\text{kg}\text{-hr}$ after 9 days at 20°C . High Ca fruits started C_2H_4 production only after

70 days storage and the magnitude of its evolution relatively small as compared with low and medium fruit Ca. Respiratory activities of the fruits were also affected by Ca levels in the fruits. The lower the fruit Ca level, the greater the total CO₂ evolution as well as the earlier they reached the climacteric peak, once transferred to 20°C. High fruit Ca delayed the onset of the climacteric. The possible Ca effect on the ACC conversion to ethylene is suggested.

Introduction

Some pear cultivars, particularly the winter types (21) have a special cold requirement before fruit ethylene production begins and normal ripening can take place at 20°C. Different cultivars have different chilling demands (12,13,14,28,47), which may vary from different growing locations (46) and from season to season (48). Anjou pear fruits normally need to be kept in -1.1°C storage for 50 to 60 days (11,12,13) although some times they initiate ethylene production after 30 days (14) and even right after harvest without been subjected to cold treatment (52).

The ripening capacity of pears after varying lengths of time in cold storage can be determined by the production of external ethylene and CO₂ at 20°C. Exposure to low temperatures activates the fruit's ability to produce ethylene by temperature dependent metabolic reactions (43). However, the physiological basis and the mechanism of low temperature induction of ripening is still unknown in pears. Ethylene, a plant hormone, is involved in and is produced in association with ripening of fruits and senescence of plant tissues (2,10,32,37,40).

Calcium is known as a retardant of senescence (35,36) and is a major factor in preventing certain physiological disorders in apples and pears (16,20,31,34,39,48,49,50). The postharvest storage life of Anjou can be extended when Ca⁺⁺ is present in high concentration in fruit tissues (38,48,49). Anjou fruits containing very low Ca⁺⁺ have been shown to produce considerable amounts of ethylene (38,48) and undergo normal ripening following harvest

without any cold treatment (48,52). It was thought that Ca^{++} may be related to the low temperature requirement of Anjou pears. This report presents evidence that Ca^{++} somehow alters the cold period required to develop ripening capacity of Anjou fruits and influences the senescence processes during storage and ripening.

Materials and Methods

Three uniform, mature d'Anjou pear trees were selected from each of 10 commercial orchards near Hood River, OR in 1983. Fruits were harvested at commercial maturity, (62 newtons firmness) and about 100 fruits per tree (300 fruits per orchard) were treated with benomyl (600 ppm), diphenylamine (1000 ppm) and keltrol (3000 ppm). Fruits were stored at -1°C and 96% RH until used for study.

Six fruits from each box (3 boxes/orchard) were extracted for Ca content by HCl extraction of fresh pears and analyzed using an inductively coupled plasma emission spectrometer. Once fruit samples from all orchards were analyzed and their Ca levels known, fruits were grouped into 3 lots: High, medium, and low, averaging 5.3; 3.3; and 1.9 mg of Ca/100 g of fresh weight, respectively.

Samples of 6 fruits (about 1 Kg), 3 replicates, were placed in 4 liter glass jars, and sealed with large rubber stoppers containing inlet and outlet tubes. Air flow rate of 150 ml per minute per container was maintained to insure that CO_2 accumulation did not exceed 0.4%. CO_2 was measured by a Beckman Model 865 infrared gas analyzer. One ml gas samples were taken from the outlet tubes of the jars and evolved C_2H_4 was measured with a Carle 311 flame ionization gas chromatograph.

To determine the number of days that Anjou pears required to be at -1°C before they produced ethylene, a number of fruits were held at 20°C for ripening, following harvest, and after 30, 40,

50, 60, and 70 days in storage. Measurements of CO_2 and C_2H_4 production were carried out every 2 days for 13 days.

To study the Ca relationship to C_2H_4 and ACC production, fruits containing medium Ca were dipped in 3% CaCl_2 , left to dry out for 1 hr then stored for 4 months, together with the medium and high Ca fruits. Low fruit Ca was not used for this study since they do not survive long periods of storage (48). C_2H_4 and ACC were measured every 2 days over an 8 day ripening period.

Endogenous ACC concentration was measured using 12 grams flesh composite of 3 fruits, replicated 4 times per treatment, homogenized in perchloric acid (final concentration 6%) with a Brinkmann polytron. The homogenate was centrifuged at 10,000 x g for 10 minutes and the supernatant passed through a column of DOWEX 50W -X4 (H^+ form). Amino acids were eluted with 2N NH_4OH . The eluent was dried under vacuum and the residue dissolved in 0.5 ml distilled water. ACC was then measured according to the method of Lizada and Yang (27).

Results and Discussion

Fruits low in Ca (i.e., 1.9 mg/100 F.W.) had already attained full ripening capacity at harvest as demonstrated by CO_2 and C_2H_4 production at 20°C (Fig.6.1), without the recommended and usual cold treatment necessary to activate the ripening mechanism of Anjou fruits (11,12,13). There was a climacteric rise in respiration from CO_2 production initially at 15 mg/Kg-hr, decreasing to a minimum climacteric after 5 days at 20°C, then rising to 18.5 mg/kg-hr after 11 days. The C_2H_4 production was already detectable at day 5, the increase paralleling CO_2 evolution and reaching 10.5 $\mu\text{l}/\text{kg-hr}$ after 11 days at 20°C. This climacteric fruit behavior at harvest was not observed in fruits having medium and high Ca; 3.3 and 5.3 mg/100 F.W., respectively.

Ethylene production by d'Anjou fruits right at harvest without any chilling treatment was previously reported for fruits with cork spot (38) a physiological disorder associated with low fruit calcium. Wang, et al (52) reported detectable amounts of ethylene production in mature Anjou fruits 7 days after harvest, which increased to about 13 $\mu\text{l}/\text{kg-hr}$ after 14 days at room temperature. Although no mention was made of the fruit's mineral levels, it is believed they were particularly low in Ca content. This might have been the reason for the unusually early and high ethylene production in d'Anjou pears without the cold treatment.

After 30 days storage at -1°C, d'Anjou fruits presented the same climacteric behaviors as at the time of harvest, with the difference that the magnitude of values for CO_2 and C_2H_4 produc-

tion were much higher (Fig. 6.2). However, those fruits with higher calcium levels were still not able to undergo normal ripening, and no detectable ethylene production was found. There was a clear difference in the amounts of CO_2 production for the fruits containing the 3 different Ca levels.

Forty days cold treatment (Fig. 6.3) showed that only the 1.9 mg Ca/100 g F.W. fruits were able to ripen, but the 3.3 and 5.3 mg Ca/100 g F.W. fruits despite producing CO_2 at slightly higher rates than at harvest or after 30 days, still did not exhibit a rise in respiration and ethylene production was below detectable limits.

The calcium effect on altering the chilling requirement was again evident after 50 days in storage at -1°C (Fig. 6.4). The low Ca fruits continued to ripen normally upon transfer to 20°C . However, the medium Ca fruits began their ethylene production after day 9 once transferred to 20°C , peaking at day 13 with 1.8 $\mu\text{l}/\text{kg}\text{-hr}$. There was a climacteric rise in respiration with the minimum also at day 9 and peaking day 13. High Ca pears showed no rise in ethylene or CO_2 .

After 60 days of cold treatment (Fig. 6.5) the 1.9 mg Ca/100 g F.W. fruits had their minimum CO_2 production shifted to day 3 at 20°C and the climacteric peak at day 11. The C_2H_4 production peak also occurred earlier. D'Anjou pears have been normally indicated to require 60 days at -1°C (11,13) to develop ripening capacity. It is evident however, that the relative Ca levels in fruit

tissues is a major factor in determining the permanence of the fruits in storage before C_2H_4 production is triggered.

Calcium is known as a retardant of senescence (30) and addition of Ca to deficient tissues reduces respiration and suppresses ethylene production (17,26,36). Our data are in agreement, since 5.3 mg Ca/100 F.W. fruits, which was considered for the season as a high Ca level, after 60 days $-1^\circ C$ did not show any detectable C_2H_4 production (Fig. 6.5) once placed at $20^\circ C$. They did not go through a climacteric rise and normal ripening. Their ripening capacity was only attained after 70 days in $-1^\circ C$ storage (Fig. 6.6) even though ethylene production was only detectable at day 5 at $20^\circ C$ and thereafter, the ethylene peak at day 13, $4.4 \mu l/kg-hr$ being much lower than for those fruits with lower Ca content.

There was a clear effect of Ca on respiration and C_2H_4 production demonstrated by the magnitude of CO_2 evolution initially and at the minimum or maximum peaks. The higher the fruit Ca the lower the respiration and C_2H_4 production of Anjou fruits. This agrees with previous reports (38,48). Calcium not only affected the total respiration and C_2H_4 evolution of fruits, but also the time to the onset of the climacteric. The lower the Ca, the faster the fruits reach their climacteric peak. Similar Ca effects were reported in apples (9,16) and in avocado fruits (45). These Ca effects on the postharvest behavior of d'Anjou fruits strongly indicates that more rapid senescence will occur if low Ca is present in fruit tissues. If fruits are to be stored successfully for long periods of time they should have high Ca content,

which slows metabolic reactions, delaying the senescence of fruit tissues.

The effect of low fruit Ca on decreasing the chilling requirement demonstrated by this study helps to explain some of the season to season as well as growing location variations in storage time of d'Anjou to undergo ripening (46,48), and Ca levels in the fruits are known to vary in similar fashion (42,48,50). The difference in fruit ripening behavior suggests that low Ca fruits have their C_2H_4 synthesizing mechanism fully developed already at harvest time. However, how this relates directly to fruit Ca concentration is still not known.

ACC serves as an intermediate in C_2H_4 biosynthesis (4,29). The pathway, methionine \rightarrow SAM \rightarrow ACC \rightarrow C_2H_4 has been well documented in plant tissues (54), and the key enzyme in this biosynthetic pathway has been demonstrated to be ACC synthase (7,53,55). There are many reports about Ca relationship to ACC and ethylene production (15,18,24,26), in fruit discs. Very few observations have been made on intact fruits as related to low temperature. Chilling exposure of cucumber fruits resulted in an increase in the synthesis of ACC, but the ACC \rightarrow C_2H_4 conversion step was inhibited while exposed to cold temperature (1,3,51).

The duration of cold storage has been suggested to be affecting either the ACC synthase activity and consequently ACC formation or the conversion of ACC to C_2H_4 (46,33). ACC was reported to increase in Anjou fruits during storage (11).

To study the relationship of fruit Ca to ACC and C_2H_4 production in intact fruits in relation to their ripening behavior, Anjou pears containing 3.3 mg Ca/100 F.W. were infiltrated with 3% $CaCl_2$ and stored for 4 months, then ACC and C_2H_4 production were measured and compared to non-infiltrated 3.3 and 5.3 mg Ca/100 F.W. fruits.

D'Anjou fruits produce low amounts of ACC, which makes it somewhat more difficult to study the Ca relationships. However, it could be seen (Fig. 6.7) that ACC seemed to accumulate in the tissues during storage to levels about 0.25 nmoles/g, increasing to 0.29 nmoles/g after 4 days at 20°C for 3.3 mg Ca/100 F.W. fruits. This increase followed the increase in C_2H_4 production. $CaCl_2$ infiltrated and 5.3 mg Ca fruits, however, had ACC content decreased slightly until day 6 when it increased again. This suggested that Ca might be affecting the conversion of ACC to C_2H_4 or at least delaying it when present in high levels. Low Ca fruit had more rapid conversion of ACC which coincided with the climacteric of the fruits after 6 days at 20°C room. Since fruits used in this observation had been stored for 4 months, and no ACC measurements were done during that time, little can be said about Ca effects on the synthesis of ACC.

A decrease in ACC concentration in d'Anjou pears on transfer from storage to 20°C, and non-parallel C_2H_4 production was reported before (46), which contrasts with this present finding. However, it could have been that the fruits used in that experiment had high Ca levels, which in a way agrees with our ACC levels

for the 3.3 + 3% CaCl₂ and 5.3 mg Ca/100 g F.W. fruits, whose Ca content was also high. In tissues producing C₂H₄ at a rapid rate, ACC formation precedes or parallels C₂H₄ evolution (5,8,23,56).

Physiologically, Ca has been implicated in maintenance of cellular integrity via membrane stabilization (36) and effects on permeability (22,41). Since the enzymatic conversion of ACC to C₂H₄ is believed to be cell wall or membrane bound (19), any change in the membranes may affect such conversion. Ca is likely to be related to this process.

It has been previously proposed that cold treatment may increase membrane permeability of subcellular organelles, allowing mixing of substrates, cofactors, and enzymes of ethylene biosynthesis, thus stimulating C₂H₄ production (43). If Ca is present at low levels in cell tissues, there may well be an increase in membrane permeability thus eliminating the otherwise necessary exposure to cold stress or shortening the time of this exposure (6,25,44).

Additional studies will be required before any conclusions can be drawn about the mechanism of cold-induced ethylene production and Ca relationships in d'Anjou pears.

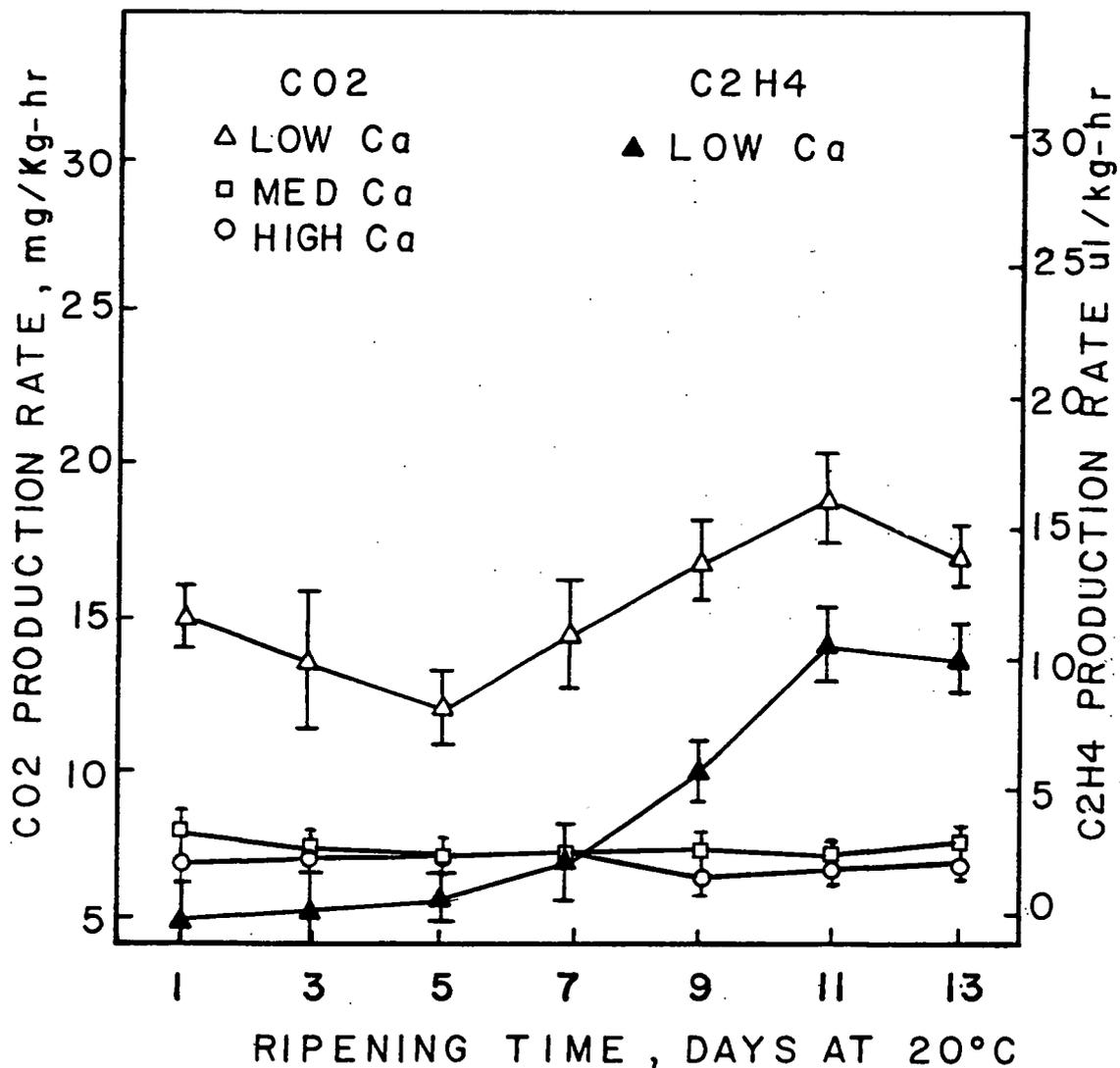


Fig.6.1 D'Anjou fruit Ca concentration and its effect on the rate of CO₂ and C₂H₄ production at harvest.

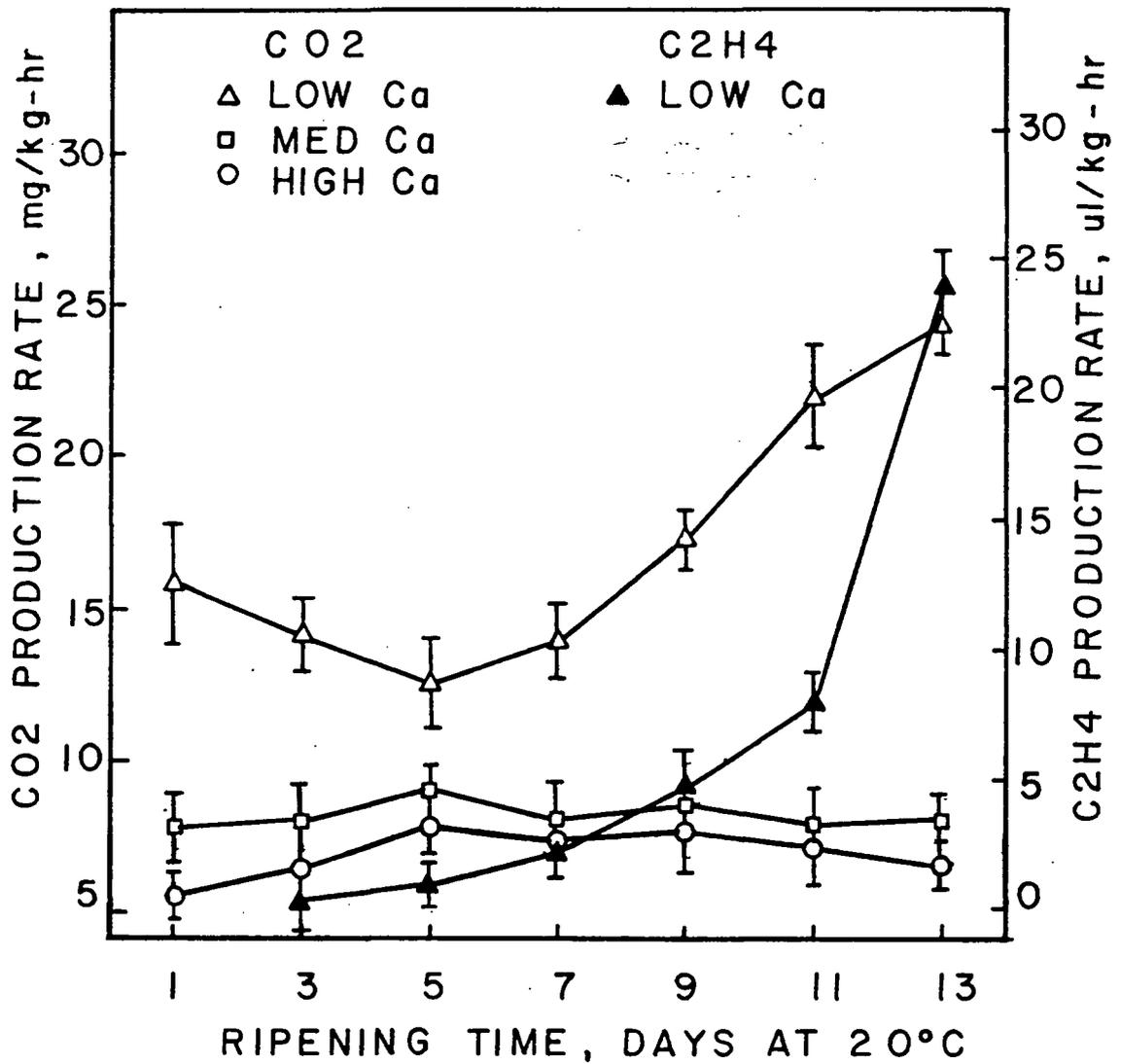


Fig 6.2 D'Anjou fruit Ca concentration and its effect on the rate of CO₂ and C₂H₄ production after 30 days at -1 °C

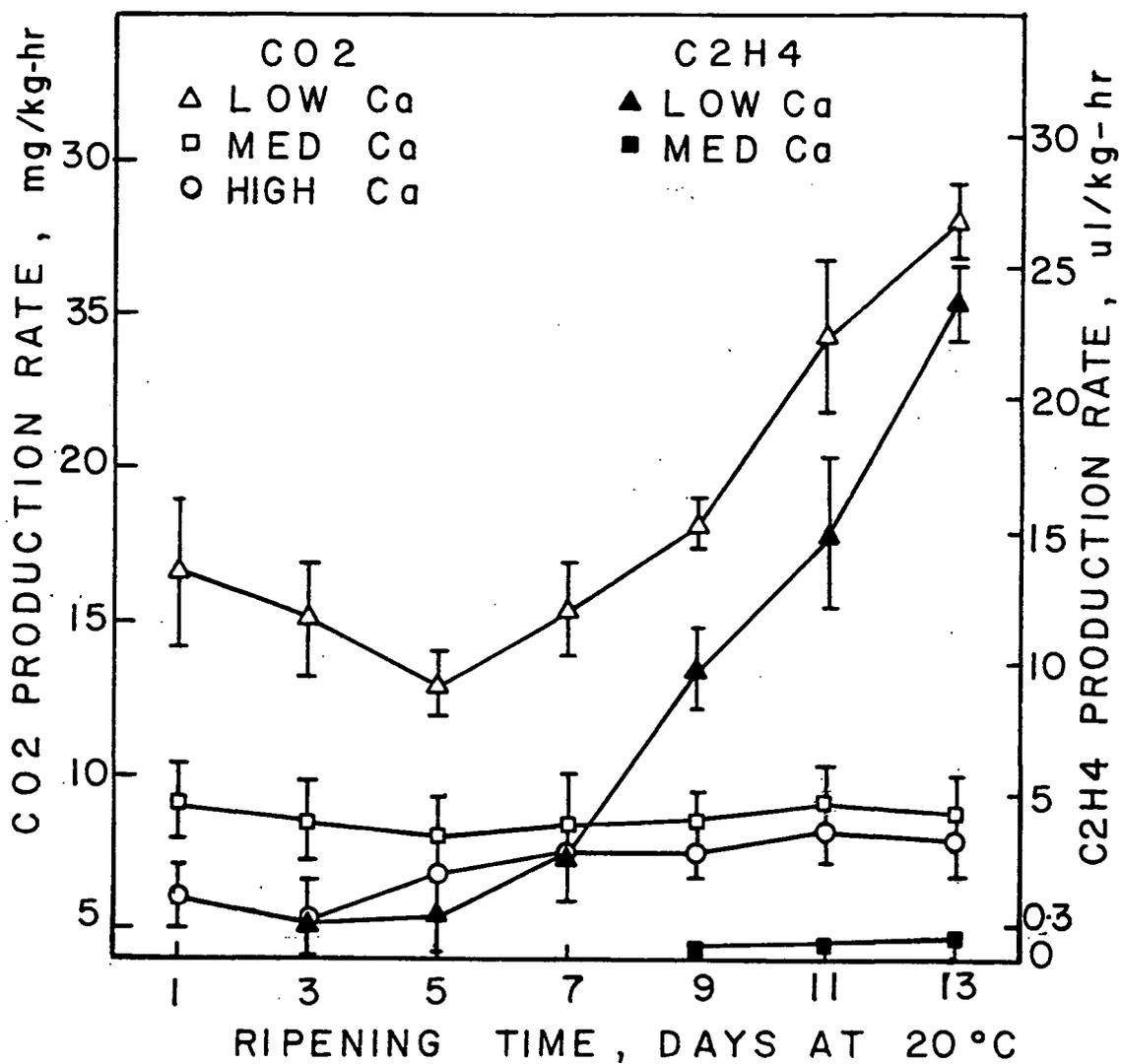


Fig 6.3 D'Anjou fruit Ca concentration and its effect on the rate of CO₂ and C₂H₄ production after 40 days at -1°C

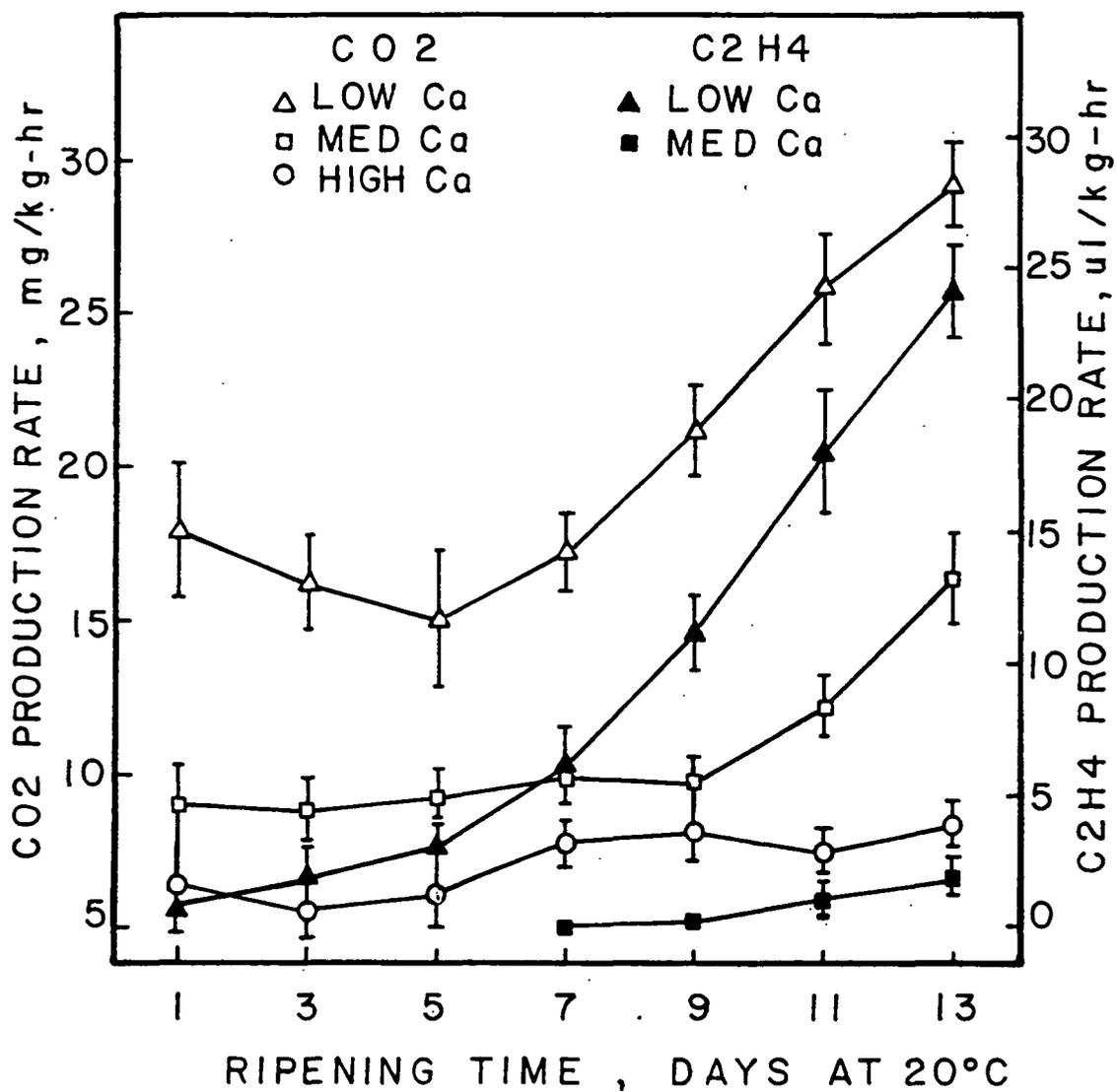


Fig 6.4 D'Anjou fruit Ca concentration and its effect on the rate of CO₂ and C₂H₄ production after 50 days at -1°C

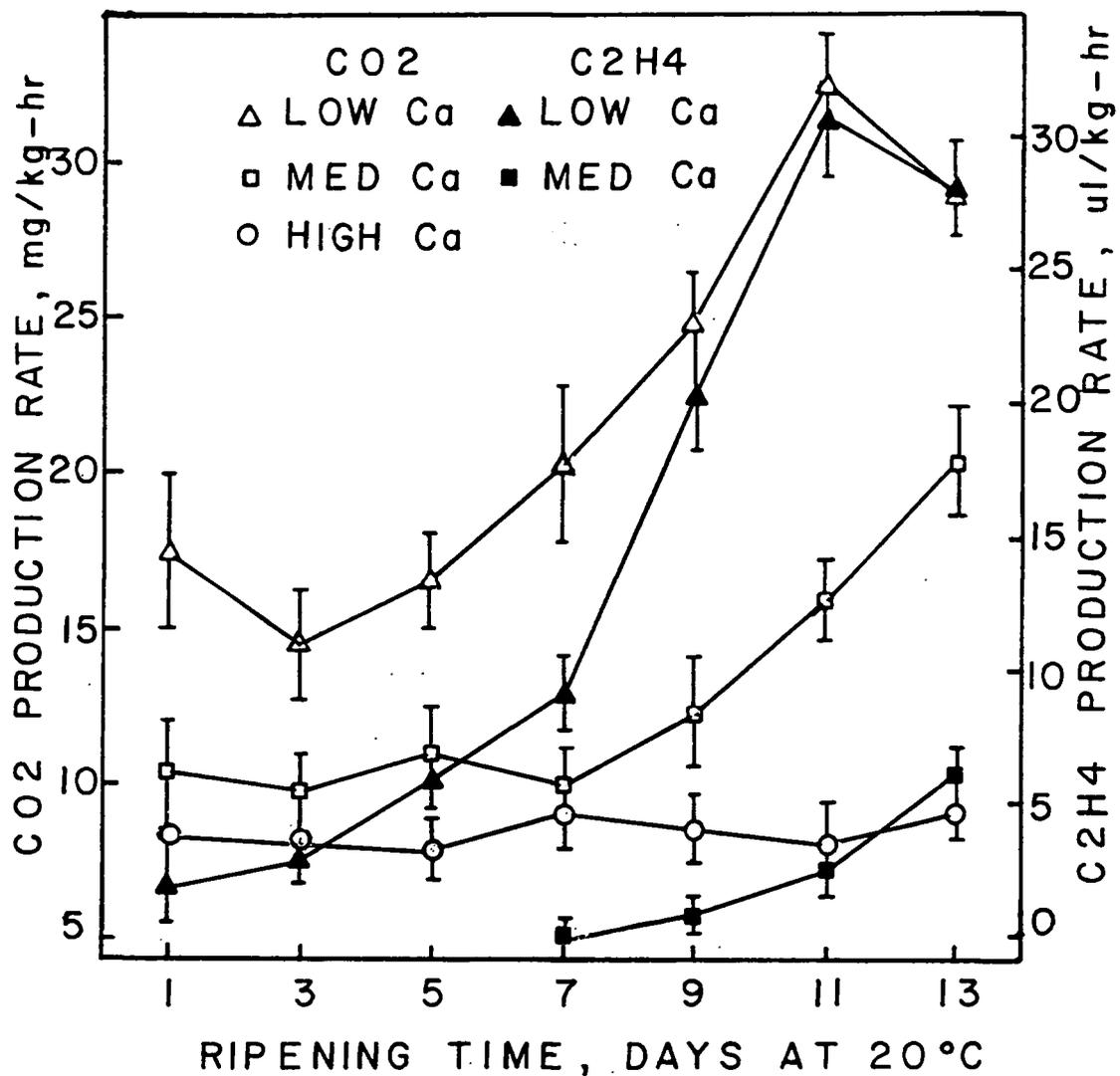


Fig 6.5 D'Anjou fruit Ca concentration and its effect on the rate of CO₂ and C₂H₄ production after 60 days at -1°C

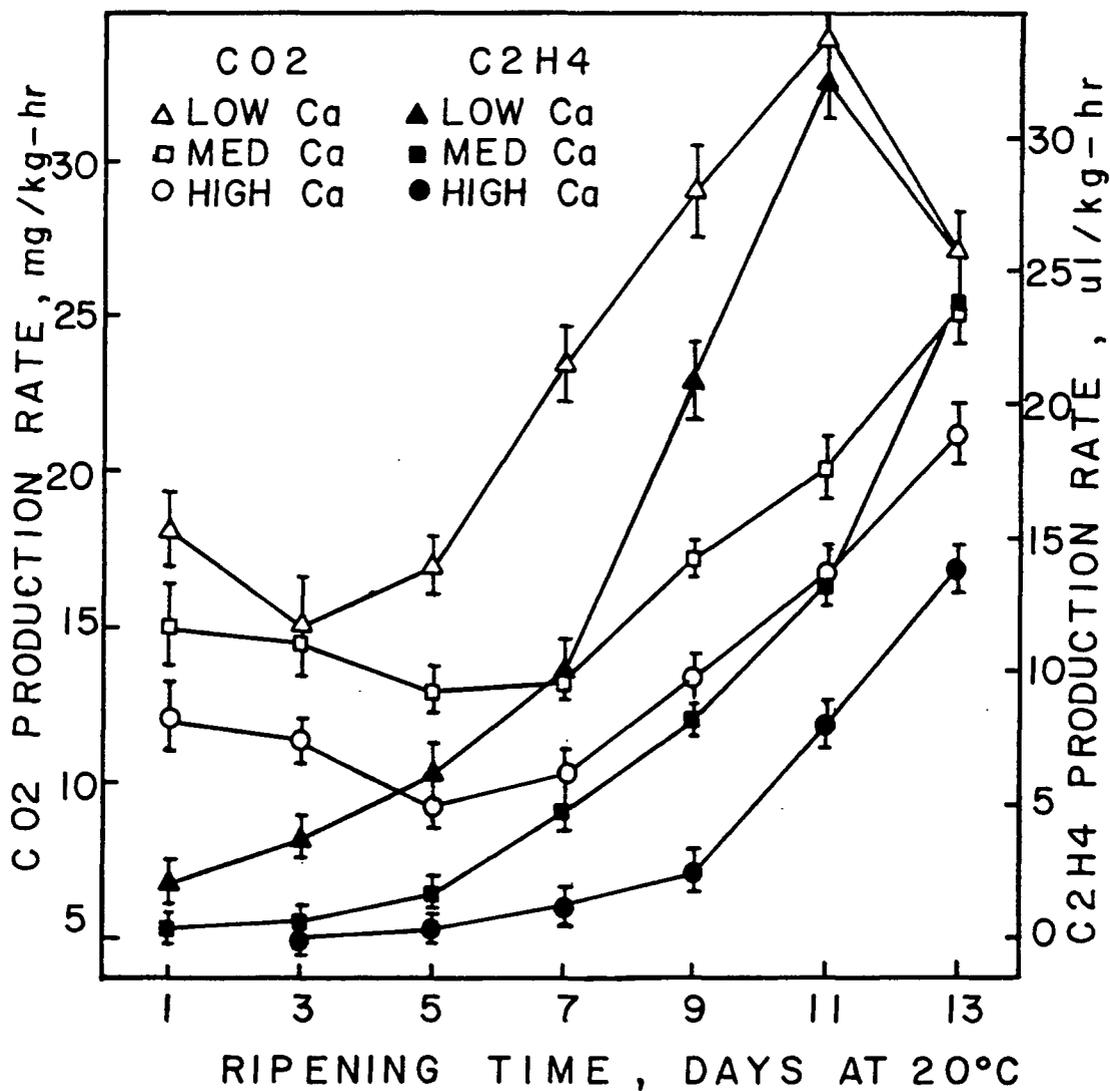


Fig 6.6 D'Anjou fruit Ca concentration and its effect on the rate of CO₂ and C₂H₄ production after 70 days at -1⁰C

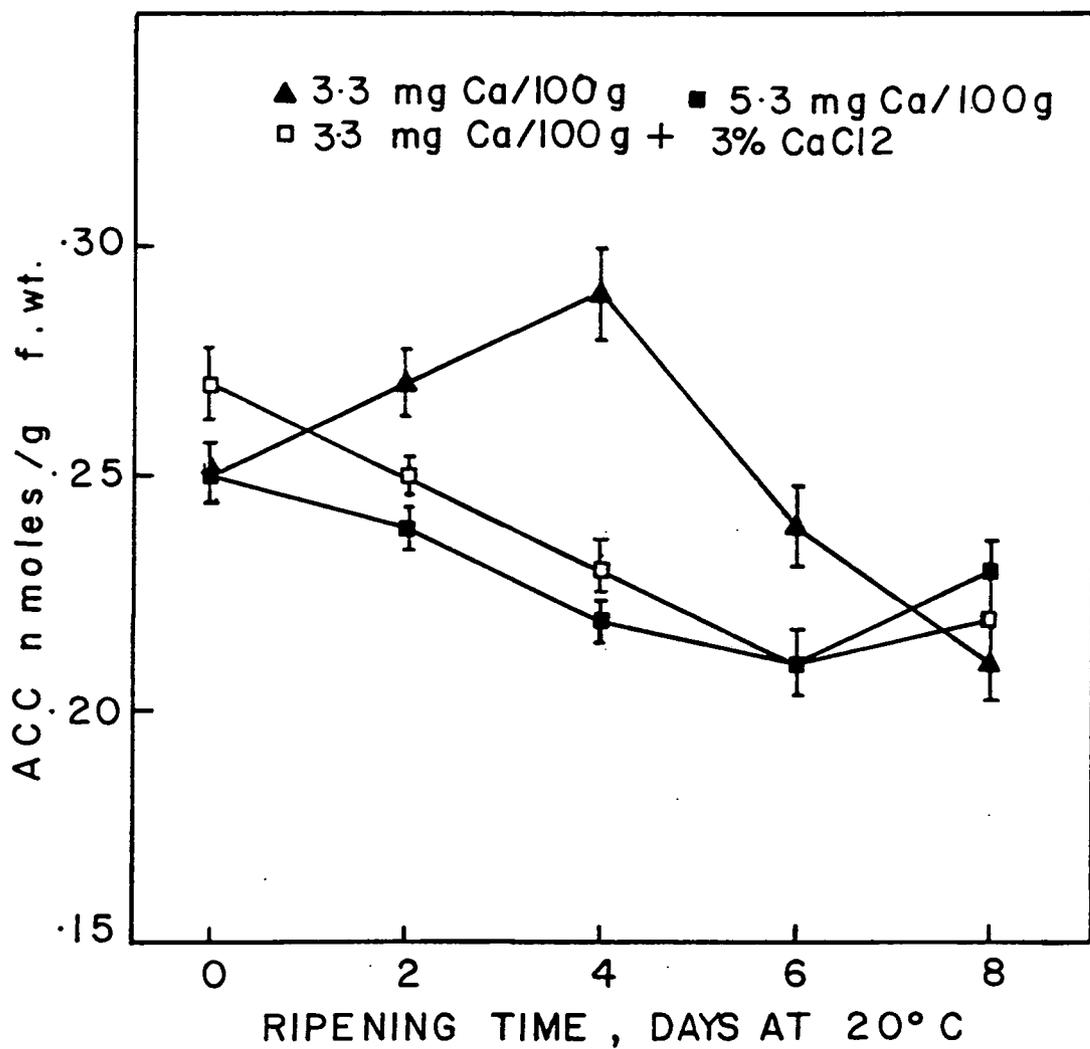


Fig 6.7 D'Anjou fruit Ca concentration and its effect on ACC levels after 120 days at -1°C

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CHAPTER VII

RELATIONSHIP OF FRUIT CALCIUM TO FIRMNESS, INTERNAL BREAKDOWN,
INCIDENCE OF ROT, GREEN COLOR RETENTION AND STORABILITY OF
D'ANJOU PEARS

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Abstract

Anjou pears sampled from commercial orchards were analyzed at harvest time and after 7 months -1°C 90% RH storage for calcium content, firmness, chlorophyll, titrable acidity and soluble solids. Firmness of Anjou pears was highly correlated with calcium, the firmer the fruit at harvest time the higher was calcium content, and also the higher the calcium the greater was the firmness retention during storage. Pear samples with greater calcium developed less internal breakdown after long-term storage. The incidence of rot was less in higher calcium fruits, and after 7 months in storage fruits remained greener and retained more acids.

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Fruits to be stored satisfactorily for long periods of time should have high amounts of calcium. Once calcium concentration is known in the fruit, it appears possible to estimate their storage potential.

Introduction

In the Pacific Northwest, despite the highly developed technology of postharvest storage in delaying senescence of pears, substantial losses of quality can occur during storage (44). Pear quality is normally defined in terms of usual commercial standards of color, size, appearance, storage potential, and organoleptic properties.

There is evidence that calcium occupies a central position in fruit nutrition and quality (7). Ca appears to have an important regulatory role in metabolism of apple and pear fruits (45).

Several investigations have found Ca to be effective in promoting firmness and delaying softening of apples (2,4,31,32,33,41) and pears (1,44) during storage. Some types of internal breakdown in apples have been indicated to be a Ca related disorder (2,6,18,20,25,32,36,40,42). The incidence of rots in apples (6,5,15,18) and pear fruits is also believed to be at least partially related to Ca, as is green color retention of apples (5,21,41) and pears (44) during prolonged storage.

The balance between minerals and organic constituents of fruits must be maintained at high concentrations if apple fruits are to be of highest storage quality (35). Fruit acidity and soluble sugars are common indicators of fruit quality and storage life (6,11,18,19,29). However, relatively few citations have discussed the relationship with calcium.

At present there appears to be a large body of data substantiating the effect of Ca on the storage life of apples. Al-

though only a few have covered the extent of the Ca relationship to the most of the fruit's quality aspects during and after storage, considering only the natural fruit Ca levels. In D'Anjou pears, little is known about this relationship. Therefore, the present investigation was aimed mainly to find out if, and to what extent, Ca is related to fruit firmness, rots, green color retention, internal breakdown, SS%, titratable acids, and postharvest losses of this pear cultivar after prolonged storage. Another paper (45) deals with the relationship of fruit Ca with cork spot, a pear storage disorder equivalent to apple bitter pit.

Materials and Methods

Three uniform and mature trees of D'Anjou pear (Pyrus communis L.) were selected in each of 10 orchards near Hood River, OR in 1983. At the initial date of commercial harvest, when fruits were 100% mature, about 300 fruits/orchard (3 boxes) were harvested, then stored in 20 kg cardboard boxes with perforated polyliners at -1°C and 96% RH for 7 months. Flesh firmness, chlorophyll content, soluble solids (SS) and titratable acids (TA) content were determined at harvest and at the end of storage when the incidence of rots and internal breakdown were also measured. Fruit firmness was also measured after 3 months in storage.

Chlorophyll. Chlorophyll measurements were used to express the green color retention of fruits throughout the storage time. Sixteen fruits were replicated in 4 subsamples. Fifteen grams of fruit skin, taken longitudinally on opposite sides of each subsample was homogenized using a Brinkmann Polytron. The chlorophyll was extracted in 80% acetone and measured in a spectrophotometer according to modifications of the methods of Bruinsma (8) and MacKinney (26), and expressed as $\mu\text{g/g}$ of peel tissue.

Firmness. Flesh firmness of fruit was determined by a UC-pressure tester (13) with an 8 mm plunger and 2 pared punches per fruit. Forty fruits from each orchard were measured each sampling period, and the firmness read as kg, then expressed as Newtons ($\text{N} = \text{kg} \times 9.84$).

Titratable acids and soluble solids. Four groups of 4 fruits from each orchard were sliced and juiced in a juicerator (Acme

Model 6001). Titrable acids were determined on 25 ml of juice to pH 7.0 using 0.1 N NaOH and calculated as milliequivalent acids per 100 ml juice. Soluble solids were measured by an ATAGO NL-1 hand refractometer and expressed as percent soluble solids.

Internal breakdown and rots. Fruits taken from storage were evaluated superficially for the incidence of rots then cut and observed for occurrence of internal breakdown (IB). The percentage was calculated from the ratio of fruits with IB or rot to the total number of fruits in the sample.

Ca analysis. Six fruits from each box were washed, then rinsed in distilled H₂O. Axial sectors were cut in 4 pieces. The pieces replicated in 3 subsamples, each containing 6 pear slices. Cores were removed prior to maceration. Ca was extracted with 12 N hydrochloric acid, filtered and analyzed on a fresh weight basis using an inductively coupled plasma emission spectrometer.

Correlation analyses were performed according to the procedures of McClave and Dietrich (28).

Results and Discussion

Flesh firmness. Although the optimum maturity stage of Anjou pear fruits at harvest has been indicated by the flesh firmness of 62.7 Newtons (N), especially if long-term storage is intended, the fruits harvested in the season 1983 were not always in that level (Fig. 7.1). High fruit Ca was firmer at harvest than lower fruit Ca. However, factors other than Ca could be related to this difference. The Ca effect is clearly evident in retaining the firmness of Anjou fruits after 3 months in storage (Fig. 7.2). There was a highly significant positive correlation ($r=.91$). The higher the Ca content, the firmer were the fruits. This effect was even more pronounced after 7 months at -1°C , when there was also a highly significant positive correlation ($r=.93$) (Fig. 7.3). Fruits containing less than $2.8\text{mgCa}/100\text{g F.W.}$ were much too soft at the end of storage to be of commercial value. Anjou pears are considered to be at the end of commercial storage life when they soften to a firmness of 44.8 N (38). Thus according to our data, low Ca levels had limited the postharvest life of D'Anjou fruits. Low Ca leads to accentuated fruit softening, thereby shortening the storage potential. Fruit with high Ca was still at 57.8 N, whereas low Ca fruit had dropped to 37.2 N demonstrating a loss in flesh firmness of 8.1 and 21.5 N respectively after 7 months in cold storage. We earlier (44) reported higher firmness values for Anjou pears after long-term storage in 1982. However, the 1982 fruit Ca levels were generally higher than for the fruits of the 1983 season which were used in this study.

This positive effect of Ca on firmness retention is in agreement with previous reports on apples (2,4,31,32,33,41,43) and pears (1,44); but contrasts with some reports where Ca was found not be significantly related to apple firmness (5,6).

The accentuated loss in flesh firmness of the low Ca fruit is most likely due to the breakdown of cell wall and cellular organization (3,9,30), resulting in more rapid senescence of the tissues (27). The loss in firmness of only 8.1 N after 7 months at -1°C of high Ca fruit suggests the very important role of this element in maintaining the texture of the cells by interaction with pectin or by improving the integrity of the cell membranes and reducing their permeability (3,9,37). Calcium may also increase fruit firmness by strengthening cell walls (1,30).

Internal breakdown. Despite scarce mention in the literature about the occurrence of the physiological disorder IB in Anjou pear fruits, considerable fruit losses were found after long-term storage (Fig. 7.4). These losses varied from 1% to 16% and were also related to the level of Ca in the fruits. Similar losses were reported before (44).

Internal breakdown which is widely recognized as a symptom of low Ca in apples (2,6,32,34,40,42), was very prevalent in samples of Anjou fruit with low Ca. There was a highly significant negative correlation ($r=-.86$) between Ca and the incidence of IB in Anjou fruits. Samples with increasing Ca concentrations had less breakdown after 7 months storage. Breakdown fruits respired faster and softened at accelerated rates (shown in previous Chapters 5

and 6), thereby greatly shortening the potential storage life. Such fruits were of very low quality which could not ripen normally once transferred to a 20°C room.

A study of fruit ultrastructural changes after harvest indicated that decreased Ca concentration in apple flesh, hastened fruit ripening (27). Therefore, since Ca is related to IB and premature senescence of D'Anjou fruit tissues (45), for long-term storage one could select for fruits naturally high in Ca content. Thus, knowledge of the fruit Ca concentration would be highly desirable for segregating fruit lots for different storage strategies.

Although the relationship of Ca to IB was demonstrated to be highly significant in the present study on pears as well as in previous reports for apples (2,6,34,36,40,42), the mechanism by which Ca prevents the development of IB in fruits is still not well characterized. Accumulation of toxic volatiles, i.e., acetaldehyde and acetic acid, has been suggested as a causative factor of IB in apples (14,46). However according to Bangerth, et al, (2) Ca inhibits IB by enhancing the uptake and compartmentation of substrates, particularly sorbitol. He proposed that sorbitol and Ca play a more important role than volatiles in the development of IB in fruits (2). More study in this subject is thus encouraged.

Chlorophyll. Green color retention of D'Anjou pear fruits after storage is also an important quality aspect which Ca seems to be affecting very closely. This relationship was expressed by

a highly significant positive correlation ($r=.87$) after 7 months in storage (Fig. 7.5). The Ca chlorophyll relationship was found to be not significant at harvest when all fruits were dark green. When Ca was present in the fruits in low amounts (less than 3 mg/100g F.W.) at the end of storage period, these fruits showed very intense yellow coloration measured by the actual amount of chlorophyll present, which was generally lower than 85 $\mu\text{g/g}$ of peel tissue. High Ca fruit peel chlorophyll was 120 $\mu\text{g/g}$ after 7 months storage. This accentuated loss of chlorophyll suggests the more advanced senescence of the fruit tissues. As the Ca concentration increases, green color was strongly retained in the fruit, resulting in much better appearance and improved quality leading to better marketable fruits. Green color retention has been used before to express the effect of Ca on the postharvest quality of apples (41) and Anjou pear (44), both reported similar Ca effects and supports the present data.

This relationship is explained indirectly since Ca has a direct influence in maintaining membrane integrity, delaying permeability changes and delaying ultrastructural changes in fruit cells (21). The effect of Ca on cell metabolism and structure not only confers great resistance to changes which precede softening, but also delays the general rate of senescence of the tissue (43), and consequently retains a greater proportion of chlorophyll originally present in the fruits.

Rots. The relationship of calcium with rots was very evident (Fig. 7.6), especially when one compares the two extremes of the

range. There was a highly significant negative correlation between Ca and the incidence of rotten fruits. With increasing Ca concentration the frequencies of rot declined sharply. At high concentration of Ca, there was a 19.5% reduction in the incidence of rot, mainly caused by Penicillium and Botrytis. The lower the fruit Ca the higher the fruit rot, suggesting an indirect relationship between this element and the susceptibility to pathogens. The results are in agreement to those found in apples (5,6,10,16, 18) and in pears (44).

The mechanism by which Ca retards fungal decay may be similar to the effect that Ca has on the mechanisms that delay ripening or senescence and softening of the fruit. Ca is known to maintain cell wall integrity (3,30,39). Since P. expansum produces enzymes that are related to cellular degradation leading to decay (17), Conway and Sams (16) suggested that Ca may maintain cell wall integrity by retarding fungal enzyme activity.

Titrateable acids and soluble solids. The relationship of Ca to TA was expressed by the highly significant positive correlation ($r=.84$). High Ca fruits retained higher levels of TA throughout the storage period, whereas those fruits where Ca was present in deficient levels (less than 3.0 mg/100g F.W.) exhibited very low amounts of TA. Retention of acids in fruits is nearly always associated with prolonged storage life (23,24). At harvest, although TA levels were higher, they did not significantly relate to Ca.

Soluble solids in the present study were not found to be significantly related to Ca either at harvest or after storage. SS content in fruits remained about the same or only slightly increased during storage

TA and SS contents are common indicators of fruit quality and storage life (1,2) which agrees with the present study. Mel-
lenthin and Wang (29) stated that Anjou pears with high acid and sugar content at harvest had better postharvest quality than those with low acid and sugar content. This is in partial agreement to our data. Higher levels are desired at harvest, but if Ca is present in deficient levels, the fruits did not maintain satisfactory TA levels throughout the storage period, rendering fruits of unacceptable quality. Fruits high in Ca and consequently high in TA content had delayed senescence as compared to those in the low Ca ranges, and quality after prolonged storage was much superior. The overall decrease in TA during the present fruit storage study agrees with previous reports (11,12,19,35). Since TA is used up more rapidly in Ca deficient fruit tissues (Fig. 7.7) as the storage duration proceeds, and these fruits are known to be at an advanced senescence stage (Figs. 7.3, 7.5, and 7.6), Ca must be affecting the TA content indirectly via preservation of cell membrane integrity (3,30) or lower cell metabolic activities (45).

Figure 7.1

D'Anjou pear fruit firmness at harvest relative to calcium concentration.

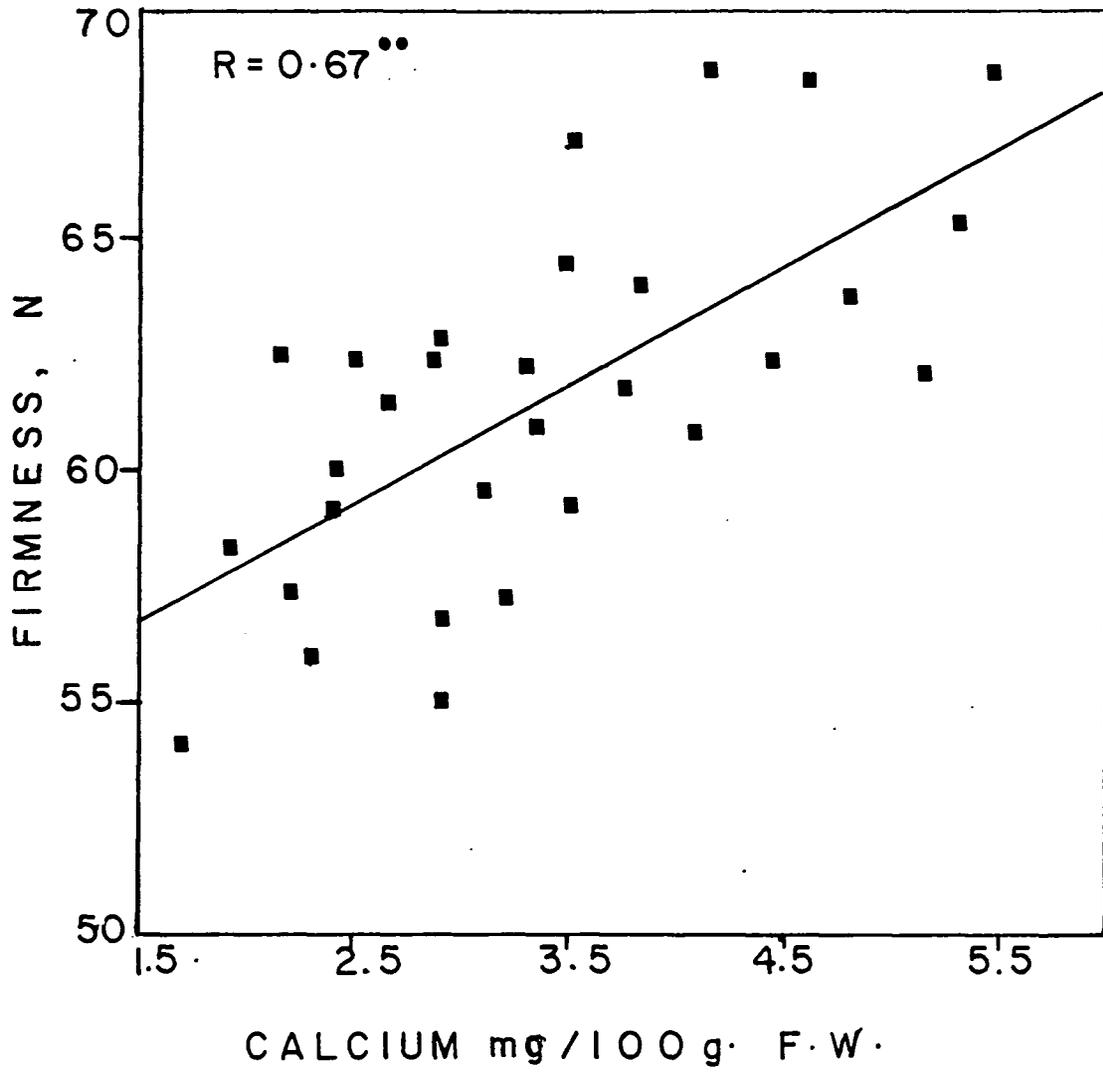


Figure 7.1

Figure 7.2

Anjou pear fruit firmness after 3 months -1°C storage relative to fruit Ca concentration.

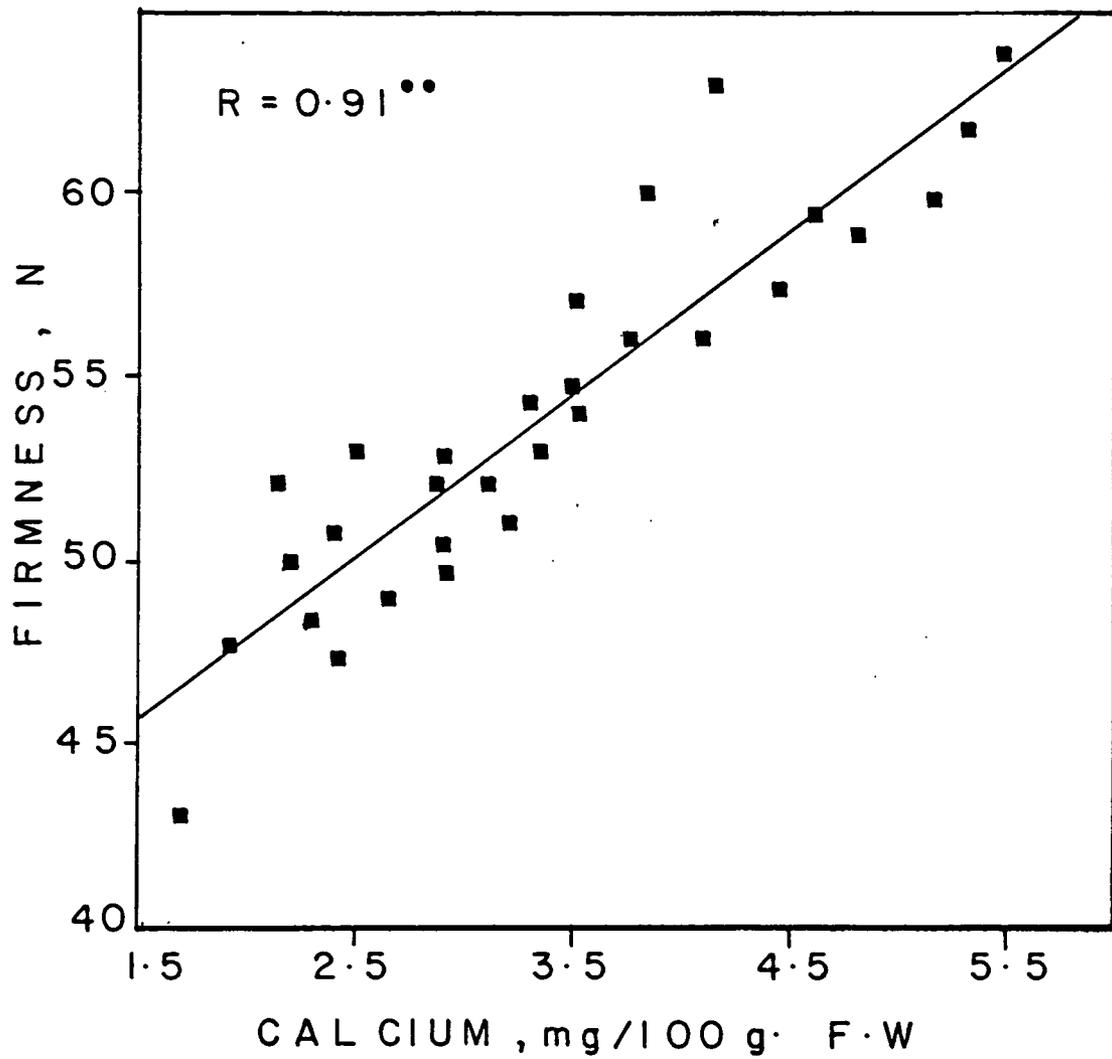


Figure 7.2

Figure 7.3

Anjou pear fruit firmness after 7 months -1°C storage relative to fruit Ca concentration.

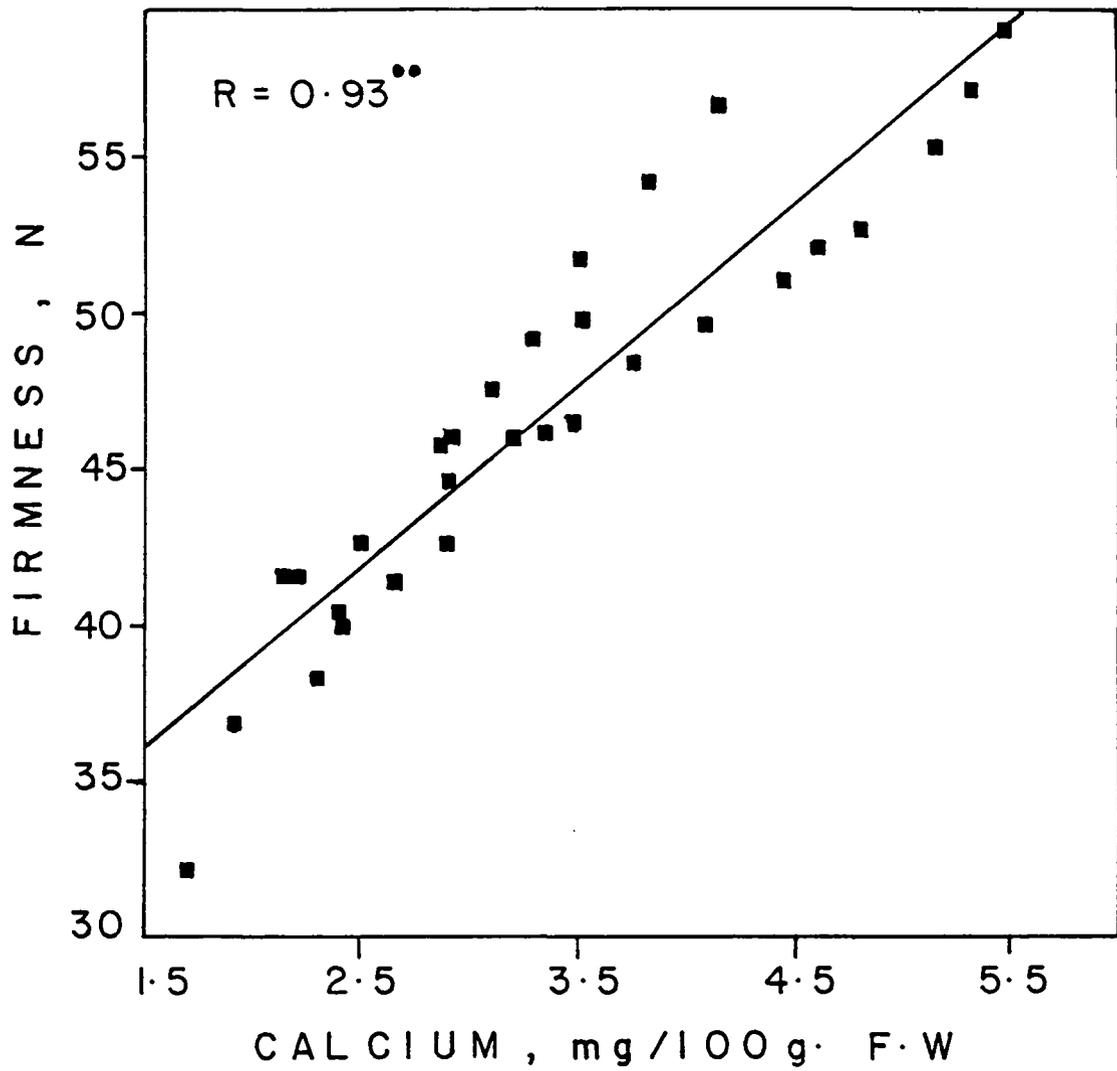


Figure 7.3

Figure 7.4

Internal breakdown of Anjou pear fruits after 7 months -1°C storage relative to fruit Ca concentration.

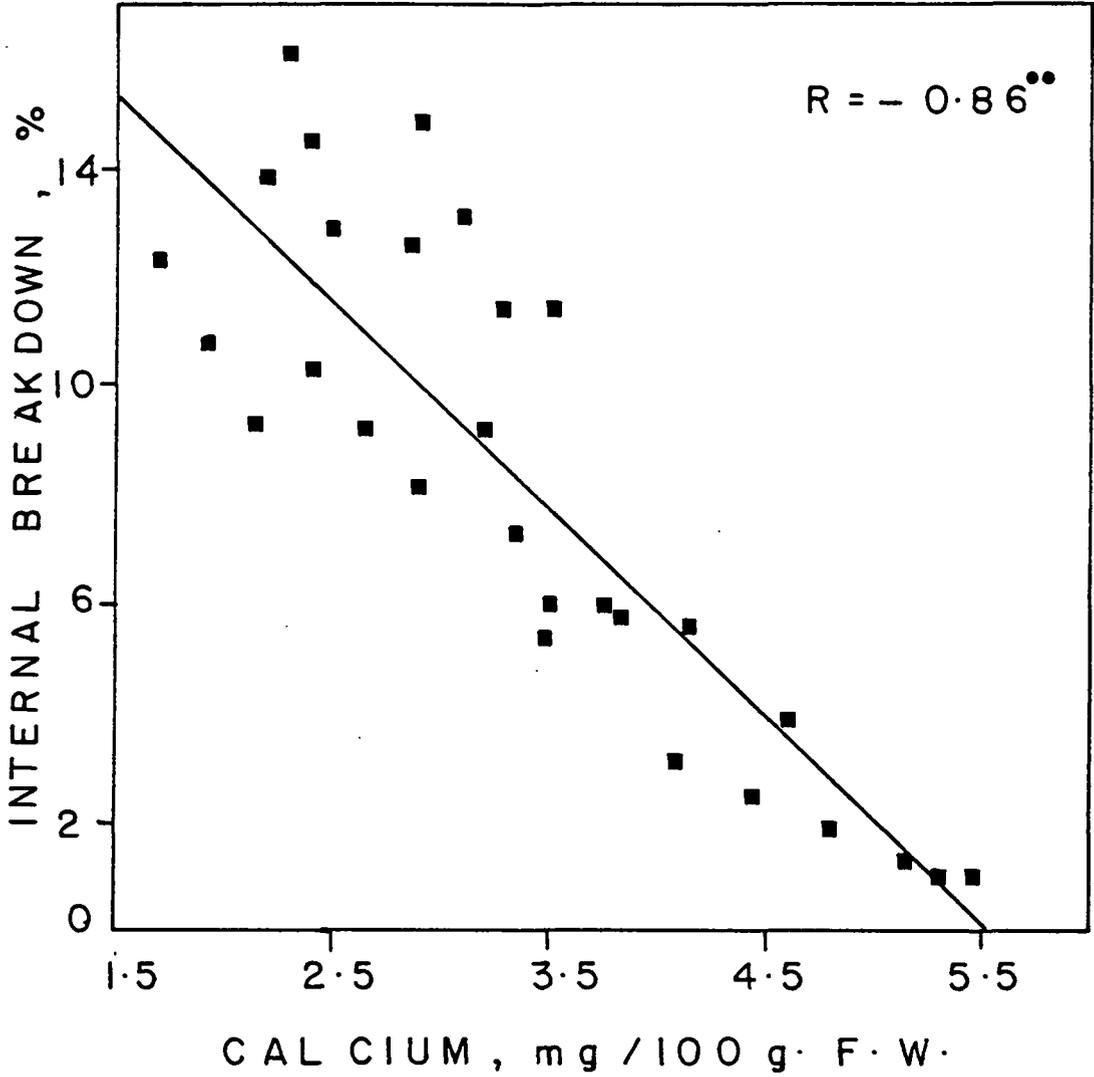


Figure 7.4

Figure 7.5

Anjou pear peel chlorophyll concentration after 7 months -1°C storage in relation to fruit Ca concentration.

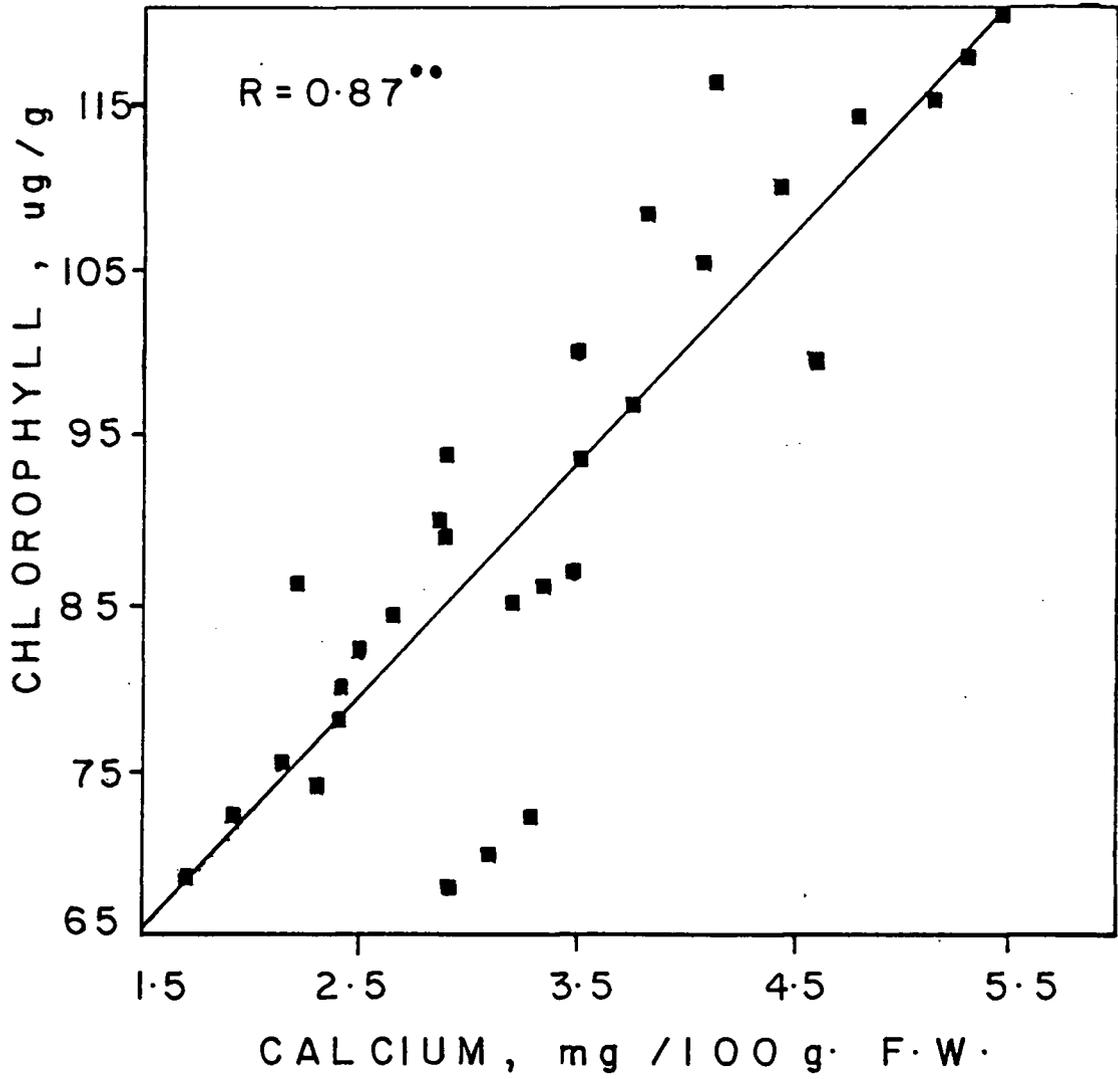


Figure 7.5

Figure 7.6

Anjou pear storage decay incidence after 7 months -1°C relative to fruit Ca concentration.

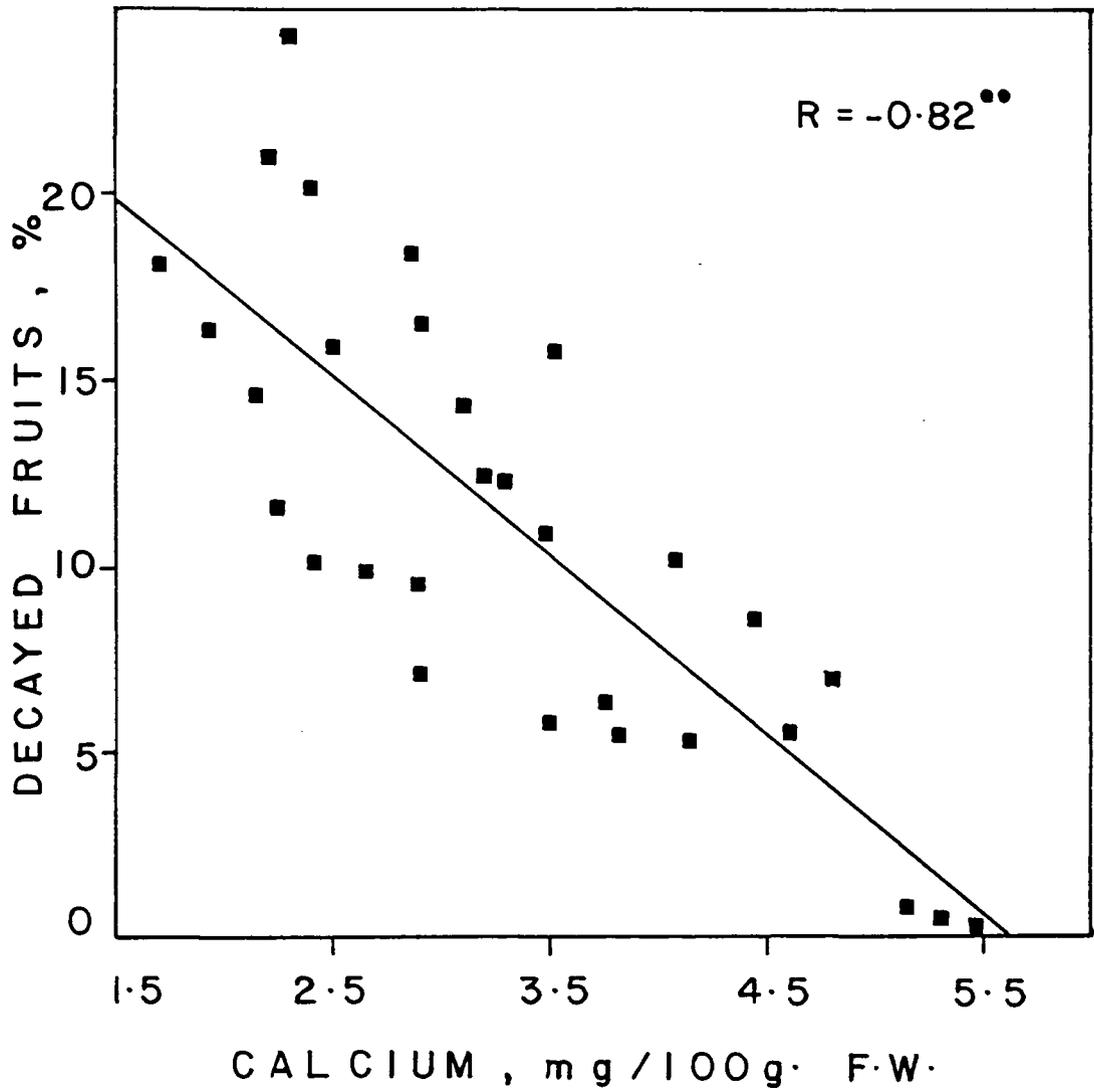


Figure 7.6

Figure 7.7

Titratable acids retention of Anjou pears after 7 months at
-1^oC relative to fruit Ca.

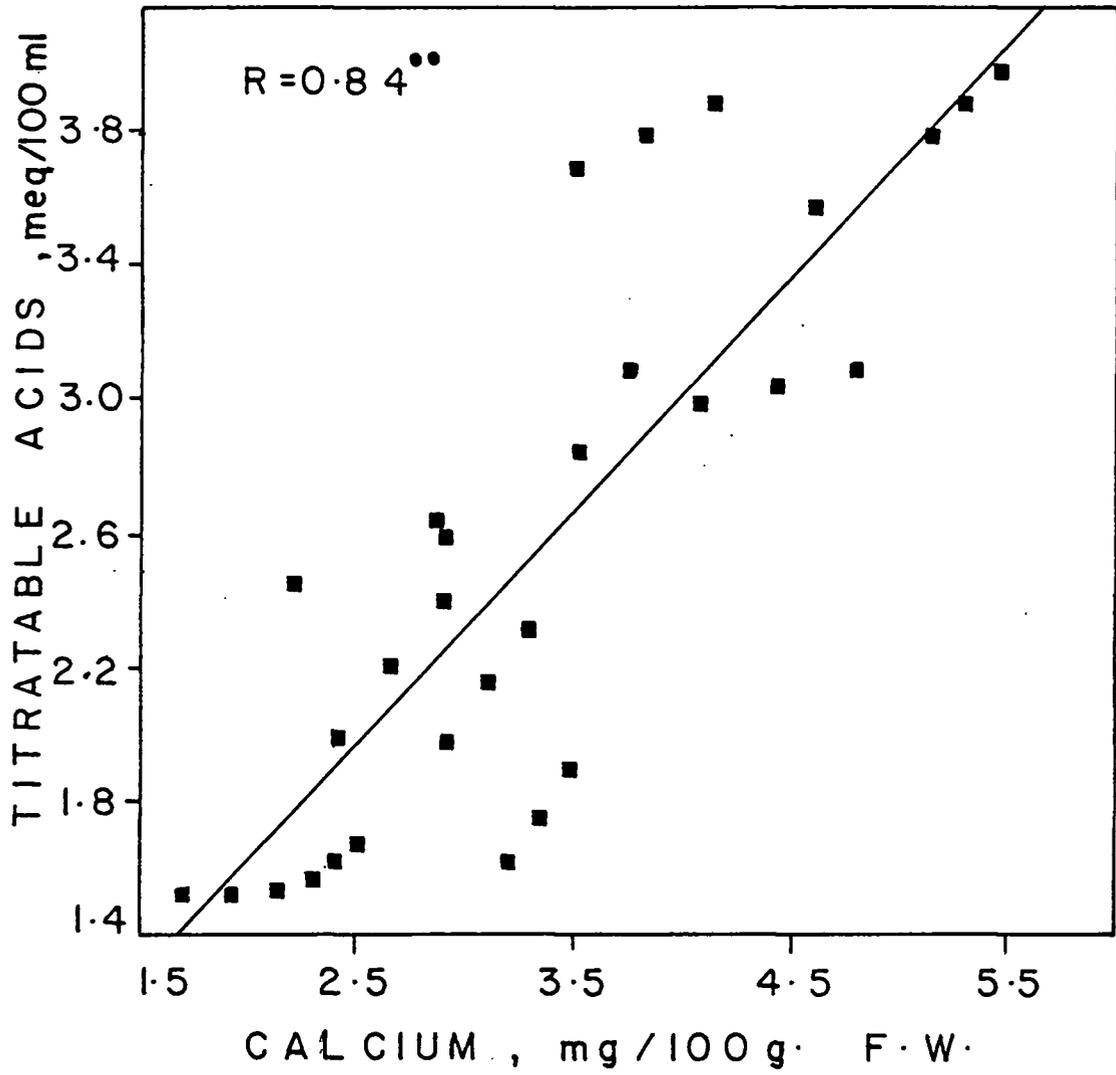


Figure 7.7

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CHAPTER VIII

RELATIONSHIP OF CALCIUM INFILTRATION OF D`ANJOU PEARS
AND POSTHARVEST FRUIT DECAY CAUSED BY PENICILLIUM EXPANSUM
AND BOTRYTIS CINEREA

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Abstract

D`Anjou pears were treated with 0, 3, 6, or 9% solutions of calcium chloride by dipping or vacuum infiltration techniques. Fruit calcium, flesh injury, and decay incidence for Penicillium expansum and Botrytis cinerea were monitored. For Penicillium studies three methods of inoculations were compared. Fruit was exposed to a spore inoculum after dipping and both prior to and after vacuum (300 torr) infiltration. Botrytis experiments were carried out concomitantly with 3 different vacuum levels, 100, 300 and 500 torr. Inoculated pear fruits were evaluated for decay incidence and fruit injury after storage for 8 mo. at -1.0°C and 96% RH. In general, the higher the fruit Ca the lower the percentage of decay. Vacuum infiltration was more efficient in moving Ca

into the fruits, but injured fruit flesh, with severity increasing with increasing vacuum. The optimum treatment, which reduced incidence of decay by 40% with minimal fruit damage was dipping fruits in 6% CaCl_2 .

Introduction

Postharvest losses of D'Anjou pears may range from 2 up to 60 per cent (18, 19, 26). Infection by fungal pathogens, especially Penicillium and Botrytis has caused considerable storage losses (3, 6, 26). In a controlled experiment, Botrytis and Penicillium was reported to cause 15% and 7%, respectively, of storage decay (3). The rotting of apple and pear tissue is associated with the loss of wall integrity (24), decreased fruit firmness (14), and reduced resistance to further attack of fungal organisms. Calcium is known to maintain cell wall integrity (1, 16), and low Ca levels were reported in rot susceptible fruits (10). The incidence of rots in apples (4, 7, 8) and pear (26) fruits has been suggested to be at least partially related to calcium.

Reductions in rot incidence have been achieved by both pre-harvest and postharvest application of calcium salts (23). Vacuum and pressure infiltration of CaCl_2 have been especially effective in getting Ca into the apple fruits (21, 22). Vacuum infiltrations have increased Ca in the fruit to about double the amount achieved with dipping treatments (8). Apples that were pressure infiltrated with CaCl_2 had considerably less decay than nontreated fruits (7, 8). More recently a 40% reduction in decay caused by Penicillium in 'Red Delicious' apple was attained with CaCl_2 pressure infiltration (20).

The objective of this study was to determine the effects of postharvest Ca treatments on decay caused by Botrytis cinerea and

Penicillium expansum on D'Anjou pear and to determine the optimum treatment method and concentration of CaCl_2 solution to use in postharvest treatment of the fruits.

Materials and Methods

D'Anjou pears (Pyrus communis) were harvested from the Hood River Experiment Station, in Oregon, at optimum commercial maturity (60N) (13). Pears were randomized and treated with calcium chloride (CaCl_2 76%), made up as 0, 3, 6, and 9% solutions. Two methods of treatment were used, vacuum infiltration and dipping.

Vacuum infiltration was performed by exposing submerged fruits in CaCl_2 solutions to 4 minutes of vacuum; 1 minute to reach the desired negative pressure, 2 minutes at the desired vacuum and 1 minute from the time of pressure release until atmospheric equilibration. Once at atmospheric pressure, the fruits were left for an additional minute in the solution after which they were rinsed with distilled water and left to drain for 1 hour before storage.

The dipping treatment was performed by immersing fruits for 5 minutes in a respective CaCl_2 containing solution. After draining for 1 hour, fruits were placed in polyethylene lined cartons in storage rooms without being rinsed.

Fruits were stored for 8 months at the usual commercial storage conditions for D'Anjou pear of -1°C and 96% RH.

Pathology portions of the study were divided into 2 parts, involving inoculation with either Botrytis cinerea or Penicillium expansum. For the experiment with Botrytis, 3 different negative pressures were used for vacuum infiltration, 100, 300 and 500 torr. The Penicillium study involved 3 methods of inoculations.

For the first treatment, fruits were immersed in a respective CaCl_2 solution, exposed to vacuum (300 torr) then transferred to another solution containing Penicillium spores. The second treatment had fruits dipped in CaCl_2 solutions that contained Penicillium spores and were then submitted to vacuum (300 torr). In the third treatment, the fruits were exposed to a spore containing solution after dipping in the CaCl_2 solution.

Inoculations were performed by immersing the fruits for 30 seconds into a conidial suspension (9.8×10^6 and 1.3×10^6 spores per milliliter for Botrytis and Penicillium, respectively) containing 0.3% Keltrol. Following immersion, fruits were left to drain for 1 hour, then stored. After 8 months of -1°C storage, fruits were removed to a 20°C room, immediately evaluated for percentage incidence of rotten fruits due to Botrytis and re-evaluated 7 days later with regard to Penicillium infection. All fruits were also evaluated for skin and flesh injury not associated with pathogenic fungi. Forty fruits were used in each of three replicates for all treatments.

At the end of the experiments, 12 fruits from each treatment were analyzed for Ca content, following the method of Perring (17) with some minor modifications. Axial sectors of the fruits were cut in 4 pieces. The pieces replicated in 3 subsamples, each containing 12 fruit slices. Cores were removed prior to maceration. Ca was extracted with 12 N HCl, filtered and analyzed on a fresh weight basis using an inductively coupled plasma emission spectrometer.

Results

There were significant differences in the amount of Ca moved into the pear fruits. Fruit Ca increased with increasing concentration of CaCl_2 solutions and negative pressures (Table 8.1). Although there was no significant difference between dipping and vacuum treatments for control concentrations, at all other concentrations, vacuum treatments had higher fruit Ca content.

D'Anjou pears inoculated with Botrytis showed an average postharvest loss of 14.5% after 8 months at -1°C storage (Table 8.2). The CaCl_2 treatments helped control Botrytis. As the CaCl_2 concentration was increased from 0 up to 9%, the incidence of decay in the fruits decreased. Vacuum infiltration also substantially reduced the incidence of rots due to Botrytis. Although differences were most apparent at higher Ca concentrations, the incidence of decayed fruit decreased with increasing vacuum. There was no difference between vacuum treatments for control concentrations. Consequently, the higher the CaCl_2 concentration and or the negative pressure, the better the control of the pathogen, since more CaCl_2 moves into the fruits.

The incidence of Penicillium infection in fruits inoculated after CaCl_2 was vacuum infiltrated was significantly reduced from 9.8% for the control treatment to as low as 2.8% (Table 8.3). The reduction in decay being relatively greater in fruits infiltrated with 3% CaCl_2 . The greatest percentage of decayed fruits resulted when CaCl_2 was mixed with Penicillium spores and then

vacuum infiltrated into the pear fruits. Although there was no statistical significance between CaCl_2 concentration levels in the reduction of decayed fruits, all calcium treatments had significantly less decay than the control treatment. Fruits dipped in CaCl_2 solution and subsequently dipped in solution containing spores of Penicillium presented decreasing incidence of decay with increasing calcium chloride concentration up to 6%.

The beneficial effect of calcium in reducing the incidence of decay by Penicillium and Botrytis was masked in both cases by fruit damage caused by the vacuum infiltration (Table 8.4). Fruits vacuum infiltrated at 300 torr and 500 torr had severe pulp injury, represented by vacuole-like empty spaces in the fruit flesh, varying in sizes and number in the fruits. At 100 torr, it still could be seen, although the lesion areas were much smaller than at 300 or 500 torr vacuum. Fruits with such disorders could not be marketed. Skin injury due to CaCl_2 was only noticed at concentration of 9%.

The optimum treatment in these tests was the dipping of fruits in 6% CaCl_2 solution, which showed a 40% reduction in the incidence of Penicillium expansum.

Discussion

Calcium chloride reduced the incidence of rotten fruits caused by Penicillium and/or Botrytis. The best control was attained with 9% CaCl_2 when vacuum up to 500 torr of negative pressure infiltration was used. As the vacuum pressure or Ca concentration increases, more Ca moves into the fruits, and reduces the incidence of decay. This is in agreement with previous reports for apples (2, 4, 7, 8) and pears (26). When Penicillium spores were added to the CaCl_2 solution then vacuum infiltrated into the fruits, the method proved efficient in moving Ca into the fruits, but it also moved the spores. The greatest infection occurred under these conditions.

Although more Ca could be added to D'Anjou pear with vacuum infiltration, it is harmful due to severe internal fruit injury. Unlike apple fruits, D'Anjou pear has different anatomical structure (11). The central core does not communicate freely with the pedicel opening and is not an avenue for gas exchange (25). When applying vacuum, even at 100 torr pressure, resistance to gas diffusion is likely. Since the symptoms resemble those caused by high CO_2 accumulation as described by Hall and Scott (12), it is possible that infiltrating processes when coupled with decreased diffusion may cause high CO_2 accumulation in the fruit flesh, generating such cavities. Dipping the fruits in CaCl_2 solution did not move as much Ca into the fruits as vacuum treatments.

However, appreciable decay control was attained at 6% CaCl_2 without flesh or skin injury.

Calcium does not reduce fungal growth in vitro (9), suggesting the effect of calcium is indirect. Infection of plants by microbial pathogens usually involved degradation of the cell wall and middle lamella of plants (2). The enzymatic basis for this phenomenon has been established. Pectolytic enzymes such as pectin esterase (PE) and polygalacturonase (PG) may be the major enzymes causing the breakdown of pectic substances in fruits inducing the subsequent softening (15) and decreased resistance to fungal pathogens (9).

The activity of PE and PG has been inhibited by Ca in tomato fruits (27) reducing the rate of cell wall breakdown. Calcium was found to inhibit PG degradation of cell wall and middle lamellae during fruit ripening (5). Therefore Ca may render higher cell wall integrity (1, 16) and according to Conway and Sams (7, 8, 9) increased resistance to enzymes produced by fungal pathogens, slowing penetration of fungus and decreasing decay. The mechanism by which Ca retards fungal decay may be similar to the mechanism that delays ripening or senescence of the fruit.

Table 8.1. Effect of Ca concentration, dipping and vacuum infiltration on the calcium concentration of D'Anjou pear fruits.

CaCl ₂ Treatment	CaCl ₂ (%)			
	0	3%	6%	9%
	mg Ca/100 g \pm SD			
dip	1.6 \pm 0.08	1.9 \pm 0.09	2.4 \pm 0.08	3.0 \pm 0.13
vacuum				
100	1.8 \pm 0.10	2.1 \pm 0.11	2.5 \pm 0.11	3.1 \pm 0.17
300	1.7 \pm 0.08	2.3 \pm 0.08	2.8 \pm 0.13	3.5 \pm 0.19
500	1.8 \pm 0.11	2.6 \pm 0.12	3.2 \pm 0.15	4.2 \pm 0.21

Table 8.2. Effect of Ca concentration and vacuum infiltration on the percentage of decayed fruits inoculated with Botrytis cinerea.

Vacuum					
torr	CaCl ₂ (%)	0	3%	6%	9%
		% Decay \pm SD			
100		14.1 \pm 2.4	10.2 \pm 0.5	8.4 \pm 0.5	6.8 \pm 0.5
300		15.3 \pm 2.0	10.5 \pm 0.8	7.2 \pm 0.3	5.3 \pm 0.3
500		14.5 \pm 2.8	8.8 \pm 0.6	6.0 \pm 0.7	4.5 \pm 0.4

Table 8.3. Effect of Ca concentration and method of treatment on decay control caused by Penicillium expansum.

CaCl ₂ (%)	CaCl ₂ + vacuum then spore	CaCl ₂ + spore then vacuum	CaCl ₂ dip then spore
	% Decay		
0	9.8 a	18.2 a	10.1 a
3	5.8 b	12.4 b	8.7 b
6	4.1 c	10.8 bc	6.1 c
9	2.8 d	11.5 bcd	5.8 cd

Mean separation in columns by Duncan's multiple range, 5% level.

Table 8.4. Visual evaluation of fruit injury caused by CaCl_2 concentration and method of application.

Method of treatment	CaCl_2 (%)	0	3%	6%	9%
		Injury Ratings			
dip		0	0	0	1
vacuum					
100 torr		2	2	2	3
300 torr		3	3	3	4
500 torr		4	4	4	4

Rating: 0=no injury, 1=very slight trace, 2=slight, 3=moderate, and 4=severe. Values are means of 30 observations.

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Chapter IX

CONCLUSIONS

In Newtown apples, fruit Ca, fruit weight and wt/Ca ratio accounted for the most consistent predictive factor. Bitter pit could be predicted 20 days before commercial harvest with about 70% probability of certainty by using any of the equations:

$$\text{Total BP} = 1.2084 - 3.1632 \text{ Ca} + 0.0822 \text{ Wt}$$

$$\text{Total BP} = -7.3179 + 0.0756 \text{ Wt} + 0.0177 \text{ Wt/Ca}$$

It appears that internal breakdown could be predicted from fruit sampling data 60 days before harvest.

In D'Anjou pears, fruit Ca and the ratios wt/Ca and N/Ca accounted for the most consistent predictive tools. Cork spot could be predicted 40 days before harvest with an accuracy of more than 70% with the equation:

$$\text{Total CS} = 18.0249 - 0.8585 \text{ Ca} - 1.8036 \text{ N/Ca} + 1.2918 \text{ Wt/Ca}$$

Respiration, ethylene evolution and internal C_2H_4 of fruits was affected with different Ca levels. Basically fruit Ca affects fruit respiration and ethylene evolution in three ways: a) it alters the time needed in cold storage to initiate the climacteric response; b) it affects the maximal rates of CO_2 and C_2H_4 produced; and c) it shifts the time to reach the climacteric peak.

The cold storage period, necessary to stimulate C_2H_4 production in D'Anjou pear, was found to be closely correlated to fruit Ca levels. The lower the fruit Ca level, the shorter the

time required in storage to initiate C_2H_4 evolution and consequently fruit ripening.

Calcium concentration in the fruits was also found to be highly correlated with fruit's flesh firmness, chlorophyll's fruit retention, titratable acidity and internal breakdown.

Vacuum infiltration and dipping the fruits in $CaCl_2$ solution proved to be satisfactory as a postharvest treatment in increasing fruit Ca concentration. High fruit Ca levels were associated with reduced incidence of rots. Vacuum treatments were found to be unpractical for D'Anjou pear fruit, which resulted in considerable internal fruit injury. Dipping the fruits in 6% $CaCl_2$ solution was effective in increasing fruit Ca with a 40% reduction in fruit infection by *Penicillium*.

Fruits to be stored satisfactorily for long periods of time should have high amounts of Ca. Once fruit Ca concentration is known, it appears possible to estimate their storage potential for better storage management and handling, with resulting reduction in postharvest fruit losses.

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APPENDICES

The figures are computer scatter diagrams.

Fig. 1. Relationship between Ca concentration and the total incidence of cork spot in D'Anjou pear fruits at the sampling date July 18, 1982.

Fig. 2. Relationship between Ca concentration and the total incidence of cork spot in D'Anjou pear fruits at the sampling date August 9, 1982.

Fig. 3. Relationship between Ca concentration and the total incidence of cork spot in D'Anjou pear fruits at the sampling date September 1, 1982.

Fig. 1

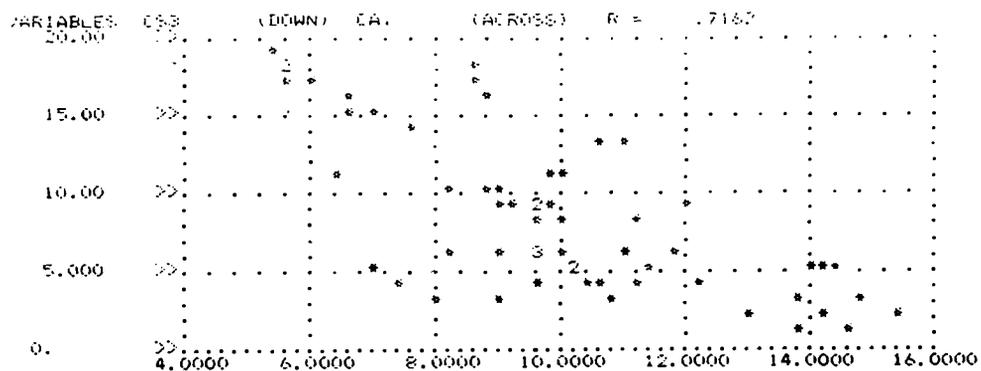


Fig. 2

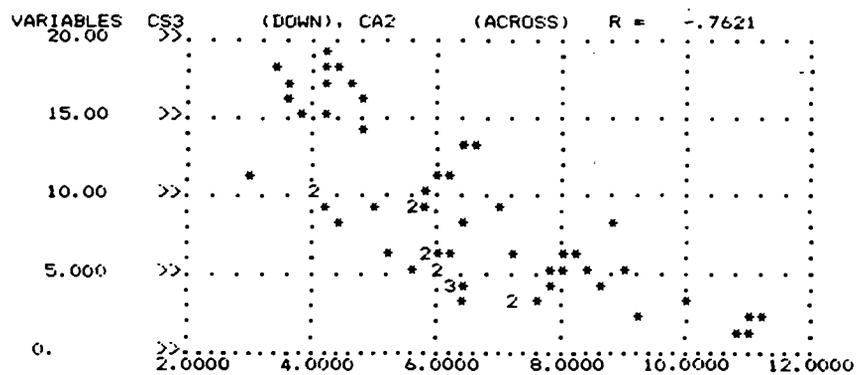


Fig.3

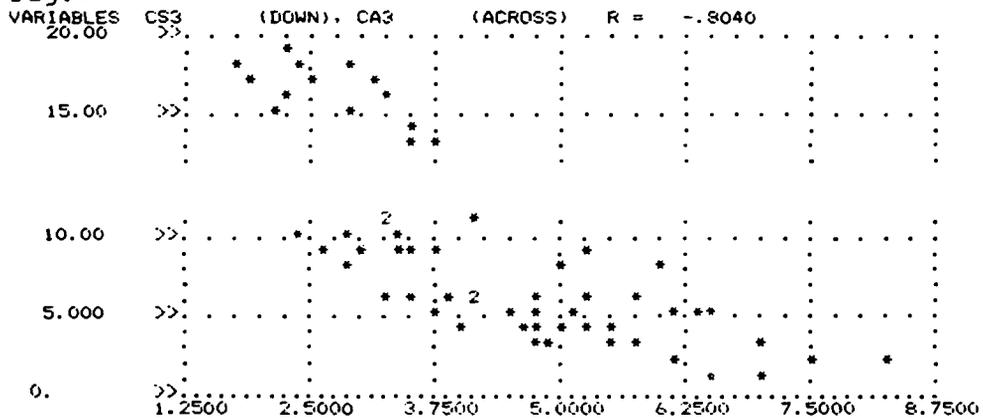


Fig. 4. Relationship between fruit weight and total cork spot in D'Anjou pear at the sampling date July 18, 1982.

Fig. 5. Relationship between fruit weight and total cork spot in D'Anjou pear at the sampling date August 9, 1982.

Fig. 6. Relationship between fruit weight and total cork spot in D'Anjou pear at the sampling date September 1, 1982.

Fig.4

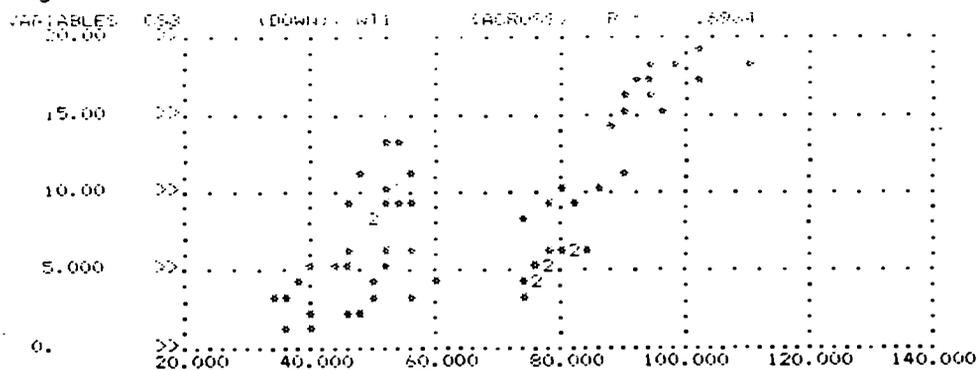


Fig.5

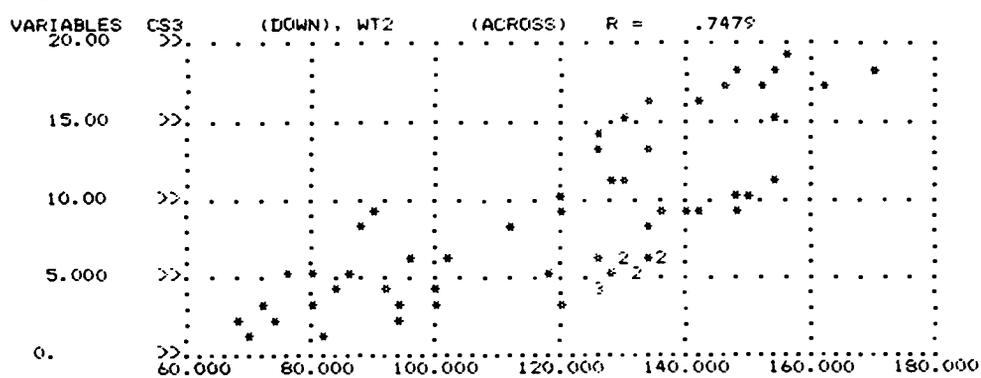


Fig.6

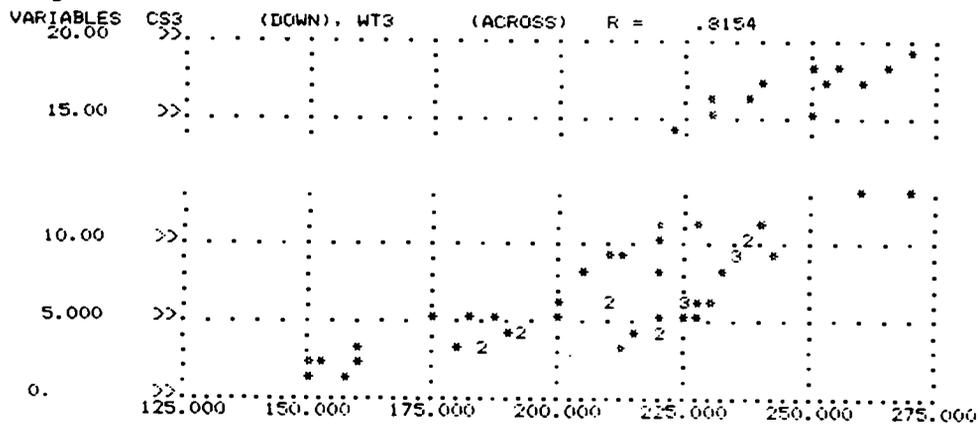


Fig. 7. Relationship between fruit Ca/wt ratio and total cork spot in D'Anjou pear at the sampling date July 18, 1982.

Fig. 8. Relationship between fruit Ca/wt ratio and total cork spot in D'Anjou pear at the sampling date August 9, 1982.

Fig. 9. Relationship between fruit Ca/wt ratio and total cork spot in D'Anjou pear at the sampling date September 1, 1982.

Fig. 7

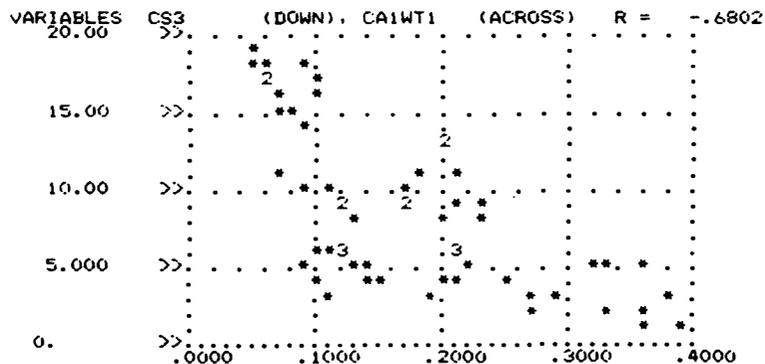


Fig. 8

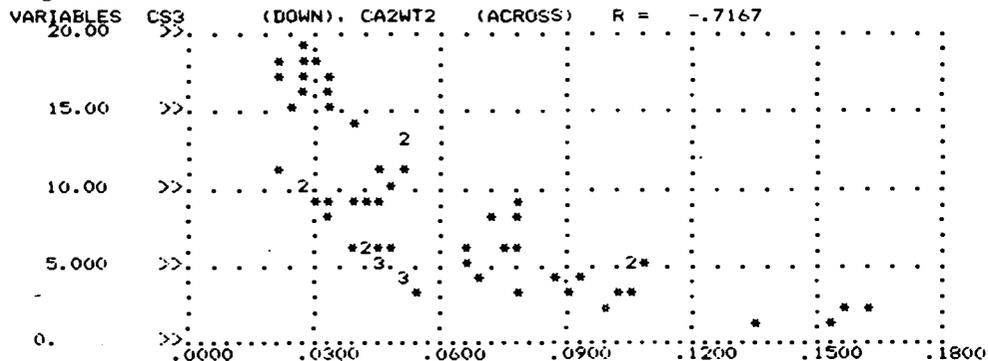


Fig. 9

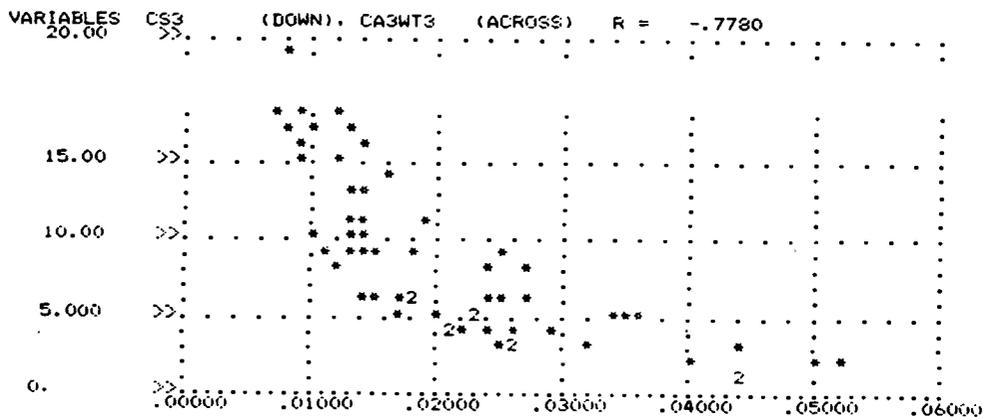


Fig.10

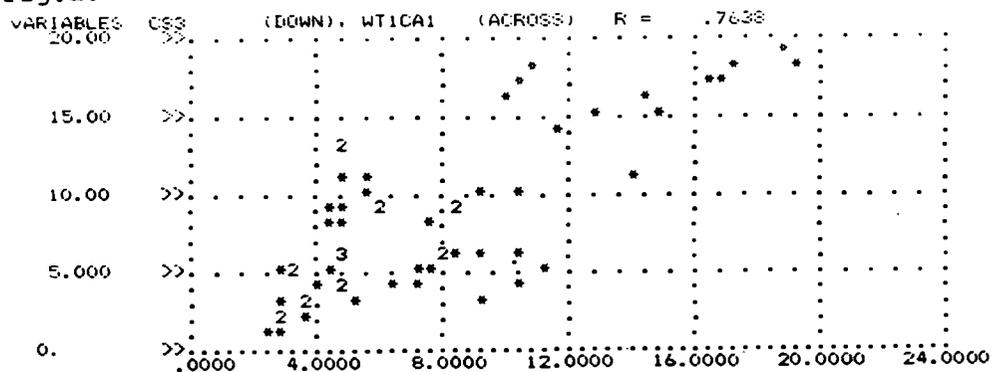


Fig. 11

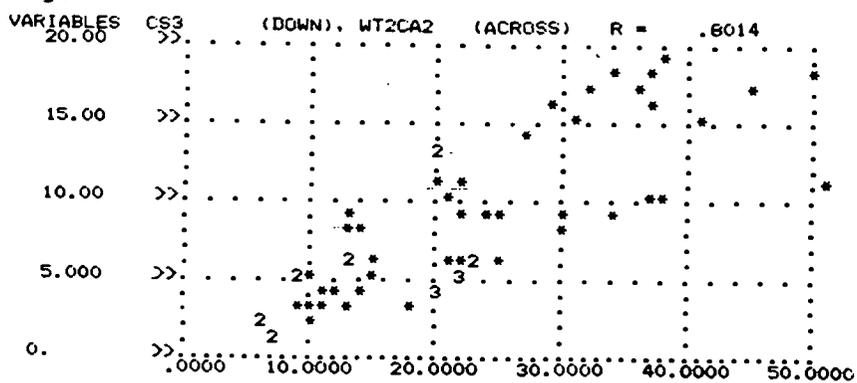


Fig. 12

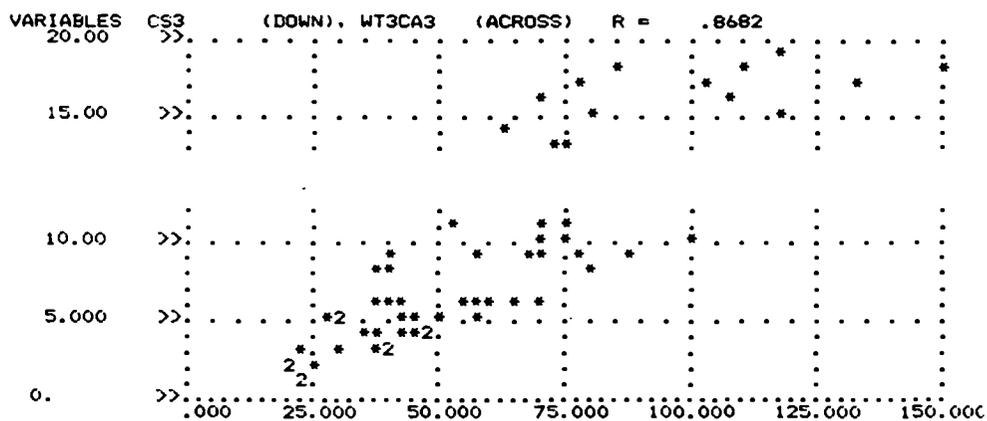


Fig. 10. Relationship between fruit wt/Ca ratio and total cork spot in D'Anjou pear at the sampling date July 18, 1982.

Fig. 11. Relationship between fruit wt/Ca ratio and total cork spot in D'Anjou pear at the sampling date August 9, 1982.

Fig. 12. Relationship between fruit wt/Ca ratio and total cork spot in D'Anjou pear at the sampling date September 1, 1982.

Fig. 13. Relationship between fruit Ca and total cork spot in D'Anjou pear at the sampling date July 1, 1983.

Fig. 14. Relationship between fruit Ca and total cork spot in D'Anjou pear at the sampling date August 9, 1983.

Fig. 15. Relationship between fruit Ca and total cork spot in D'Anjou pear at sampling date September 1, 1983.

Fig. 13

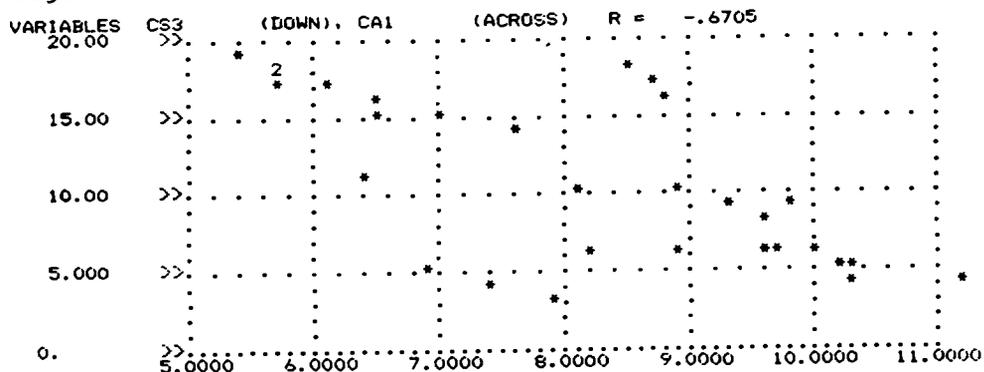


Fig. 14

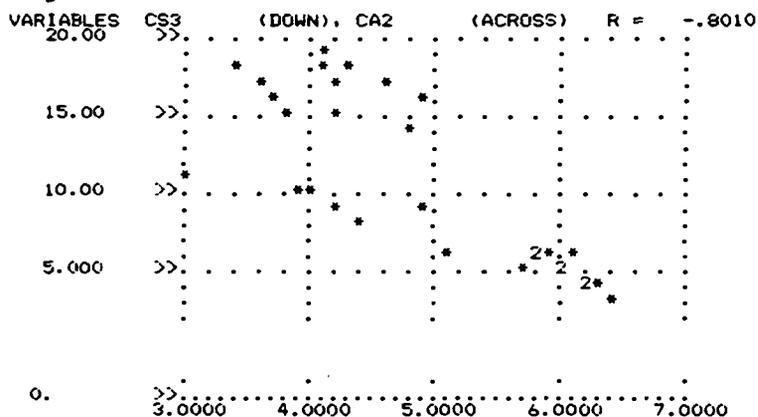


Fig. 15

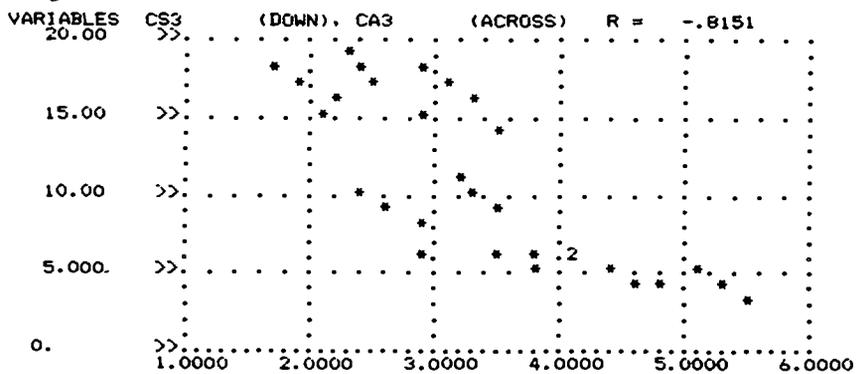


Fig. 16. Relationship between fruit K and total cork spot in D'Anjou pear at the sampling date August 9, 1983.

Fig. 17. Relationship between fruit K and total cork spot in D'Anjou pear at the sampling date September 1, 1983.

Fig. 18. Relationship between fruit Mg and total cork spot in D'Anjou pear at the sampling date August 9, 1983.

Fig. 19. Relationship between fruit Mg and total cork spot in D'Anjou pear at the sampling date September 1, 1983.

Fig. 16

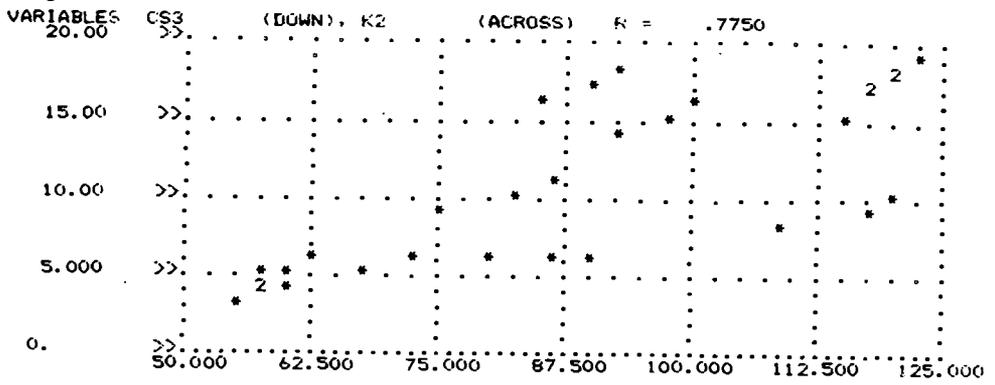


Fig. 17

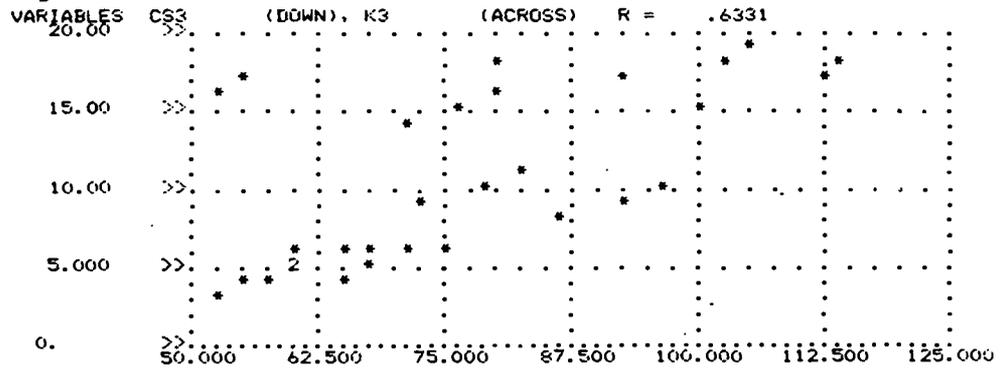


Fig. 18

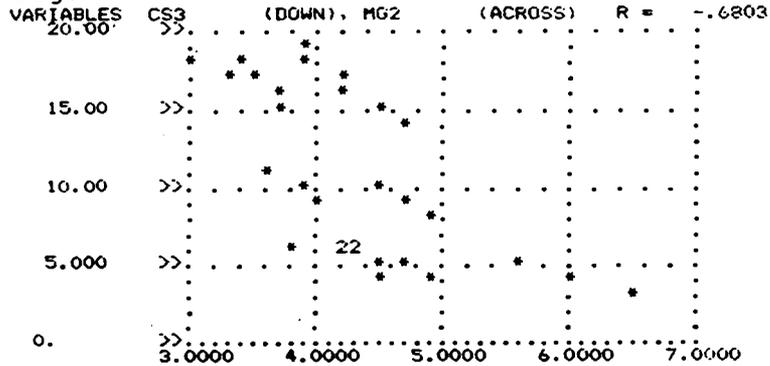


Fig. 19

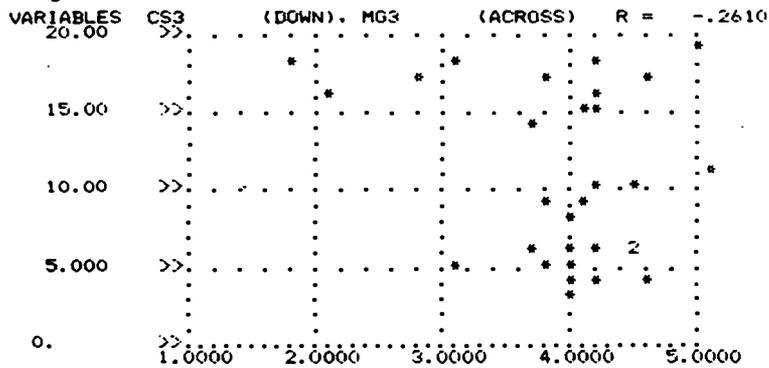


Fig. 20. Relationship between fruit K/Ca ratio and total cork spot in D'Anjou pear at the sampling date July 18, 1983.

Fig. 21. Relationship between fruit K/Ca ratio and total cork spot in D'Anjou pear at the sampling date August 9, 1983.

Fig. 22. Relationship between fruit K/Ca ratio and total cork spot in D'Anjou pear at the sampling date September 1, 1983.

Fig. 20

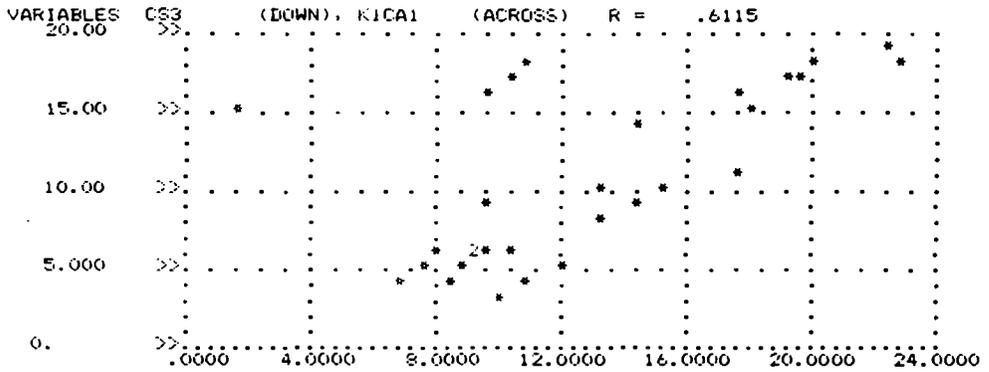


Fig. 21

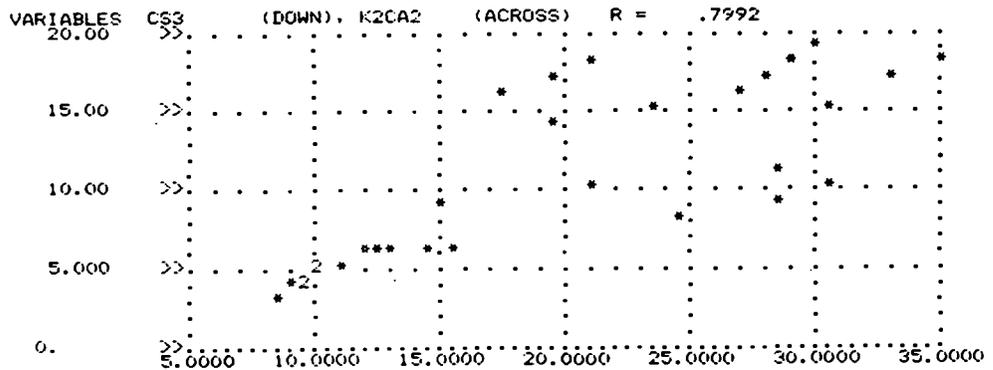


Fig. 22

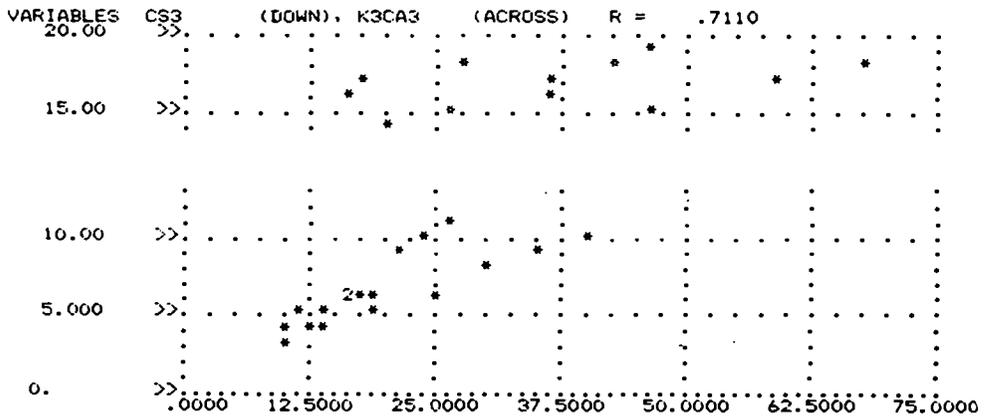


Fig. 23. Relationship between fruit P and total cork spot in D'Anjou pear at the sampling date July 18, 1983.

Fig. 24. Relationship between fruit P and total cork spot in D'Anjou pear at the sampling date August 9, 1983.

Fig. 25. Relationship between fruit P and total cork spot in D'Anjou pear at the sampling date September 1, 1983.

Fig. 23

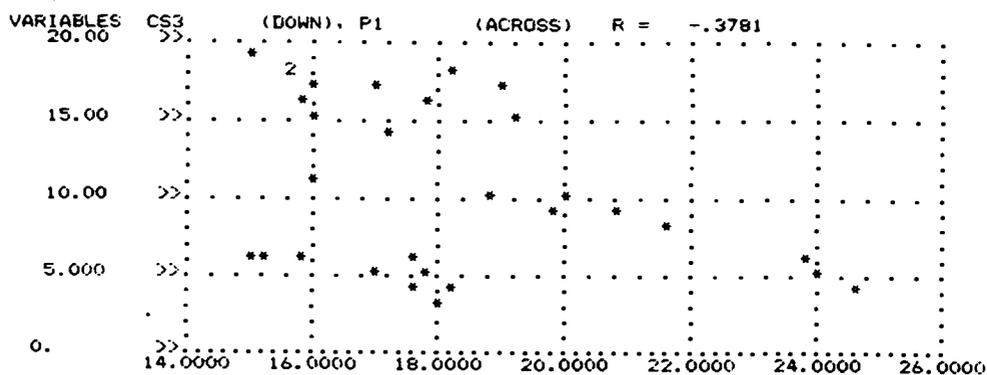


Fig. 24

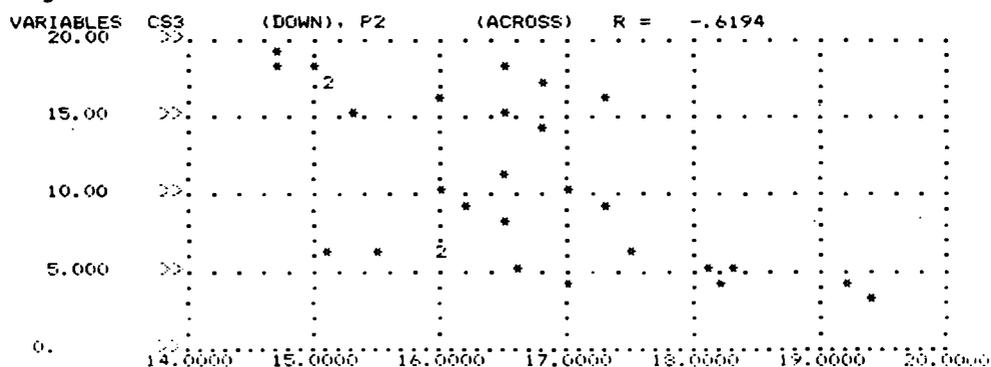


Fig. 25

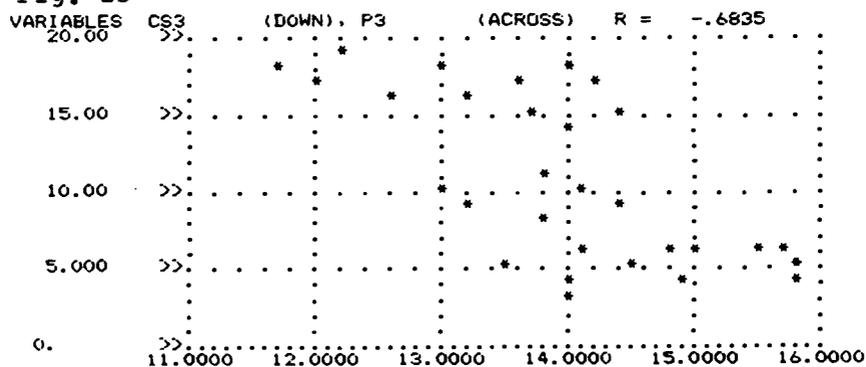


Fig. 26. Relationship between fruit P/Ca ratio and cork spot at the sampling date July 18, 1983.

Fig. 27. Relationship between fruit P/Ca ratio and cork spot at the sampling date August 9, 1983.

Fig. 28. Relationship between fruit P/Ca ratio and cork spot at the sampling date September 1, 1983.

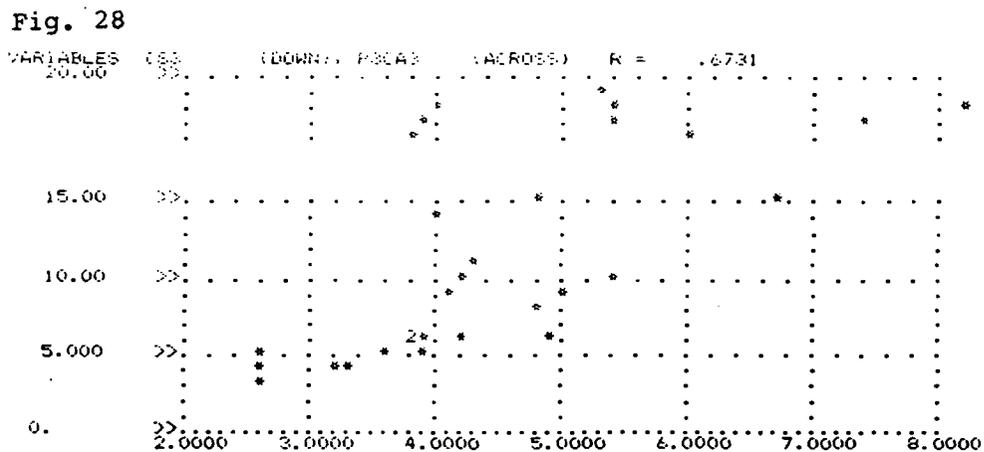
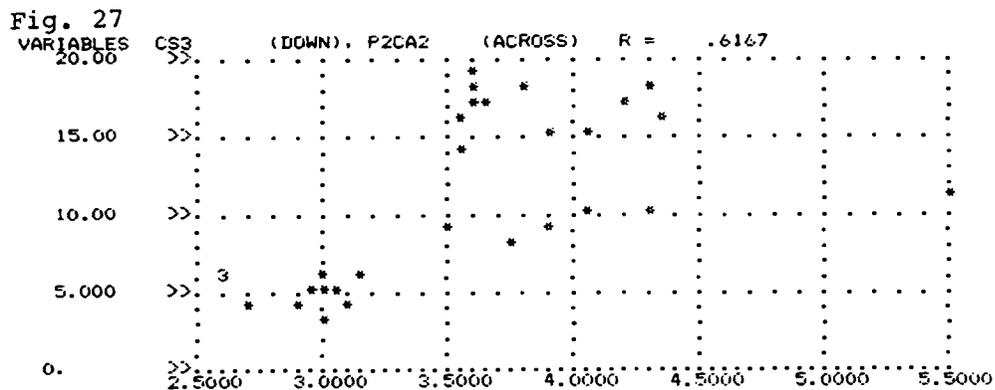
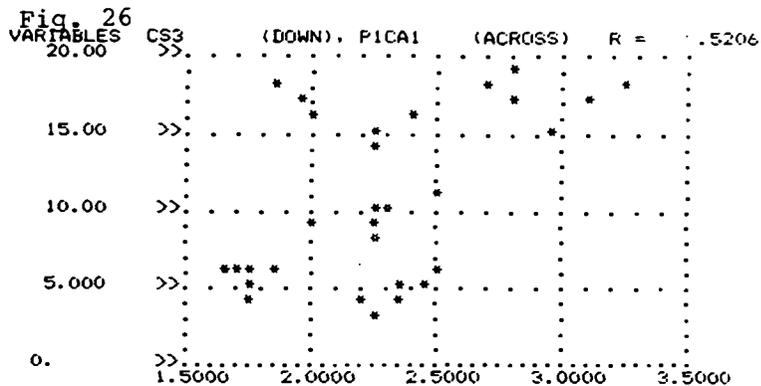


Fig. 29. Relationship between fruit weight and cork spot in D'Anjou pear at the sampling date July 1, 1983.

Fig. 30. Relationship between fruit weight and cork spot in D'Anjou pear at the sampling date August 9, 1983.

Fig. 31. Relationship between fruit weight and cork spot in D'Anjou pear at the sampling date September 1, 1983.

Fig. 29

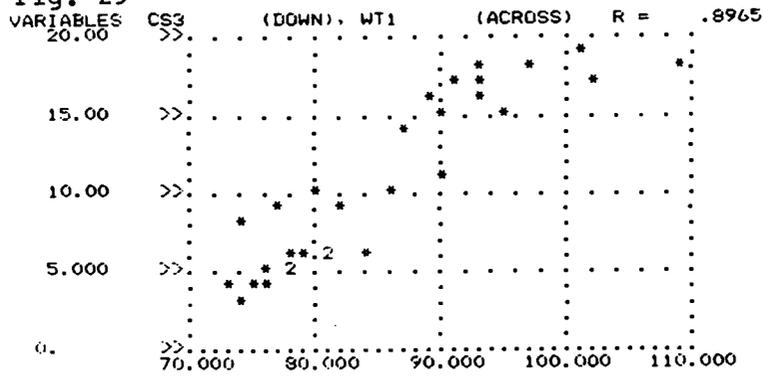


Fig. 30

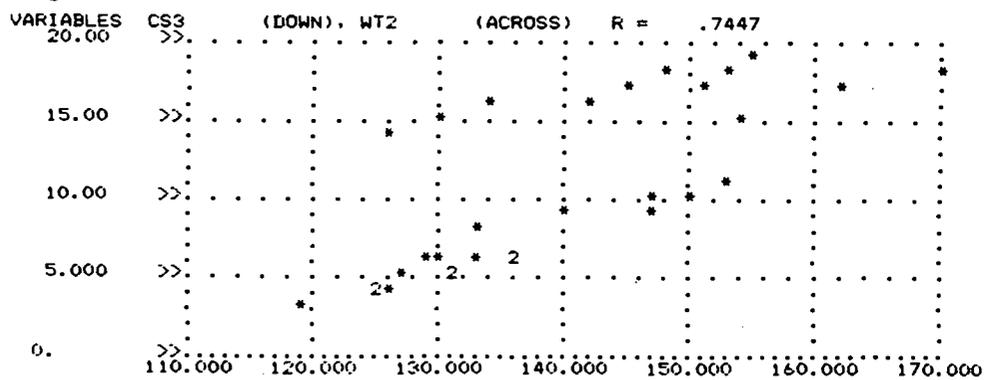


Fig.31

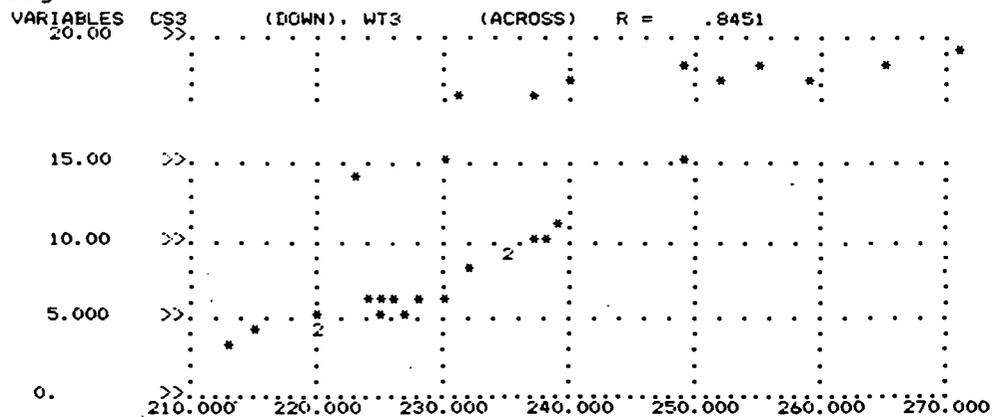


Fig. 32. Relationship between fruit wt/Ca ratio and total cork spot in D'Anjou pear at the sampling date July 18, 1983.

Fig. 33. Relationship between fruit wt/Ca ratio and total cork spot in D'Anjou pear at the sampling date August 9, 1983.

Fig. 34. Relationship between fruit wt/Ca ratio and total cork spot in D'anjou pear at the sampling date September 1, 1983.

Fig. 32

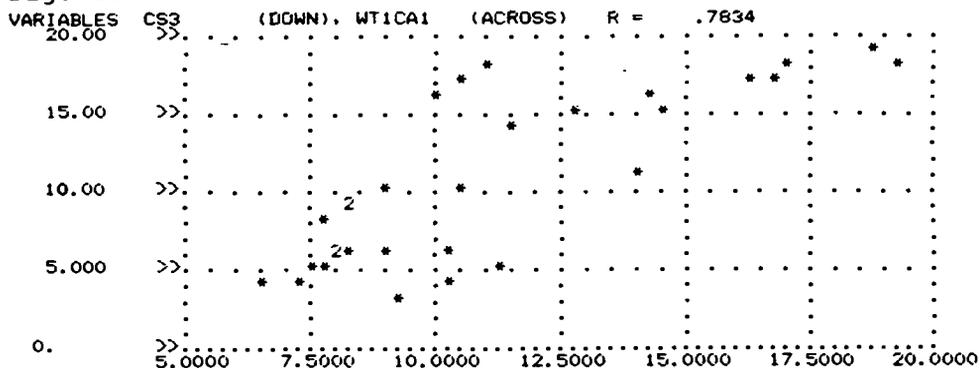


Fig.33

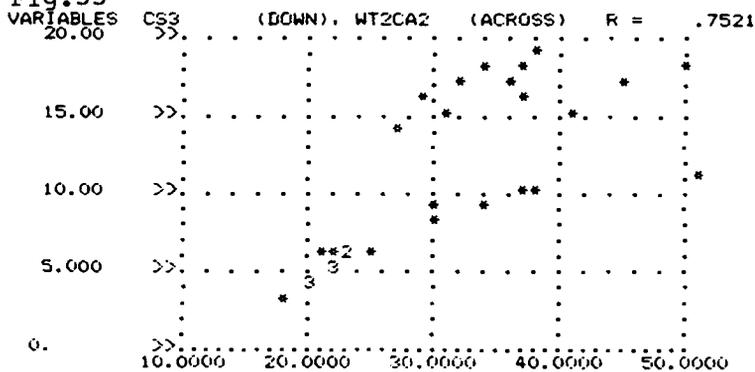


Fig.34

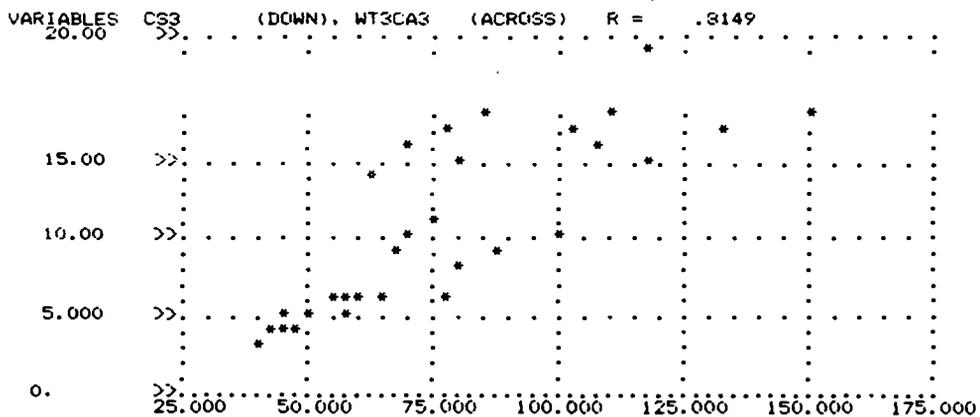


Fig. 35. Relationship between fruit N/Ca and total cork spot in D'Anjou pear at the sampling date July 18, 1983.

Fig. 36. Relationship between fruit N/Ca and total cork spot in D'Anjou pear at the sampling date August 9, 1983.

Fig. 37. Relationship between N/Ca and total cork spot in D'Anjou pear at the sampling date September 1, 1983.

Fig. 35

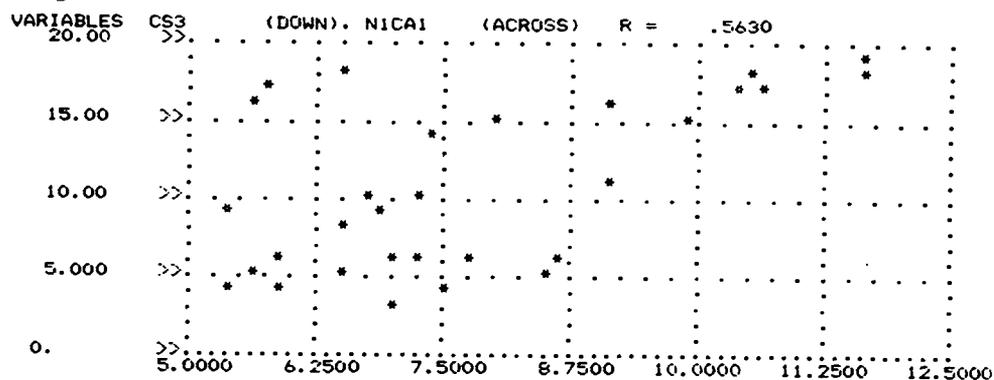


Fig.36

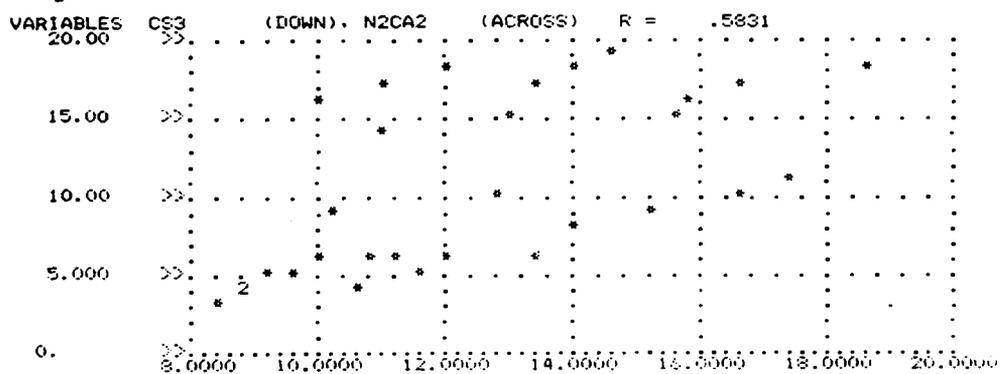


Fig.37

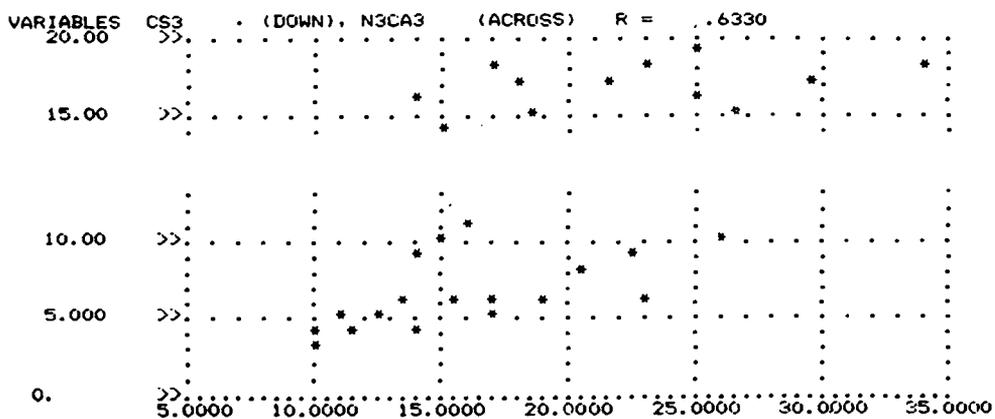


Fig. 38. Relationship between fruit $\text{Ca}/(\text{K} + \text{Mg})$ ratio and total cork spot in D'Anjou pear at the sampling date July 18, 1983.

Fig. 39. Relationship between fruit $\text{Ca}/(\text{K} + \text{Mg})$ ratio and total cork spot in D'Anjou pear at the sampling date August 9, 1983.

Fig. 40. Relationship between fruit $(\text{K} + \text{Mg})/\text{Ca}$ ratio and total cork spot in D'Anjou pear at the sampling date August 9, 1983.

Fig. 41. Relationship between fruit $(\text{K} + \text{Mg})/\text{Ca}$ ratio and total cork spot in D'Anjou pear at the sampling date September 1, 1983.

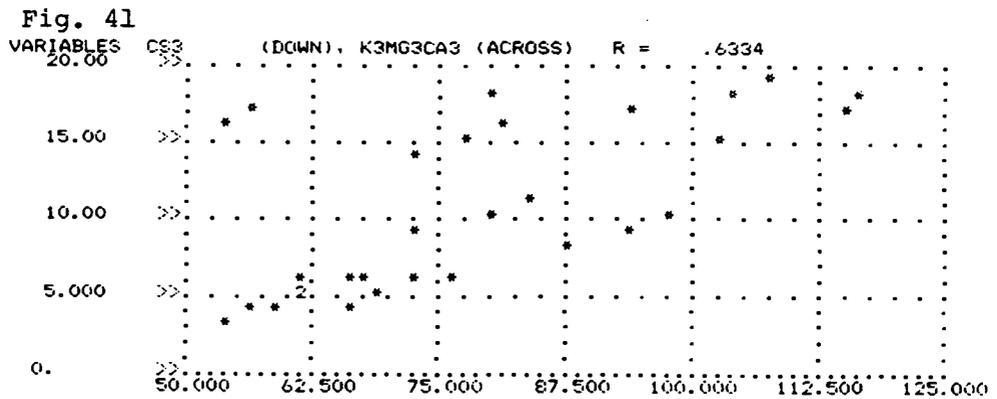
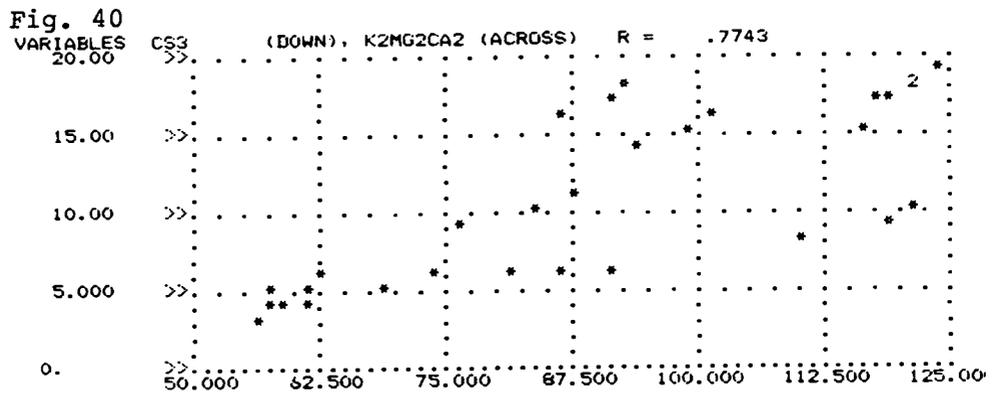
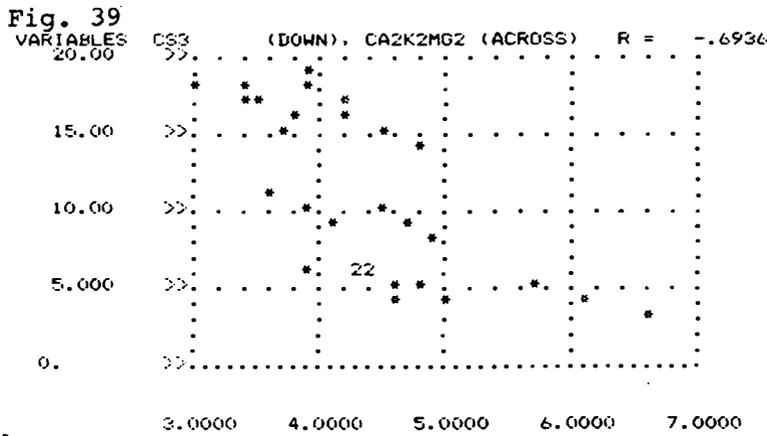
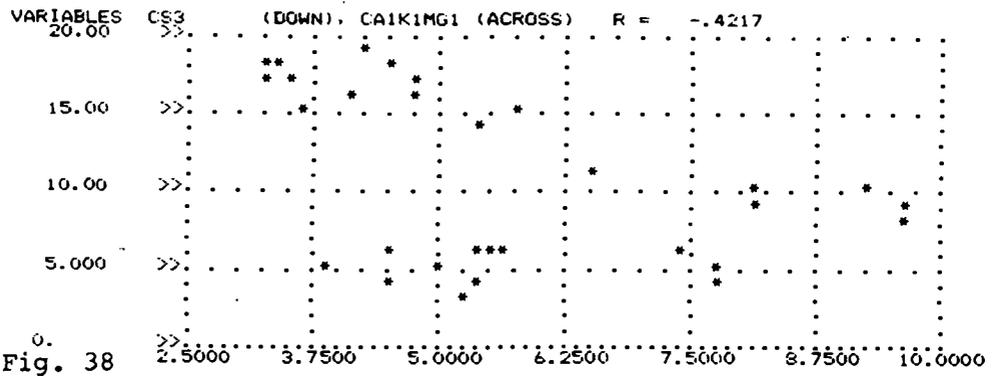


Fig. 42. Relationship between fruit Ca and total bitter pit in Newtown apple at the sampling date July 19, 1982.

Fig. 43. Relationship between fruit Ca and total bitter pit in Newtown apple at the sampling date August 13, 1982.

Fig. 44. Relationship between fruit Ca and total bitter pit in Newtown apple at the sampling date September 6, 1982.

Fig. 45. Relationship between fruit Ca and total bitter pit in Newtown apple at the sampling date September 27, 1982.

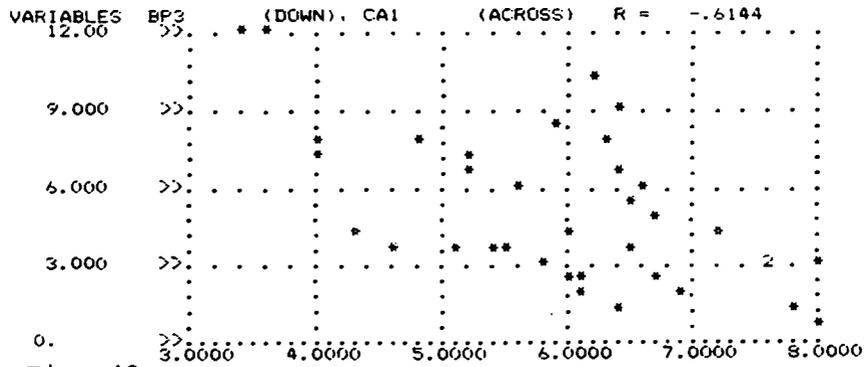


Fig. 42
Fig. 43

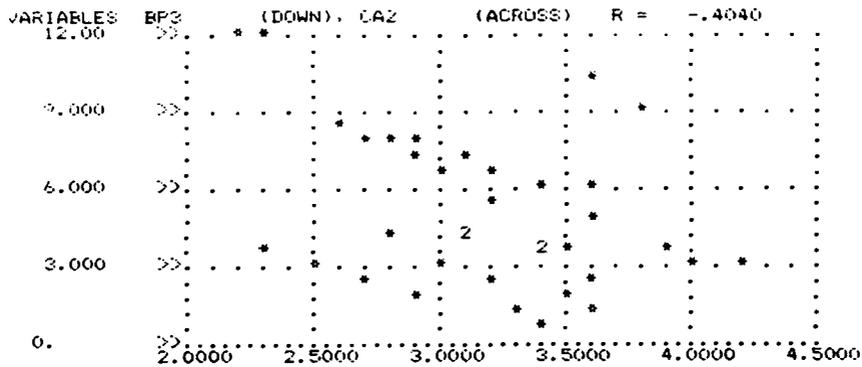


Fig.44

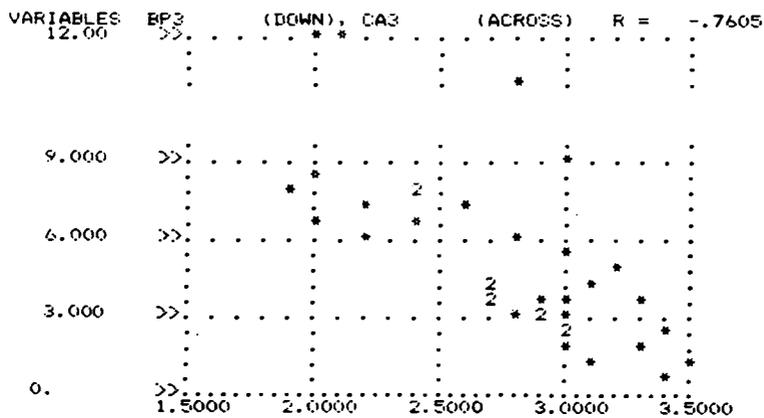


Fig.45

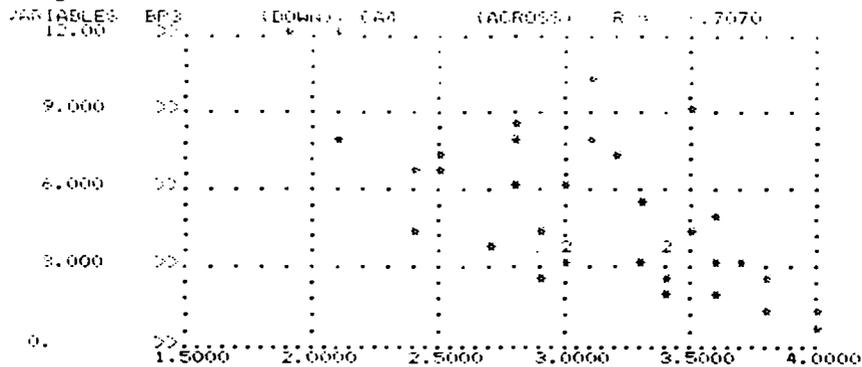


Fig. 46. Relationship between fruit weight and total bitter pit in Newtown apple at the sampling date July 19, 1982.

Fig. 47. Relationship between fruit weight and total bitter pit in Newtown apple at the sampling date August 13, 1982.

Fig. 48. Relationship between fruit weight and total bitter pit in Newtown apple at the sampling date September 6, 1982.

Fig. 49. Relationship between fruit weight and total bitter pit in Newtown apple at the sampling date September 27, 1982.

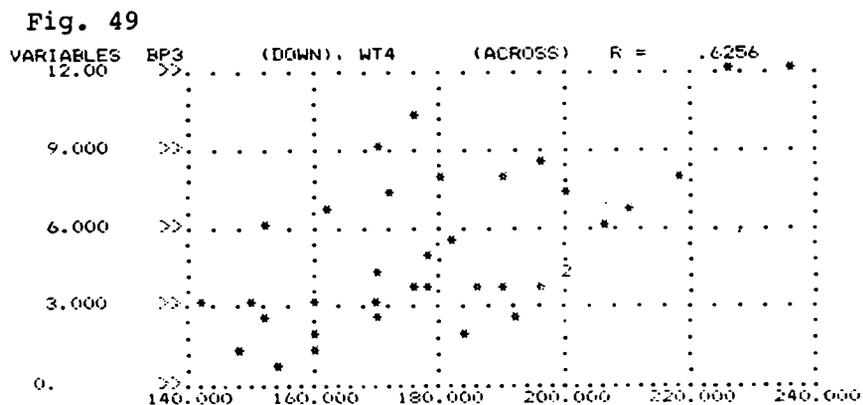
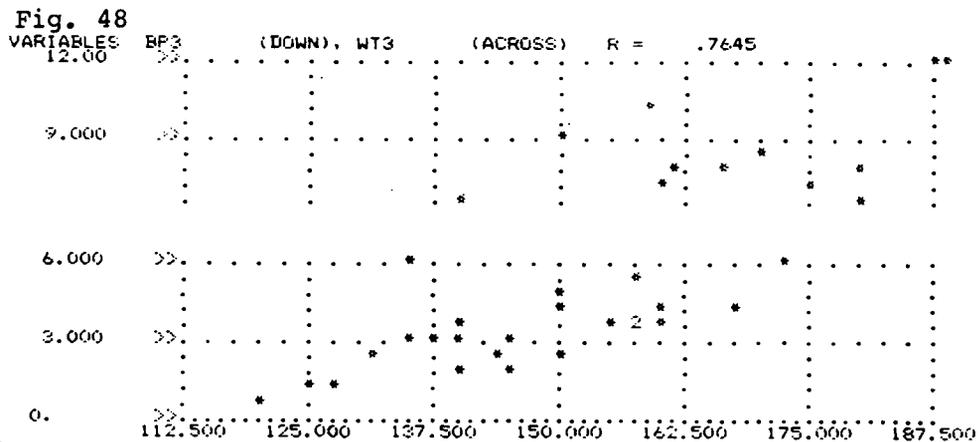
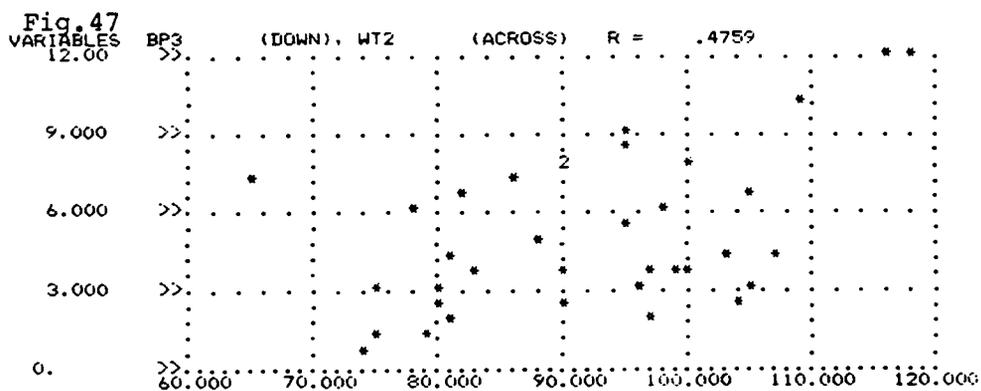
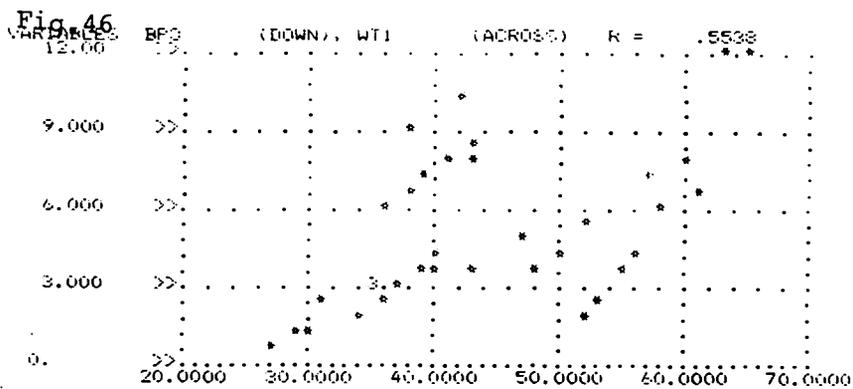


Fig. 50. Relationship between fruit wt/Ca ratio and total bitter pit in Newtown apple at the sampling date July 19, 1982.

Fig. 51. Relationship between fruit wt/Ca ratio and total bitter pit in Newtown apple at the sampling date August 13, 1982.

Fig. 52. Relationship between fruit wt/Ca ratio and total bitter pit in Newtown apple at the sampling date September 6, 1982.

Fig. 53. Relationship between fruit wt/Ca ratio and total bitter pit in Newtown apple at the sampling date September 27, 1982.

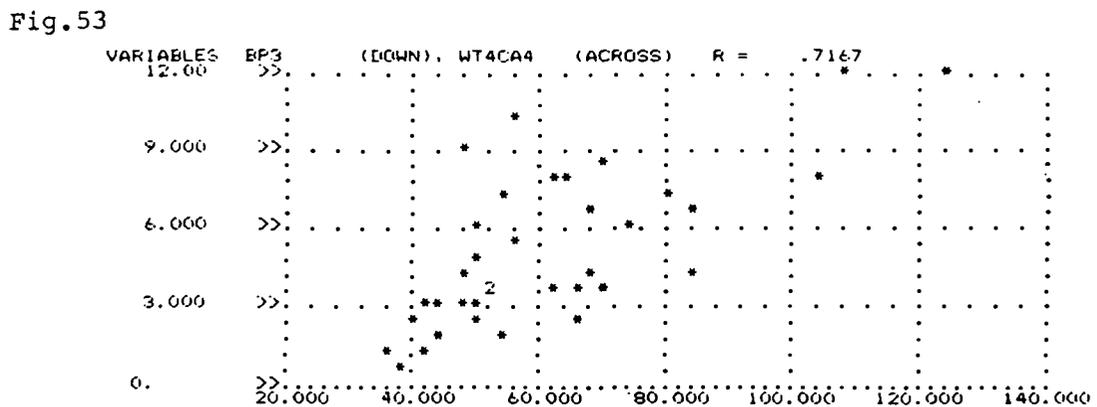
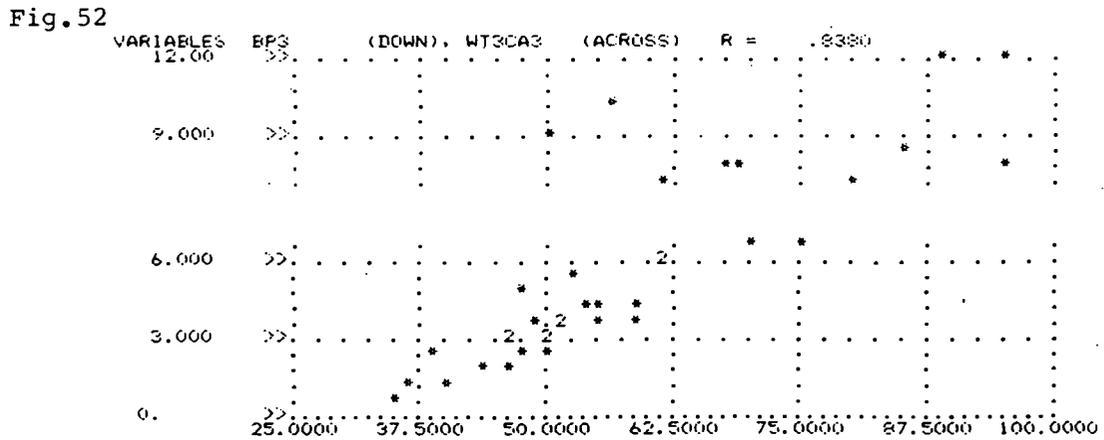
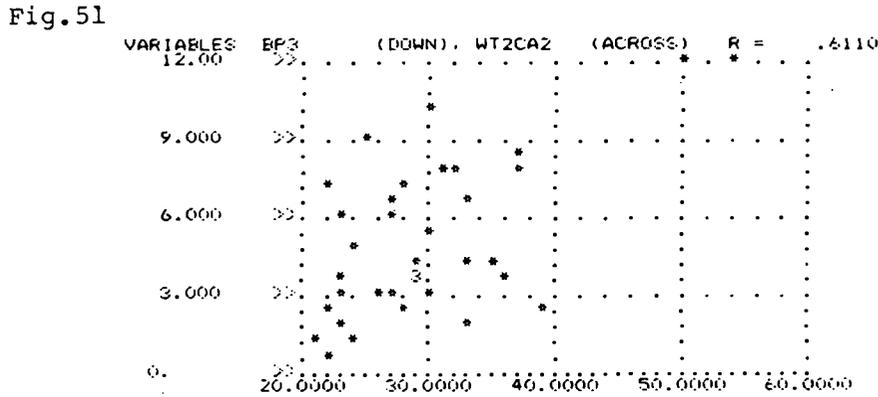
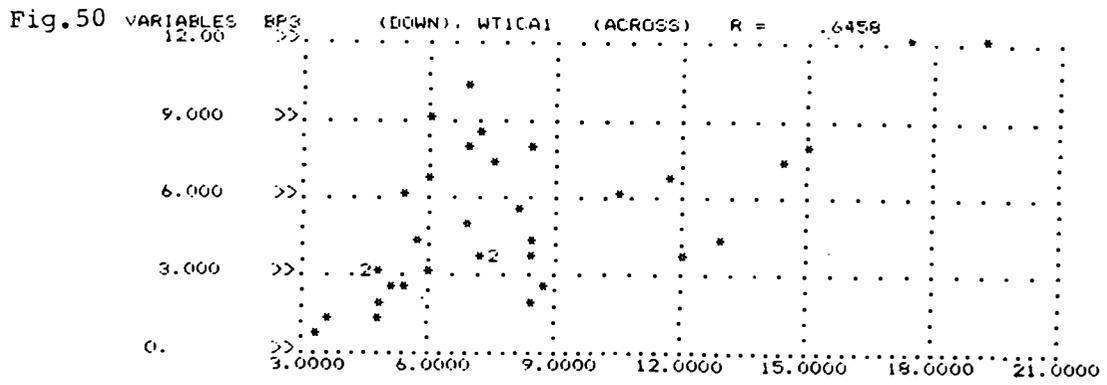


Fig. 54. Relationship between fruit Ca and total bitter pit in Newtown apple at the sampling date July 20, 1983.

Fig. 55. Relationship between fruit Ca and total bitter pit in Newtown apple at the sampling date August 10, 1983.

Fig. 56. Relationship between fruit Ca and total bitter pit in Newtown apple at the sampling date August 29, 1983.

Fig. 57. Relationship between fruit Ca and total bitter pit in Newtown apple at the sampling date September 19, 1983.

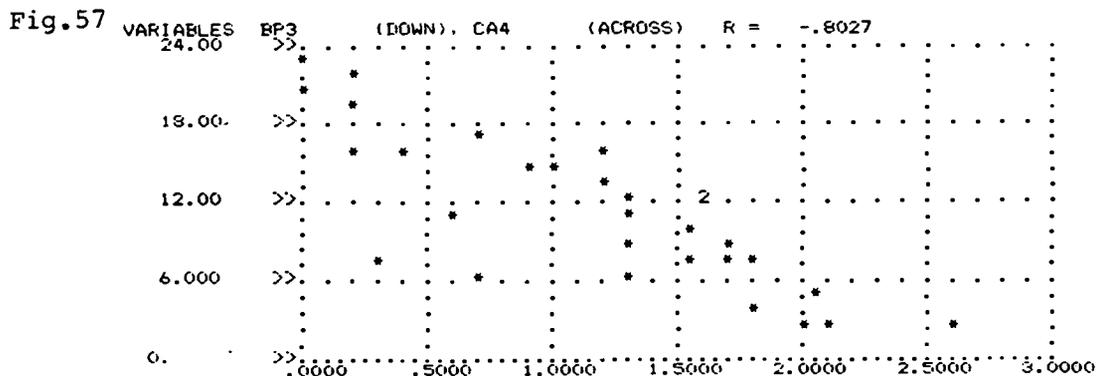
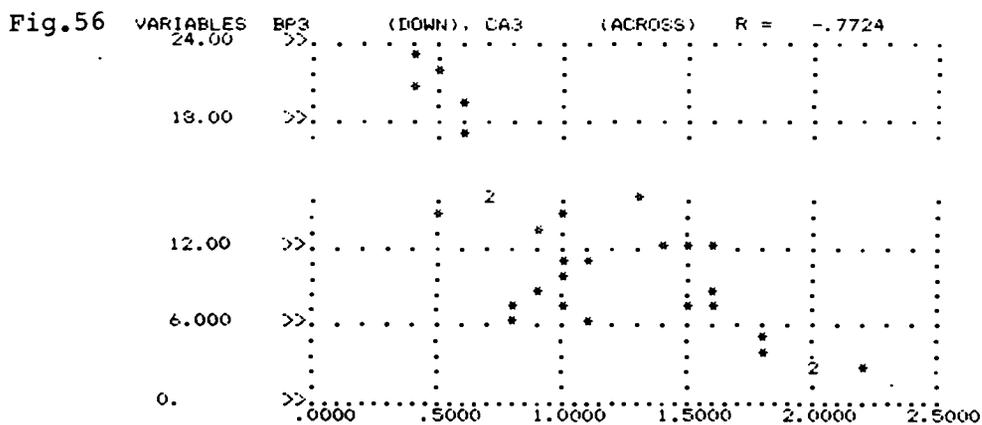
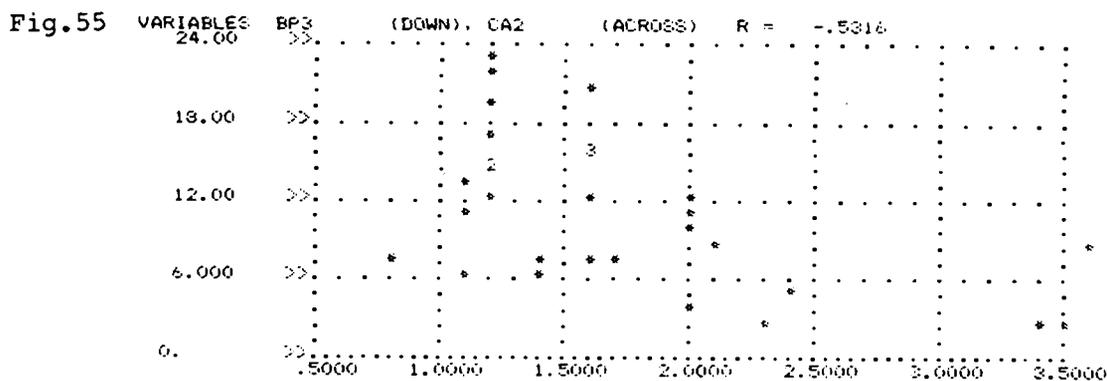
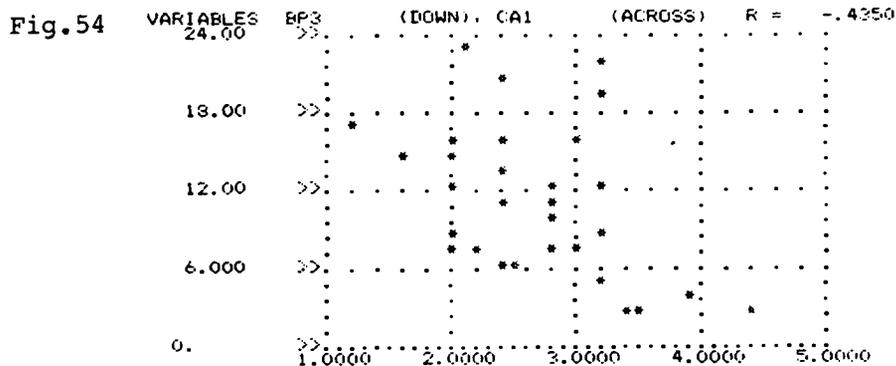


Fig. 58. Relationship between fruit weight and total bitter pit in Newtown apple at the sampling date July 20, 1983.

Fig. 59. Relationship between fruit weight and total bitter pit in Newtown apple at the sampling date August 10, 1983.

Fig. 60. Relationship between fruit weight and total bitter pit in Newtown apple at the sampling date August 29, 1983.

Fig. 61. Relationship between fruit weight and total bitter pit in Newtown apple at the sampling date September 19, 1983.

Fig.58

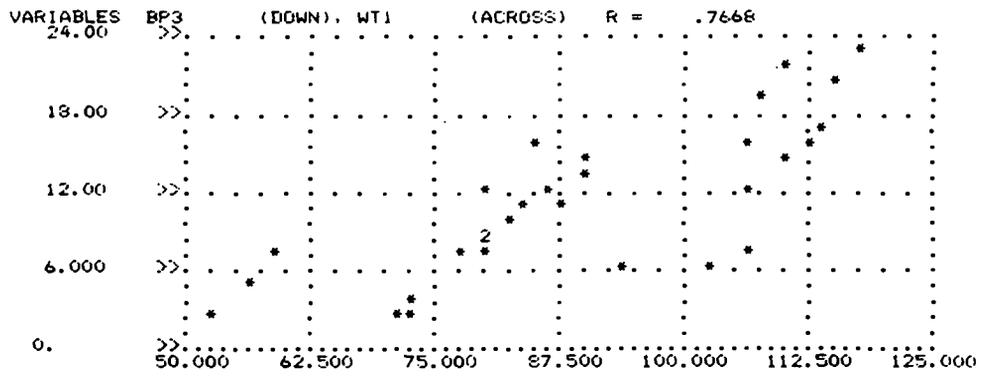


Fig.59

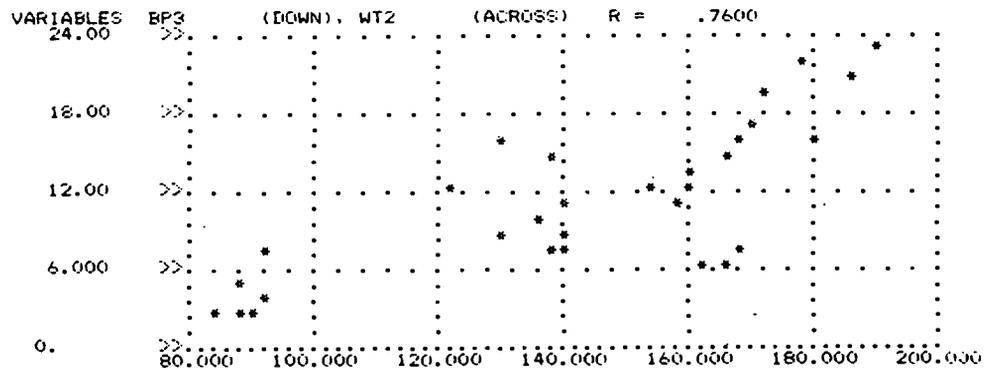


Fig.60

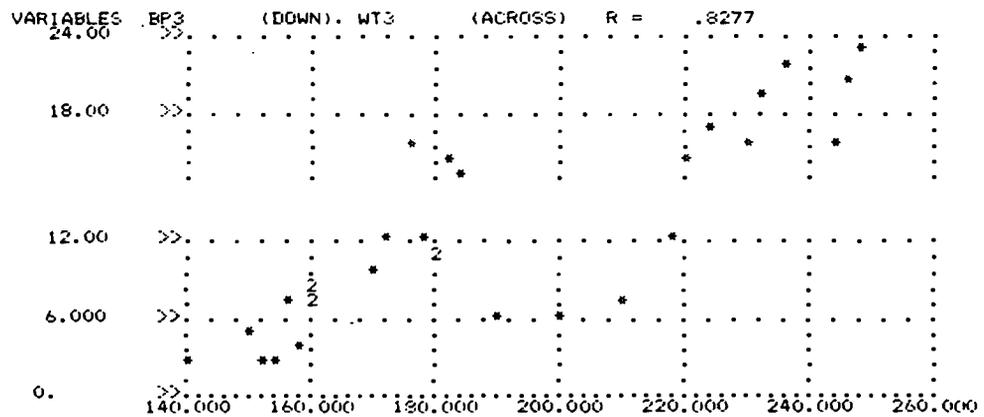


Fig.61

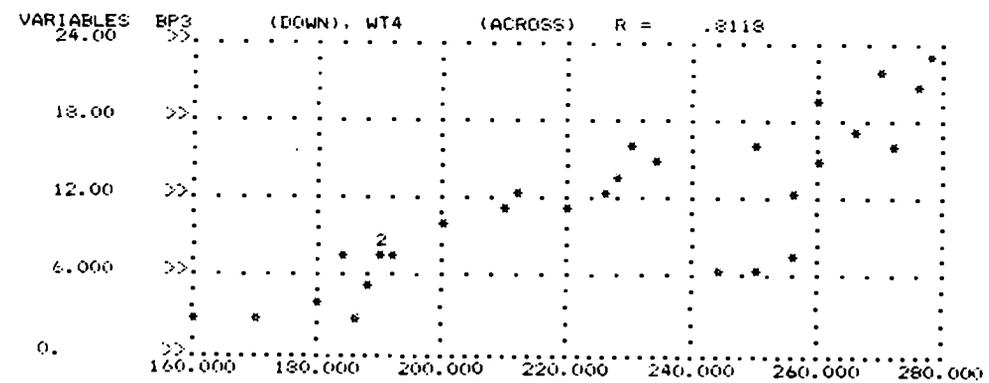


Fig. 62. Relationship between fruit wt/Ca ratio and total bitter pit in Newtown apple at the sampling date July 20, 1983.

Fig. 63. Relationship between fruit wt/Ca ratio and total bitter pit in Newtown apple at the sampling date August 10, 1983.

Fig. 64. Relationship between fruit wt/Ca ratio and total bitter pit in Newtown apple at the sampling date August 29, 1983.

Fig. 65. Relationship between fruit wt/Ca ratio and total bitter pit in Newtown apple at the sampling date September 19, 1983.

Fig.62

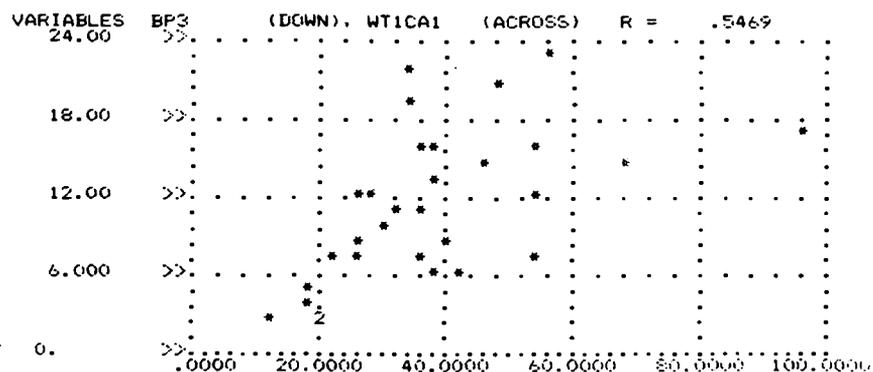


Fig.63

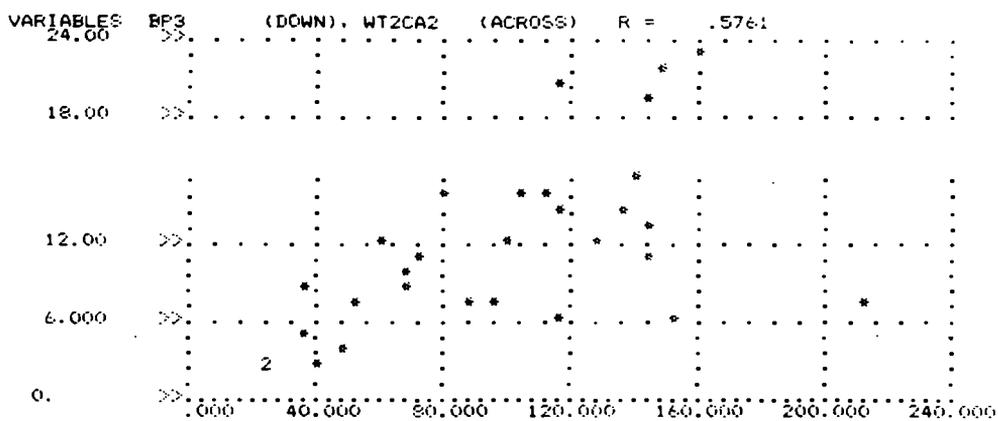


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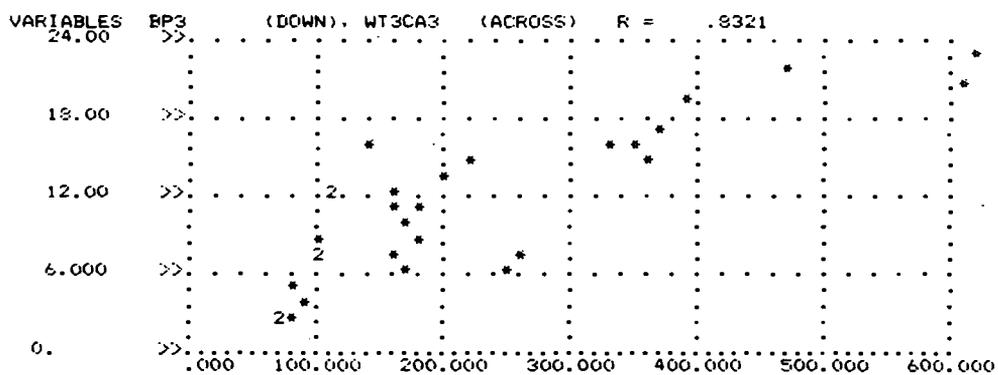


Fig.65

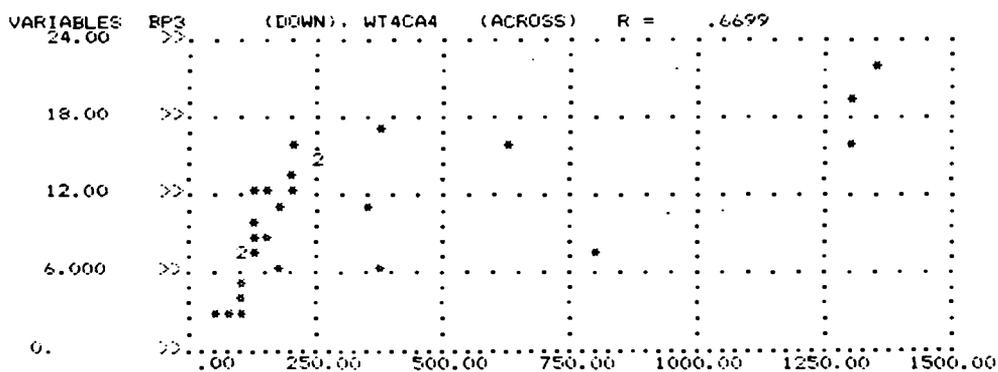


Fig. 66. Relationship between fruit N/Ca ratio and total bitter pit in Newtown apple at the sampling date July 20, 1983.

Fig. 67. Relationship between fruit N/Ca ratio and total bitter pit in Newtown apple at the sampling date August 10, 1983.

Fig. 68. Relationship between fruit N/Ca ratio and total bitter pit in Newtown apple at the sampling date August 29, 1983.

Fig. 69. Relationship between fruit N/Ca ratio and total bitter pit in Newtown apple at the sampling date September 19, 1983.

Fig.66

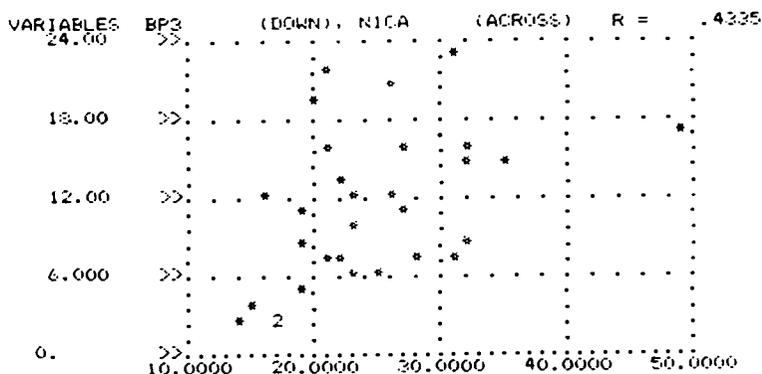


Fig.67

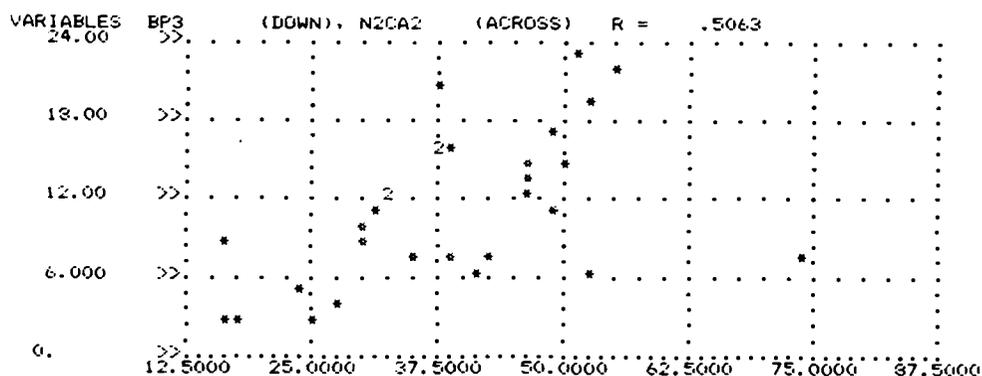


Fig.68

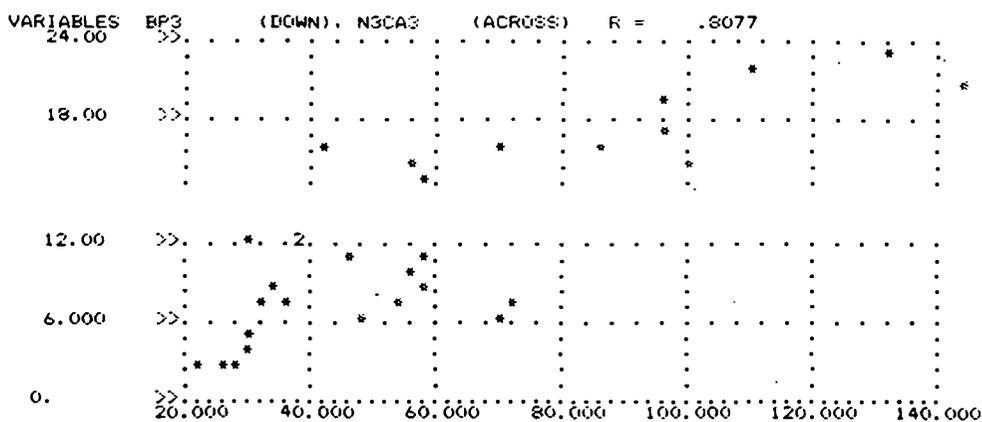


Fig.69

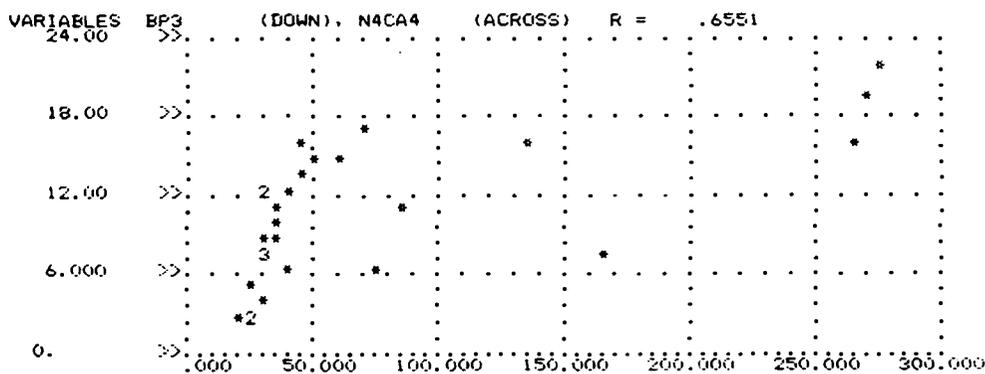


Fig. 70. Relationship between fruit P and total bitter pit in Newtown apple at sampling date August 29, 1983.

Fig. 71. Relationship between fruit P and total bitter pit in Newtown apple at sampling date September 19, 1983.

Fig. 70

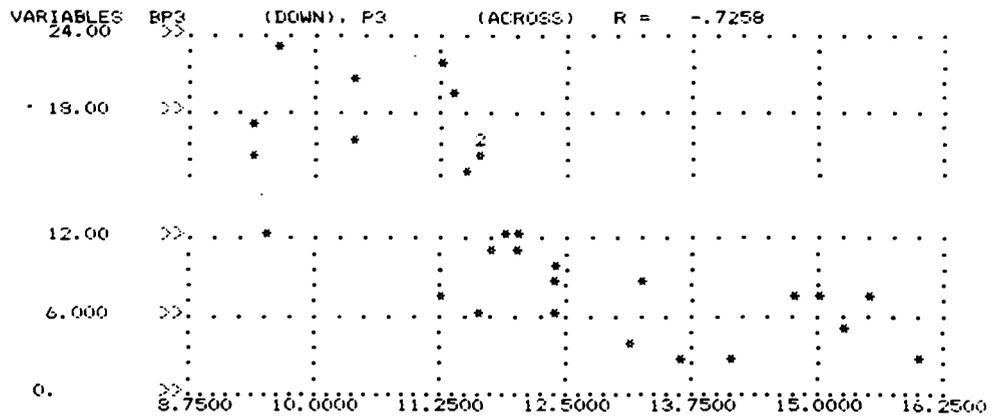


Fig. 71

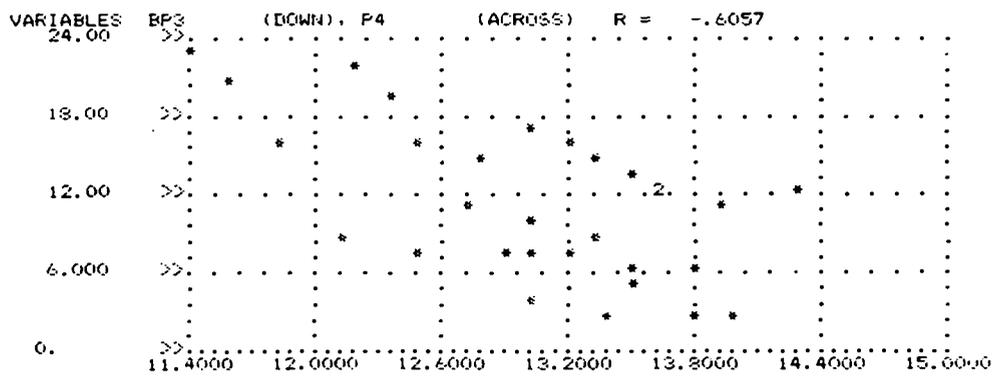


Fig. 72. Relationship between fruit P/Ca ratio and total bitter pit in Newtown apple at sampling date August 10, 1983.

Fig. 73. Relationship between fruit P/Ca ratio and total bitter pit in Newtown apple at sampling date August 29, 1983.

Fig. 74. Relationship between fruit P/Ca ratio and total bitter pit in Newtown apple at sampling date September 19, 1983.

Fig.72

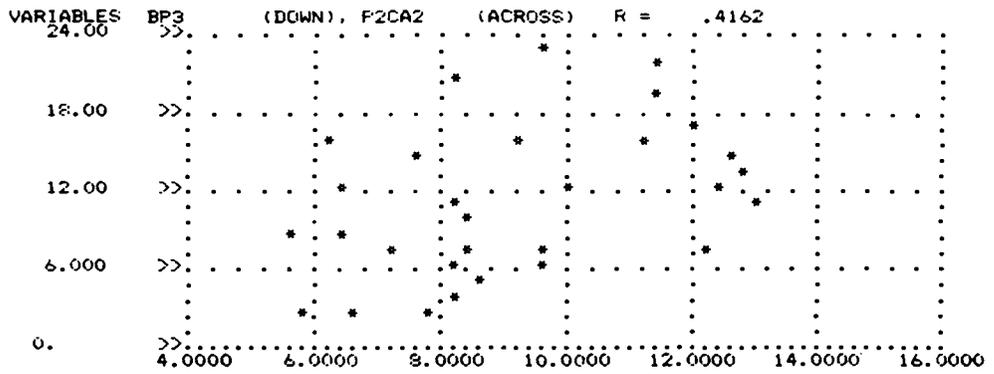


fig.73

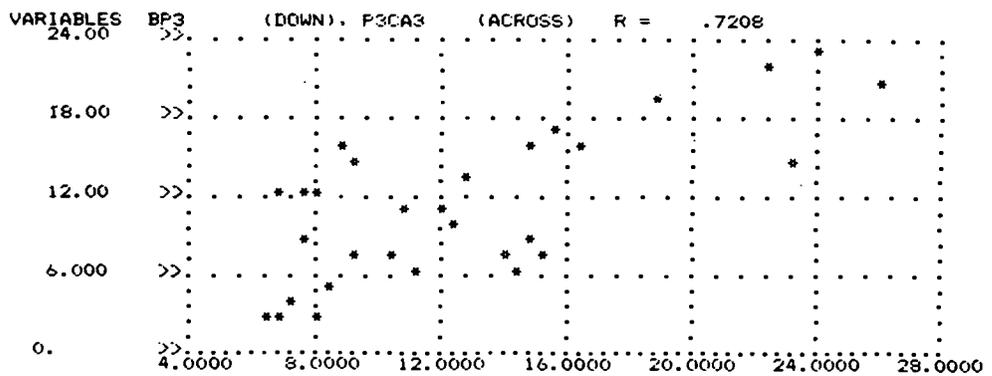


Fig.74

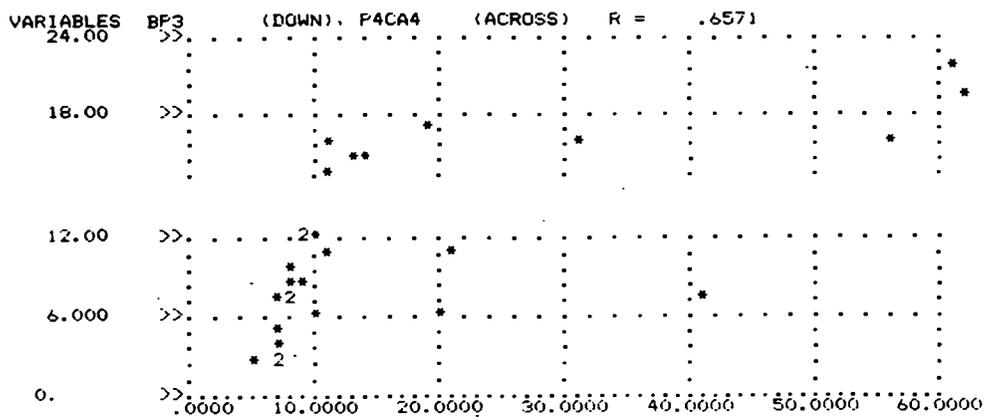


Fig. 75. Relationship between fruit K and total bitter pit in Newtown apple at sampling date July 20, 1983.

Fig. 76. Relationship between fruit K and total bitter pit in Newtown apple at sampling date August 29, 1983.

Fig. 77. Relationship between fruit K and total bitter pit in Newtown apple at sampling date September 19, 1983.

Fig.75

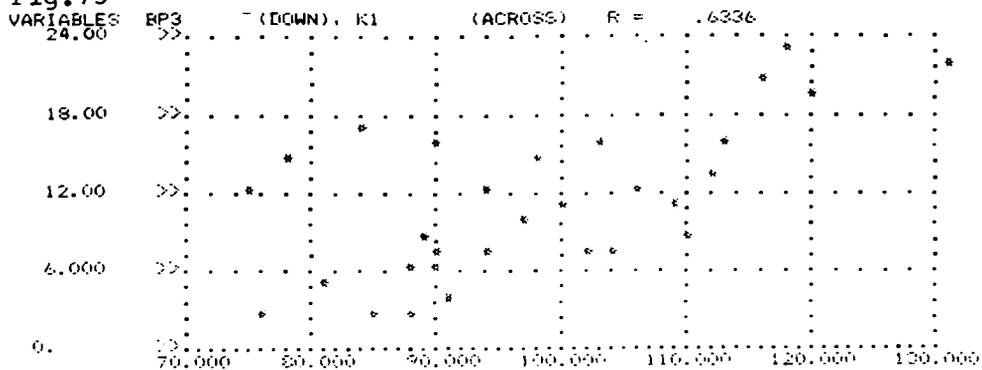


Fig.76

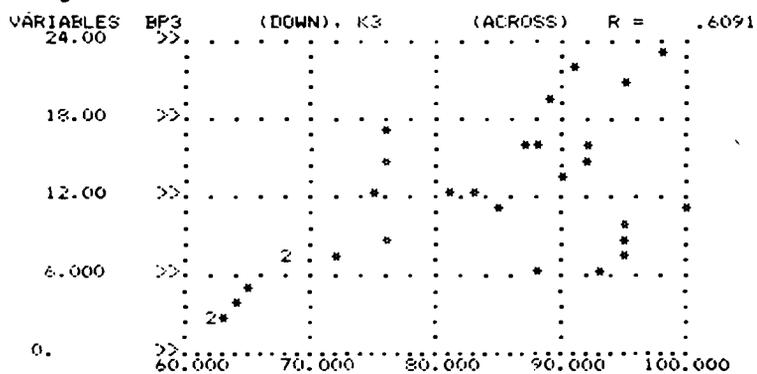


Fig.77

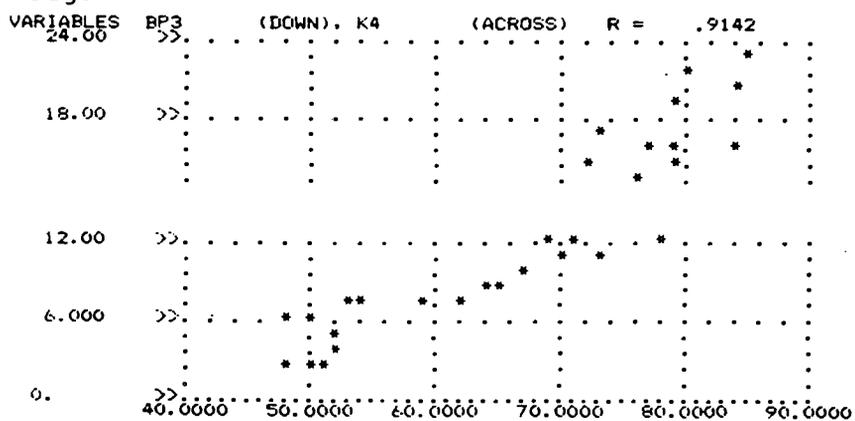


Fig. 78. Relationship between fruit K/Ca ratio and total bitter pit in Newtown apple at sampling date August 10, 1983.

Fig. 79. Relationship between fruit K/Ca ratio and total bitter pit in Newtown apple at sampling date August 29, 1983.

Fig. 80. Relationship between fruit K/Ca ratio and total bitter pit in Newtown apple at sampling date September 19, 1983.

Fig.78

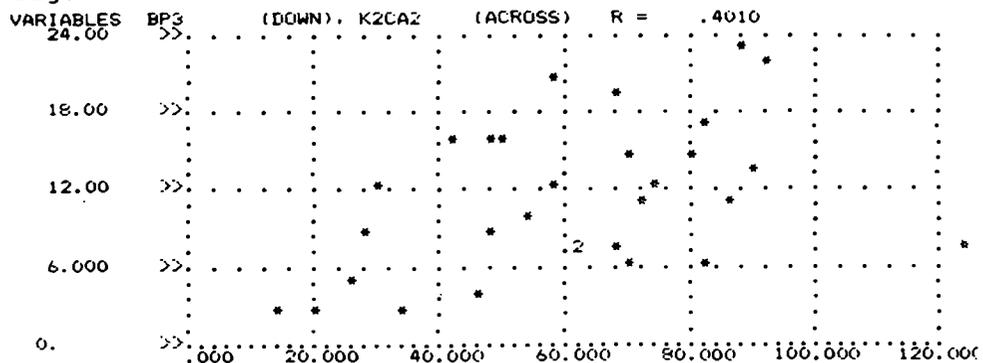
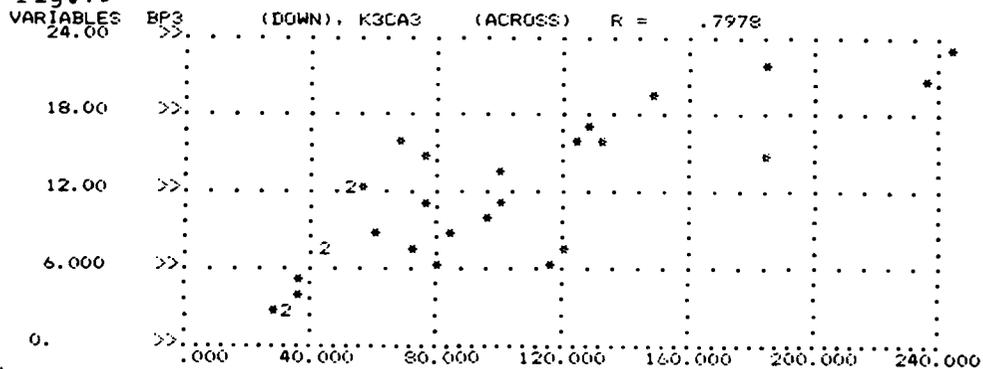


Fig.79



?

Fig.80

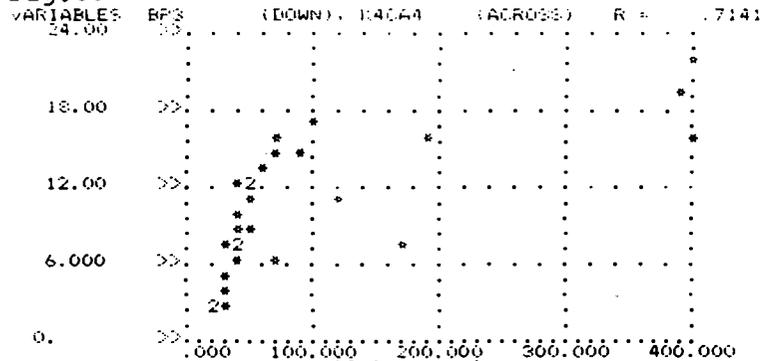


Fig. 81. Relationship between fruit $\text{Ca}/(\text{K} + \text{Mg})$ ratio and total bitter pit in Newtown apple at sampling date July 20, 1983.

Fig. 82. Relationship between fruit $\text{Ca}/(\text{K} + \text{Mg})$ ratio and total bitter pit in Newtown apple at sampling date August 10, 1983.

Fig. 83. Relationship between fruit $\text{Ca}/(\text{K} + \text{Mg})$ ratio and total bitter pit in Newtown apple at sampling date August 29, 1983.

Fig. 84. Relationship between fruit $\text{Ca}/(\text{K} + \text{Mg})$ ratio and total bitter pit in Newtown apple at sampling date September 19, 1983.

Fig.81

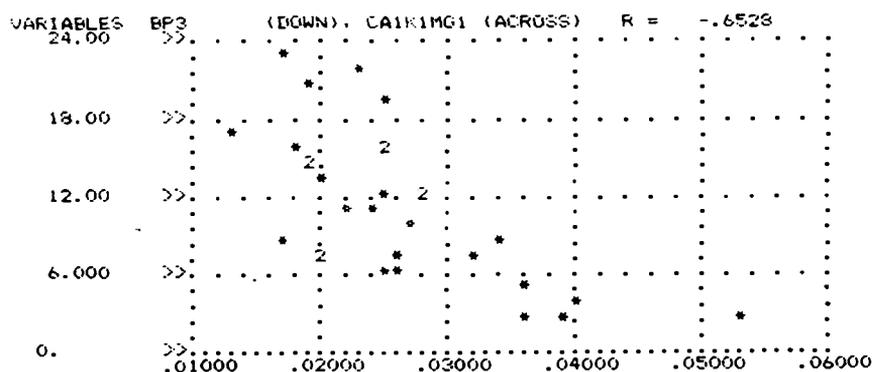


Fig.82

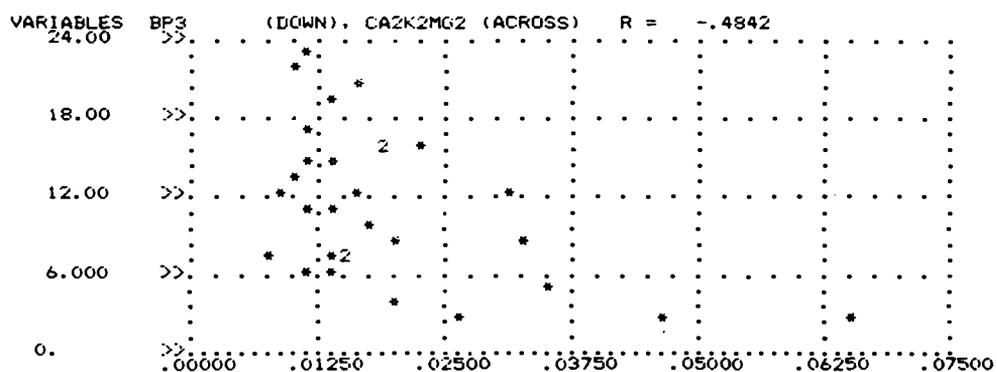


Fig.83

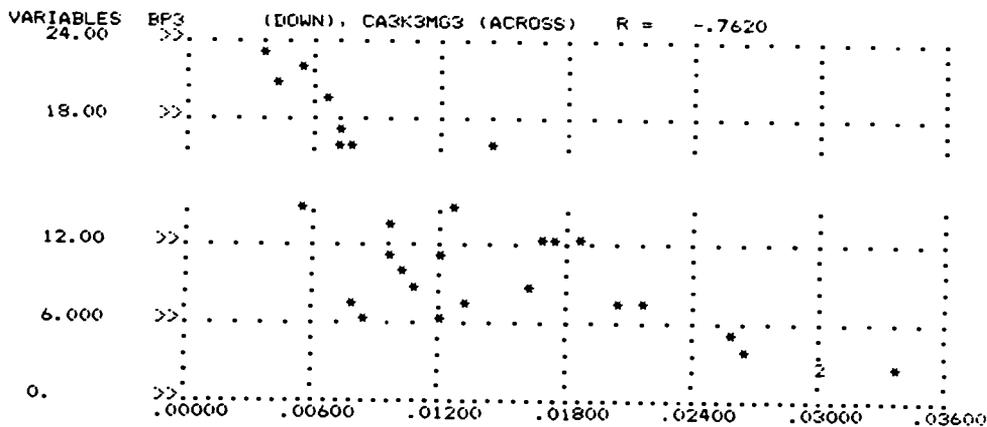


Fig.84

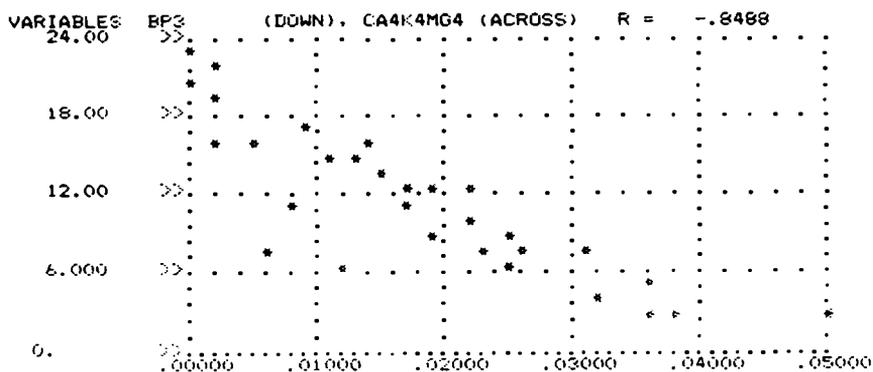


Fig. 85. Relationship between fruit $(K + Mg)/Ca$ ratio and total bitter pit in Newtown apple at sampling date July 20, 1983.

Fig. 86. Relationship between fruit $(K + Mg)/Ca$ ratio and total bitter pit in Newtown apple at sampling date August 10, 1983.

Fig. 87. Relationship between fruit $(K + Mg)/Ca$ ratio and total bitter pit in Newtown apple at sampling date August 29, 1983.

Fig. 88. Relationship between fruit $(K + Mg)/Ca$ ratio and total bitter pit in Newtown apple at sampling date September 19, 1983.

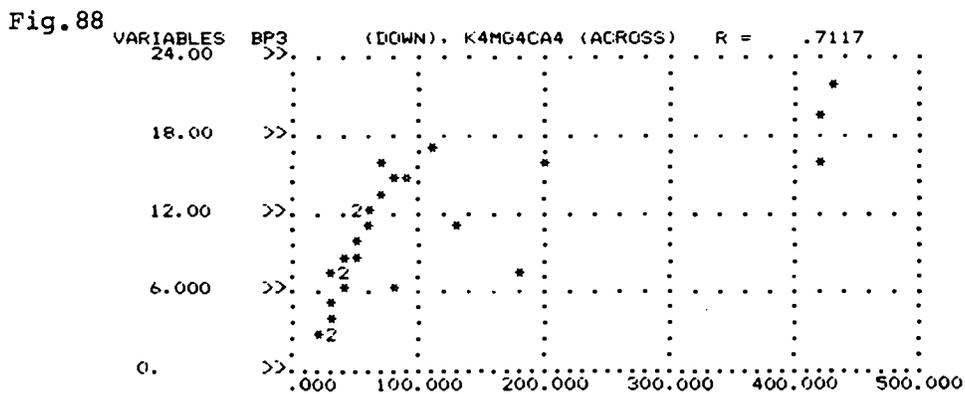
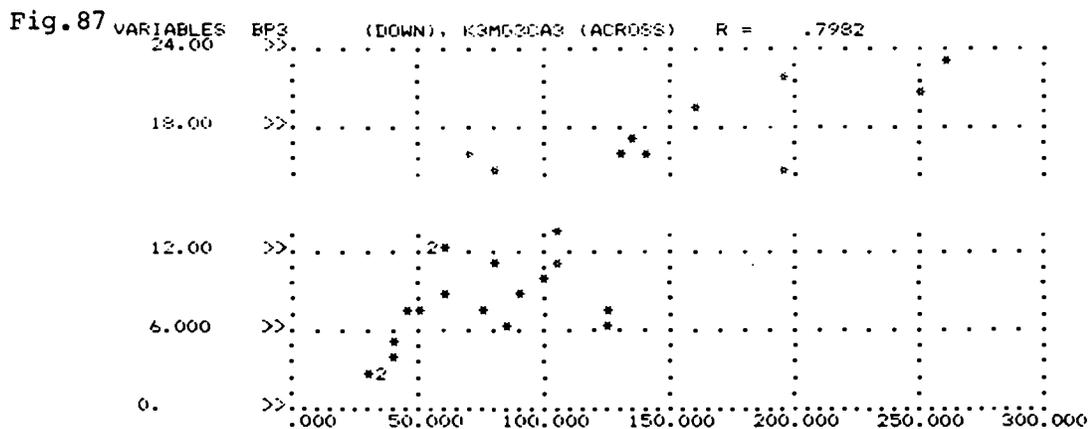
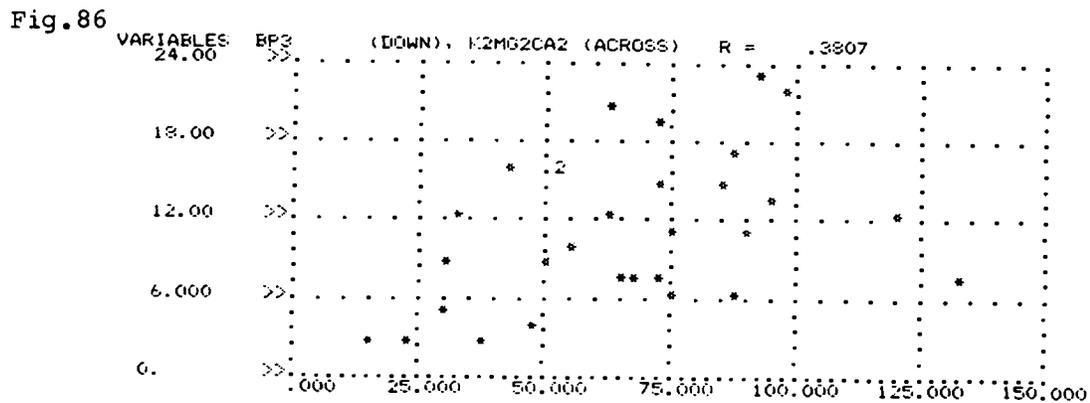
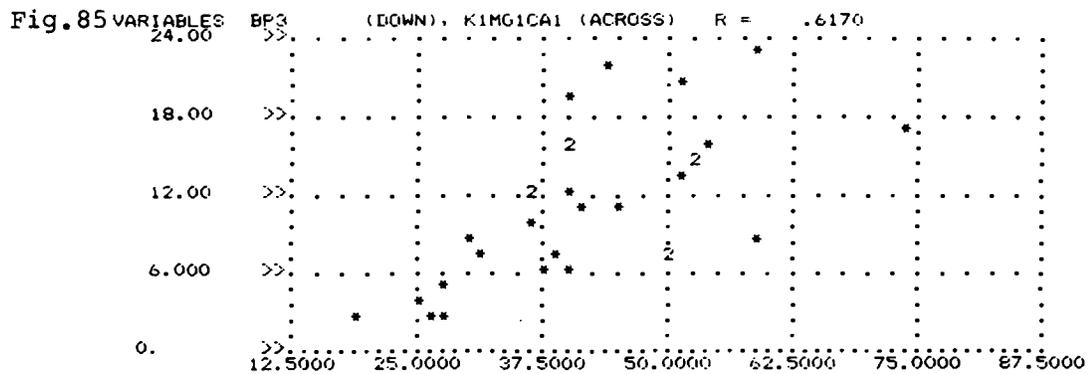


Fig. 89. Relationship between fruit Ca and internal breakdown in Newtown apple at sampling date July 19, 1982.

Fig. 90. Relationship between fruit Ca and internal breakdown in Newtown apple at sampling date August 13, 1982.

Fig. 91. Relationship between fruit Ca and internal breakdown in Newtown apple at sampling date September 6, 1982.

Fig. 92. Relationship between fruit Ca and internal breakdown in Newtown apple at sampling date September 27, 1982.

Fig.89

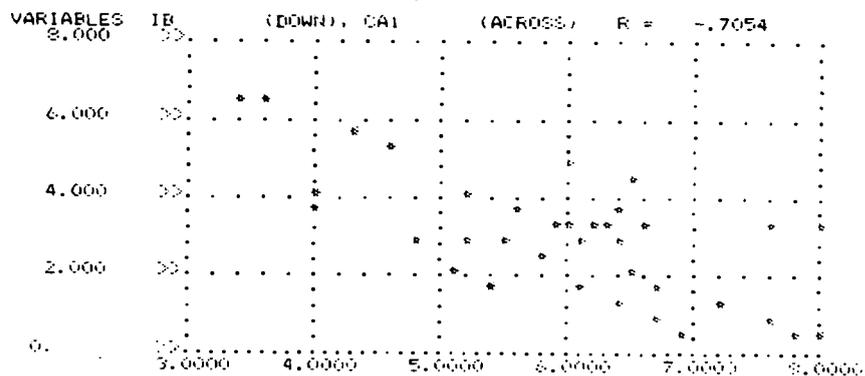


Fig.90

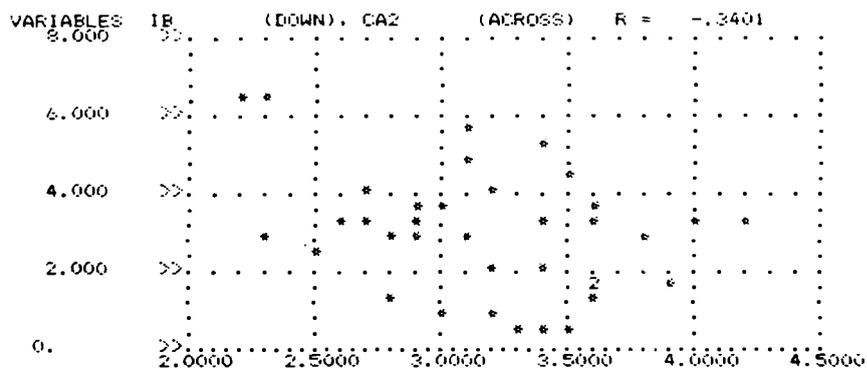


Fig.91

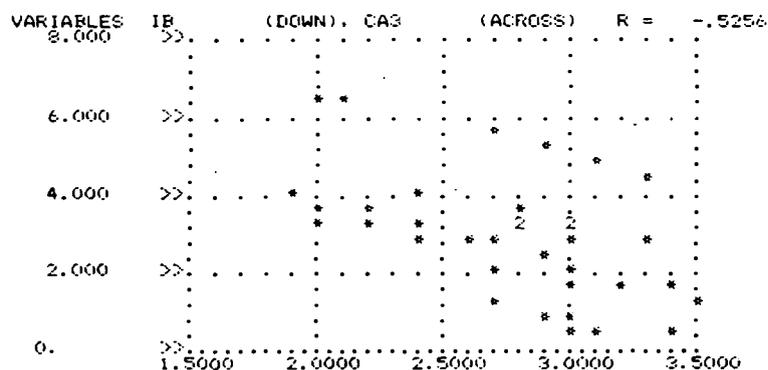


Fig.92

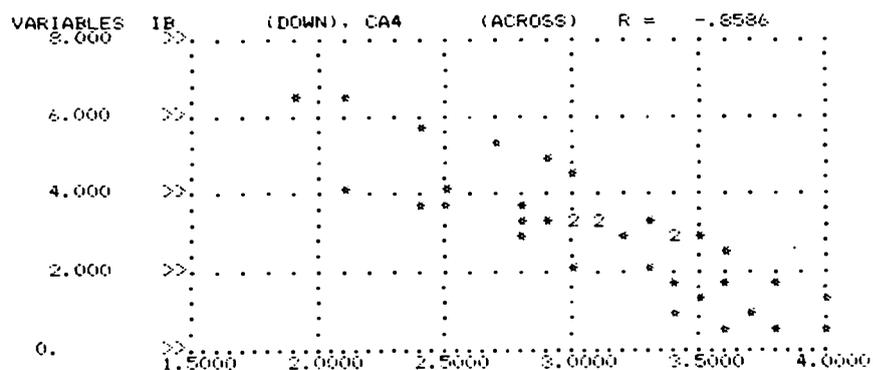
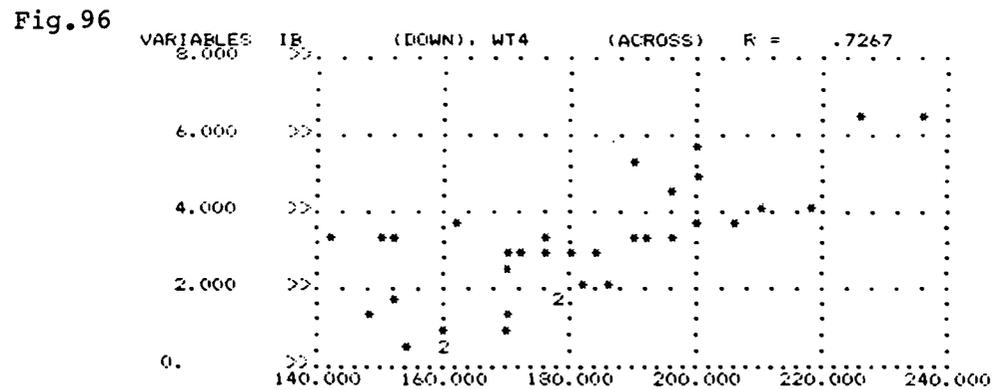
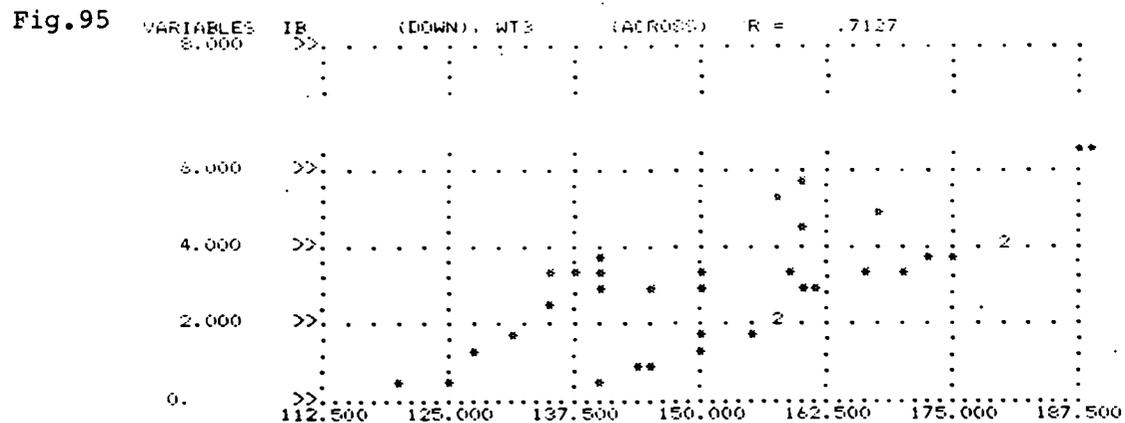
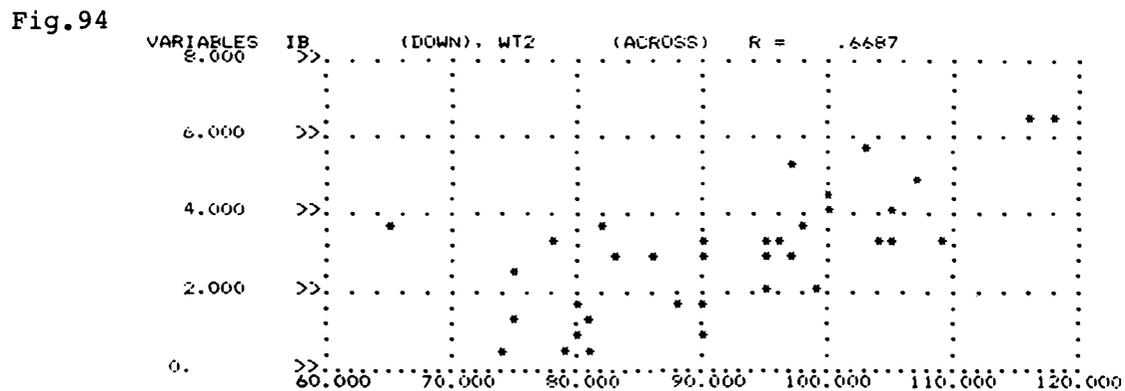
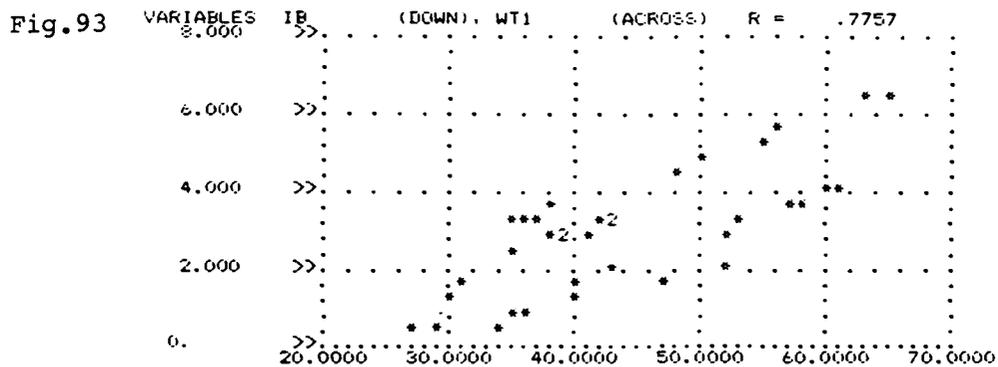


Fig. 93. Relationship between fruit weight and internal breakdown in Newtown apple at sampling date July 19, 1982.

Fig. 94. Relationship between fruit weight and internal breakdown in Newtown apple at sampling date August 13, 1982.

Fig. 95. Relationship between fruit weight and internal breakdown in Newtown apple at sampling date September 6, 1982.

Fig. 96. Relationship between fruit weight and internal breakdown in Newtown apple at sampling date September 27, 1982.



- Fig. 97. Relationship between fruit wt/Ca ratio and internal breakdown in Newtown apple at sampling date July 19, 1982.
- Fig. 98. Relationship between fruit wt/Ca ratio and internal breakdown in Newtown apple at sampling date August 13, 1982.
- Fig. 99. Relationship between fruit wt/Ca ratio and internal breakdown in Newtown apple at sampling date September 6, 1982.
- Fig. 100. Relationship between fruit wt/Ca ratio and internal breakdown in Newtown apple at sampling date September 27, 1982.

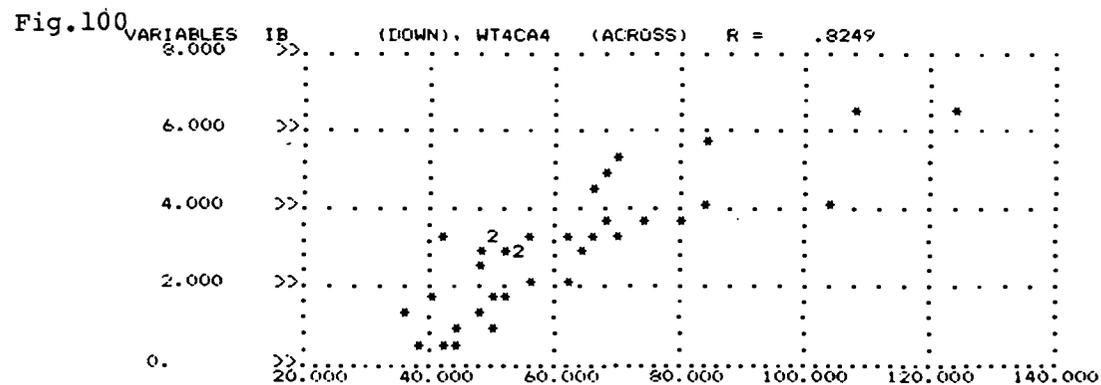
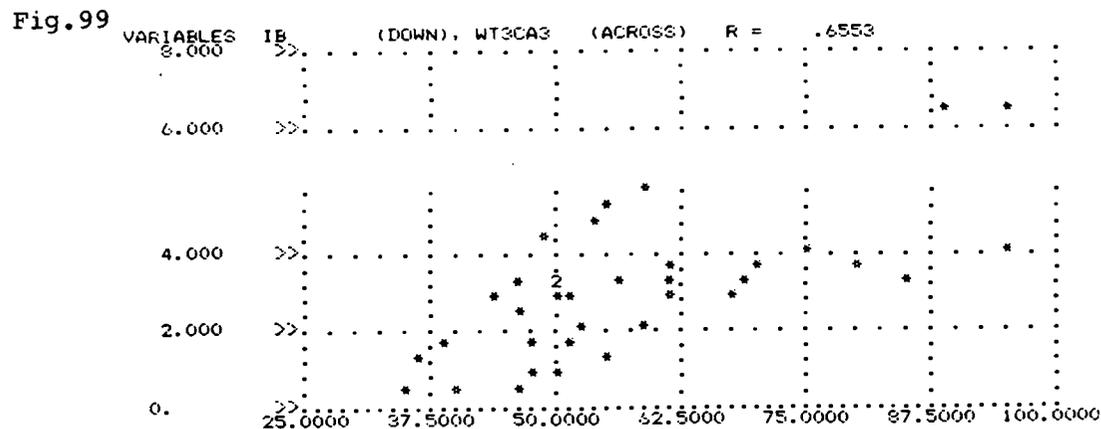
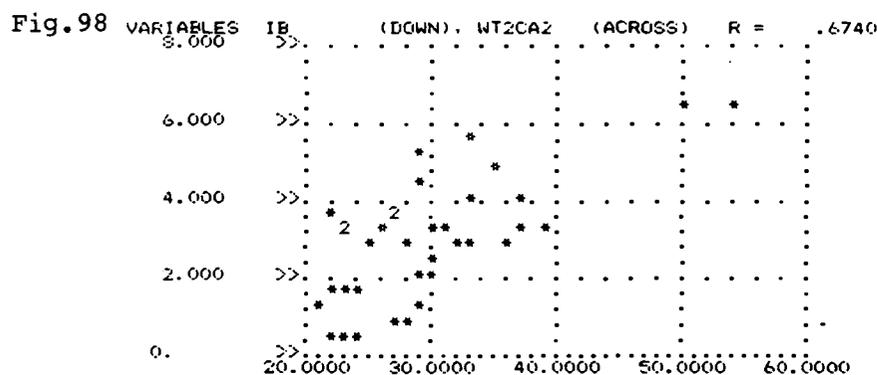
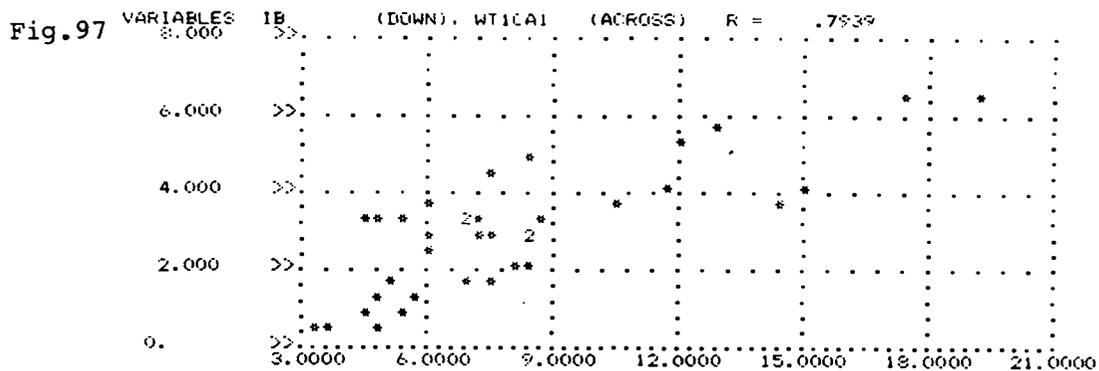


Fig. 101. Relationship between fruit Ca and internal breakdown in Newtown apple at the sampling date July 20, 1983.

Fig. 102. Relationship between fruit Ca and internal breakdown in Newtown apple at the sampling date August 10, 1983.

Fig. 103. Relationship between fruit Ca and internal breakdown in Newtown apple at the sampling date August 29, 1983.

Fig. 104. Relationship between fruit Ca and internal breakdown in Newtown apple at the sampling date September 19, 1983.

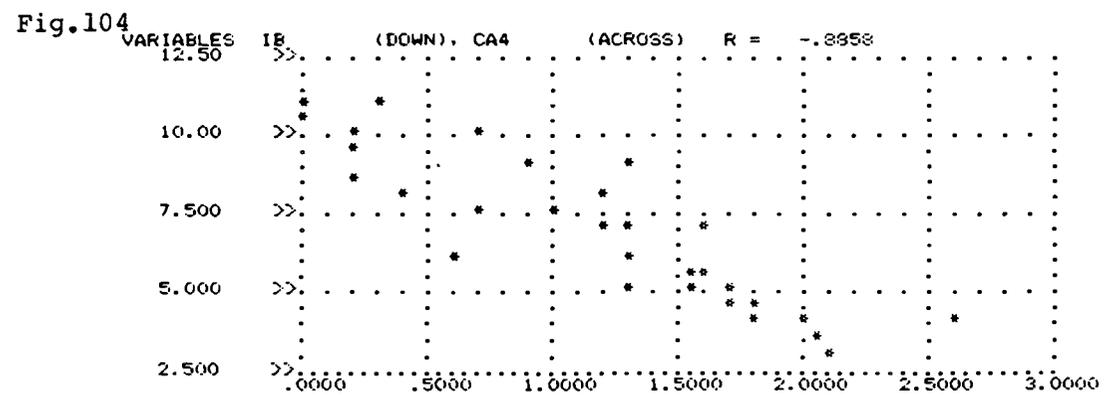
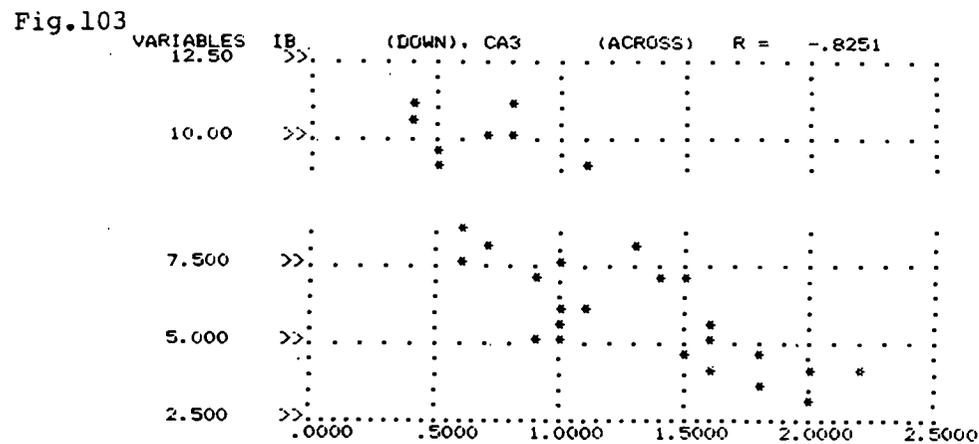
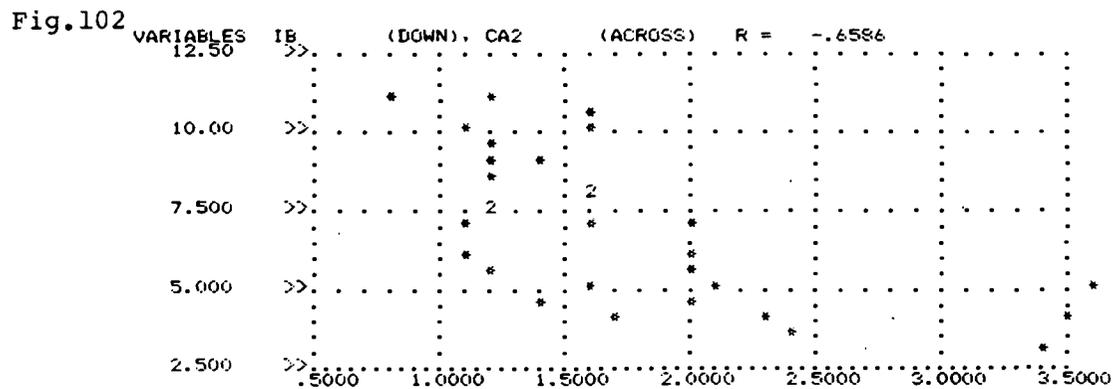
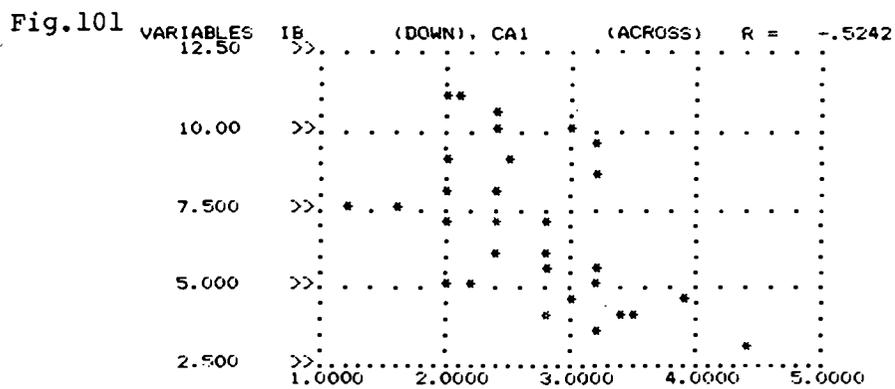


Fig. 105. Relationship between fruit Mg and internal breakdown in Newtown apple at the sampling date August 29, 1983.

Fig. 106. Relationship between fruit Mg and internal breakdown in Newtown apple at the sampling date September 19, 1983.

Fig. 107. Relationship between fruit Mg and internal breakdown in Newtown apple at the harvest date August 29, 1983.

Fig. 108. Relationship between fruit Mg and internal breakdown in Newtown apple at the sampling date September 19, 1983.

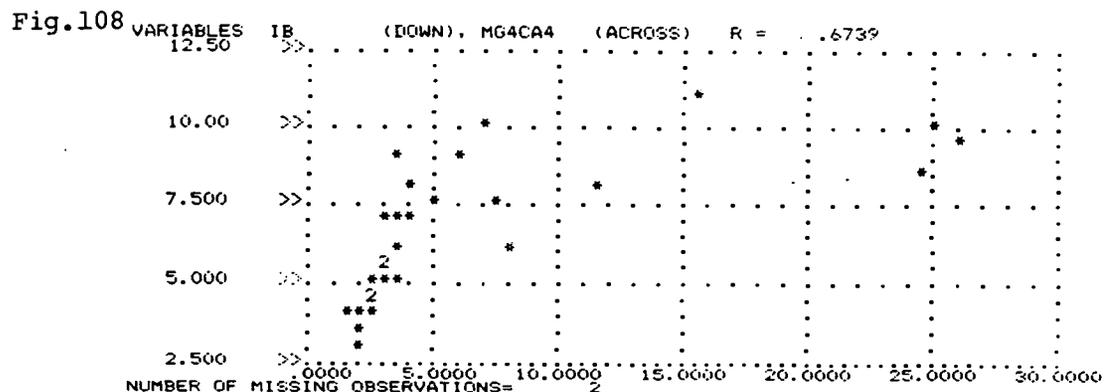
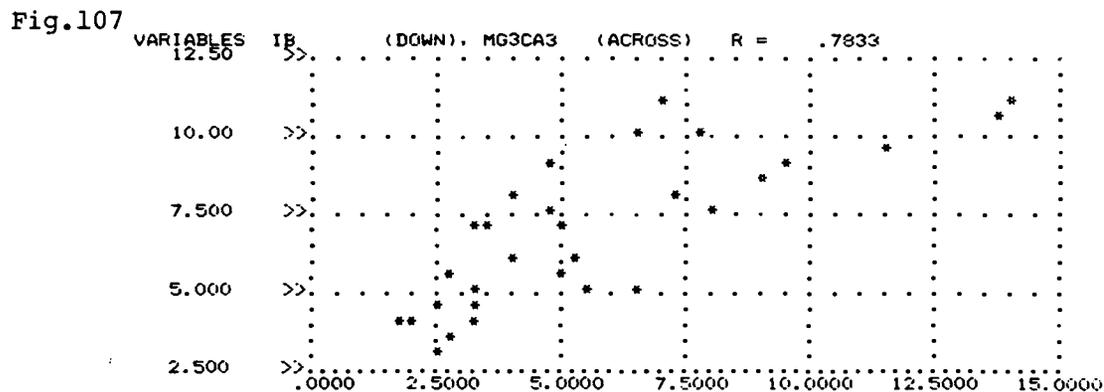
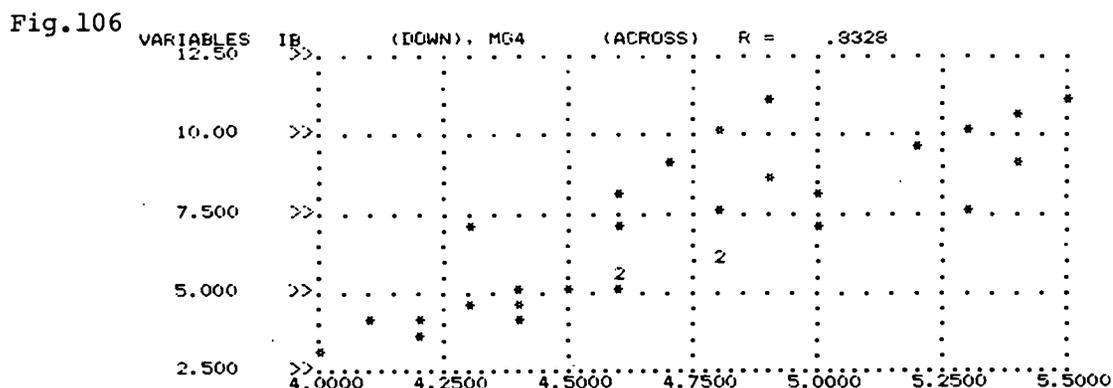
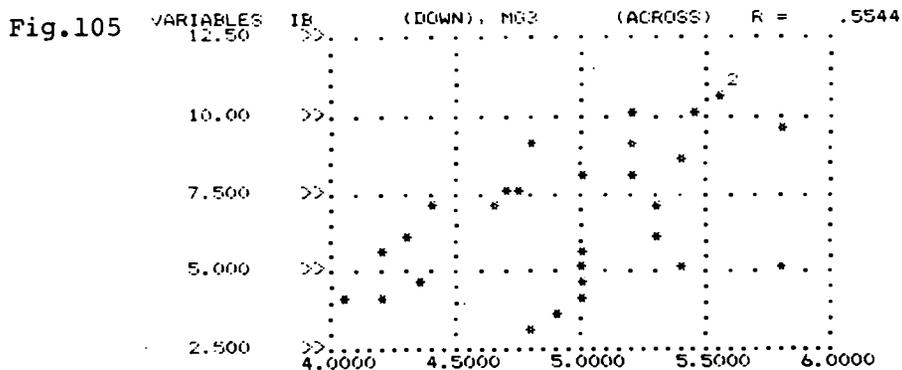


Fig. 109. Relationship between fruit P and internal breakdown in Newtown apple at the sampling date July 20, 1983.

Fig. 110. Relationship between fruit P and internal breakdown in Newtown apple at the sampling date August 10, 1983.

Fig. 111. Relationship between fruit P and internal breakdown in Newtown apple at the sampling date August 29, 1983.

Fig.109

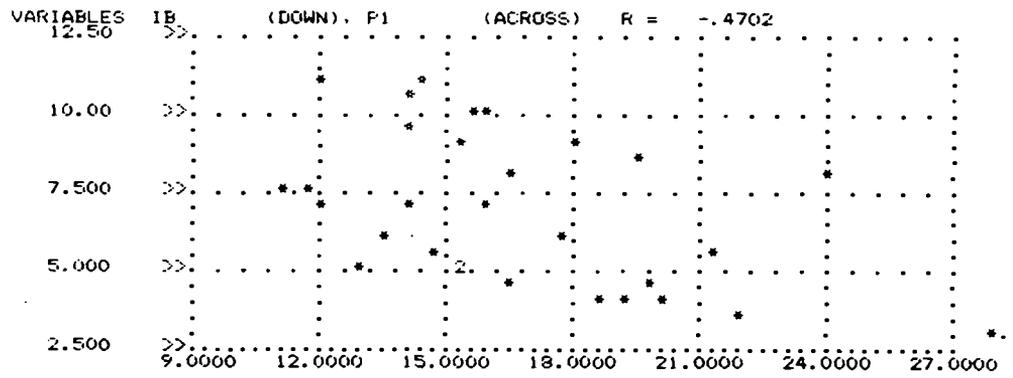


Fig.110

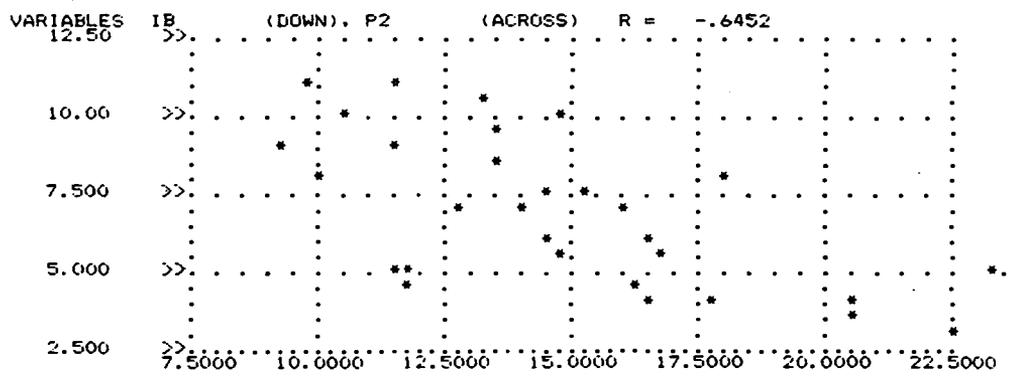


Fig.111

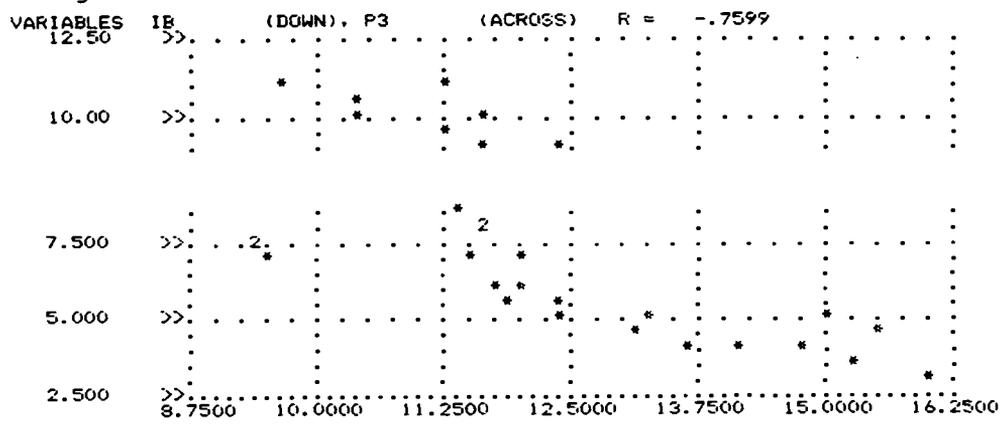


Fig. 112. Relationship between P/Ca ratio and internal breakdown in Newtown apple at the sampling date August 29, 1983.

Fig. 113. Relationship between fruit P/Ca ratio and internal breakdown in Newtown apple at the sampling date September 19, 1983.

Fig. 114. Relationship between fruit weight and internal breakdown in Newtown apple at the sampling date July 20, 1983.

Fig. 115. Relationship between fruit weight and internal breakdown in Newtown apple at the sampling date August 10, 1983.

Fig. 116. Relationship between fruit weight and internal breakdown in Newtown apple at the sampling date August 29, 1983.

Fig. 117. Relationship between fruit weight and internal breakdown in Newtown apple at the sampling date September 19, 1983.

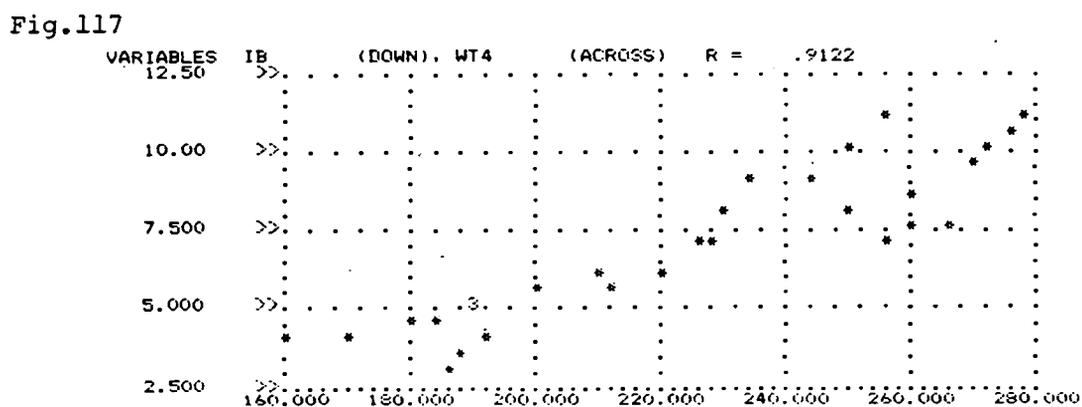
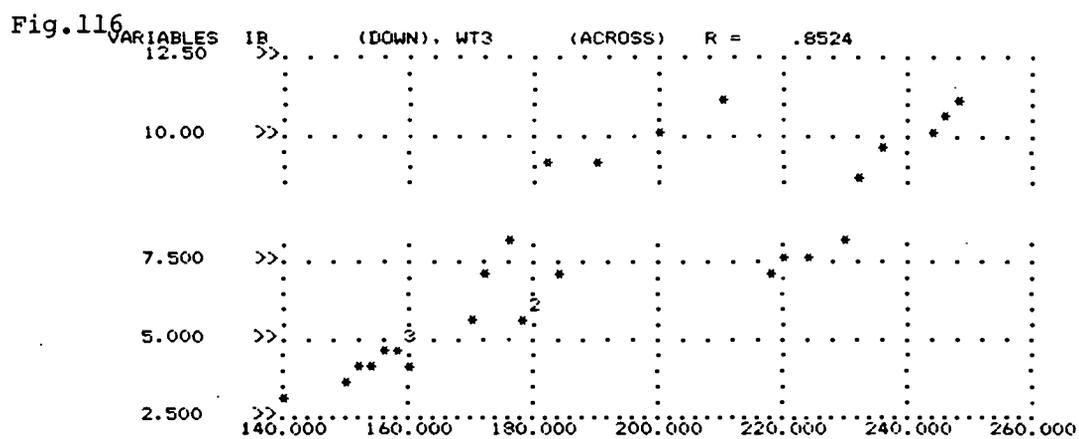
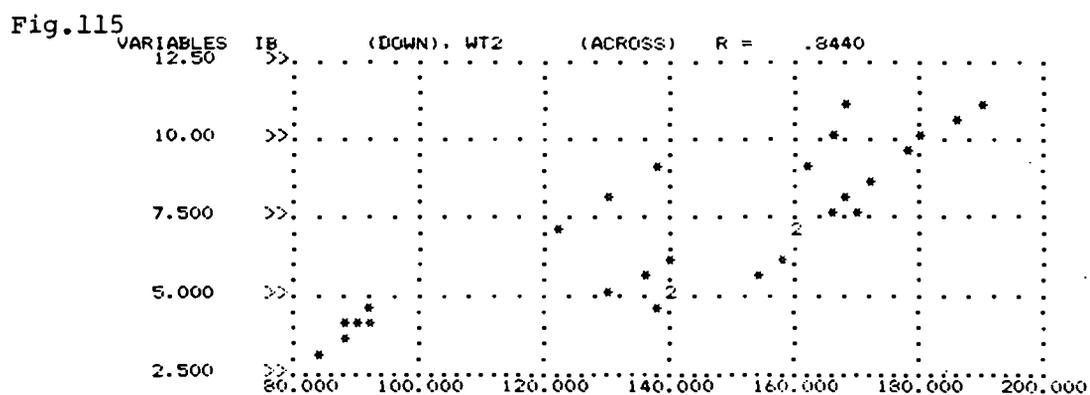
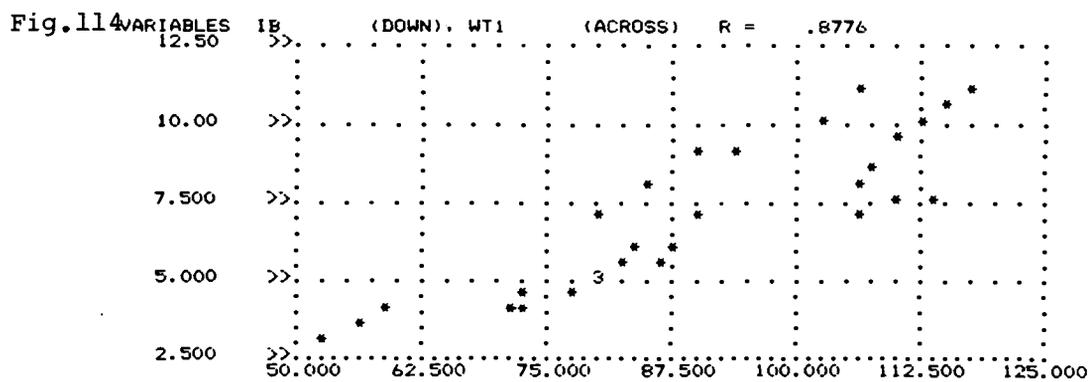


Fig. 118. Relationship between fruit wt/Ca ratio and internal breakdown in Newtown apples at the sampling date July 20, 1983.

Fig. 119. Relationship between fruit wt/Ca ratio and internal breakdown in Newtown apples at the sampling date August 10, 1983.

Fig. 120. Relationship between fruit wt/Ca ratio and internal breakdown in Newtown apples at the sampling date August 29, 1983.

Fig. 121. Relationship between fruit wt/Ca ratio and internal breakdown in Newtown apple at the sampling date September 19, 1983.

Fig.118

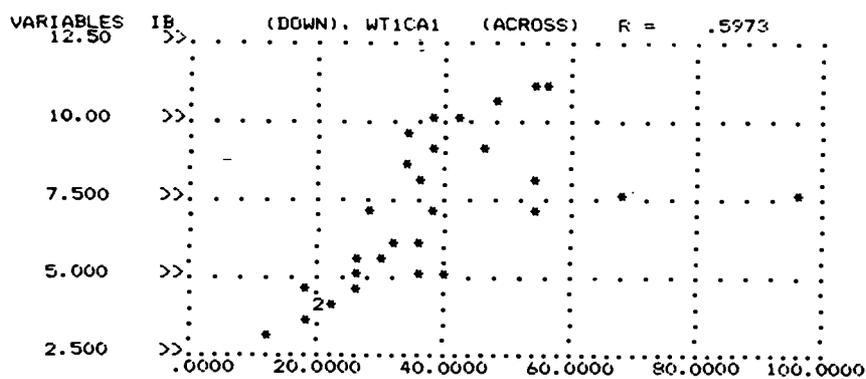


Fig.119

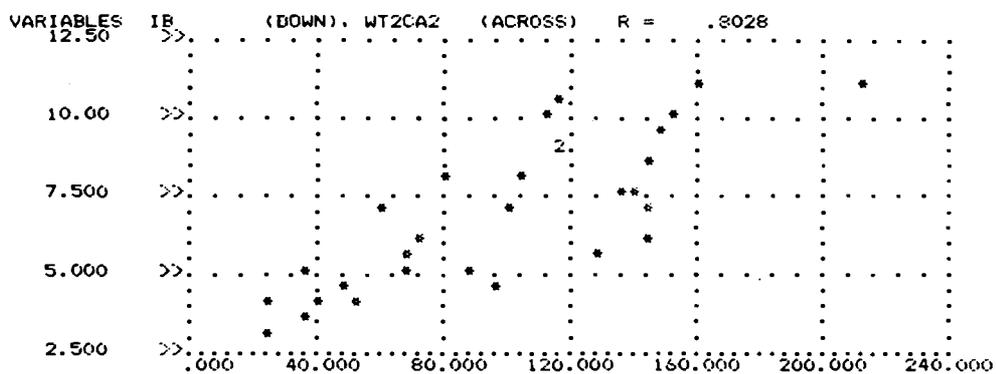


Fig.120

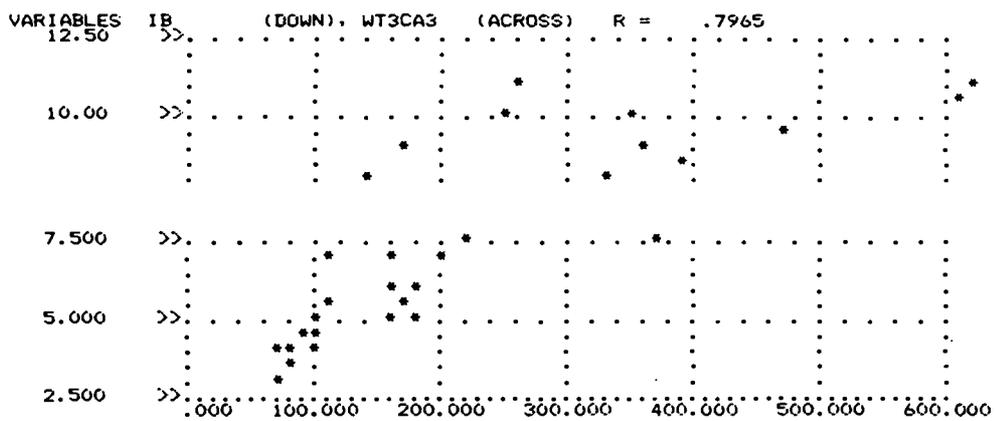


Fig.121

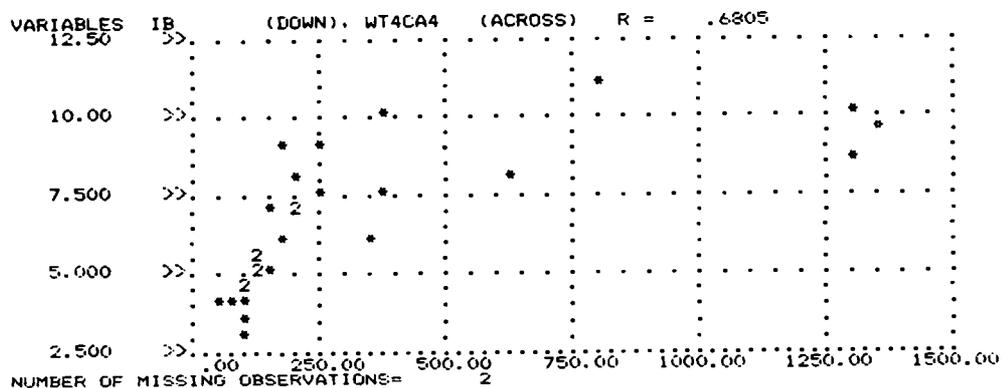


Fig. 122. Relationship between fruit K and internal breakdown in Newtown apple at the sampling date August 29, 1983.

Fig. 123. Relationship between fruit K and internal breakdown in Newtown apple at the sampling date September 19, 1983.

Fig. 124. Relationship between fruit B and internal breakdown in Newtown apple at the sampling date August 29, 1983.

Fig. 125. Relationship between fruit B and internal breakdown in Newtown apple at the sampling date September 19, 1983.

Fig. 122

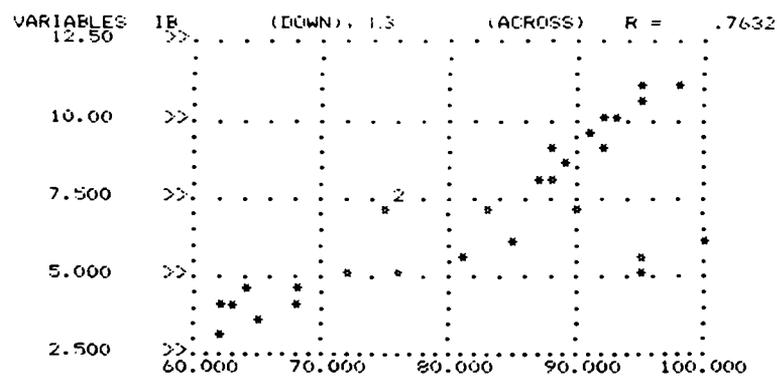


Fig.123

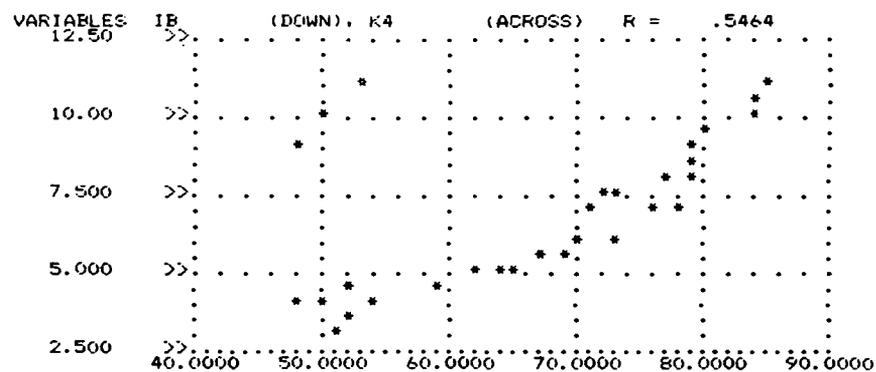


Fig.124

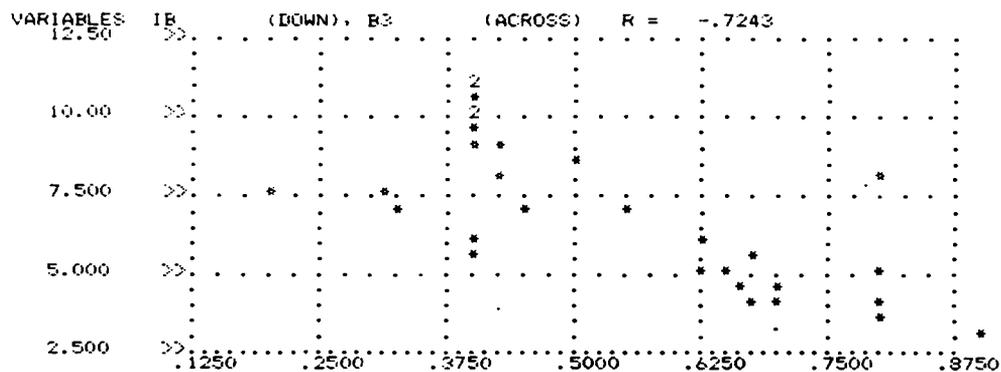


Fig.125

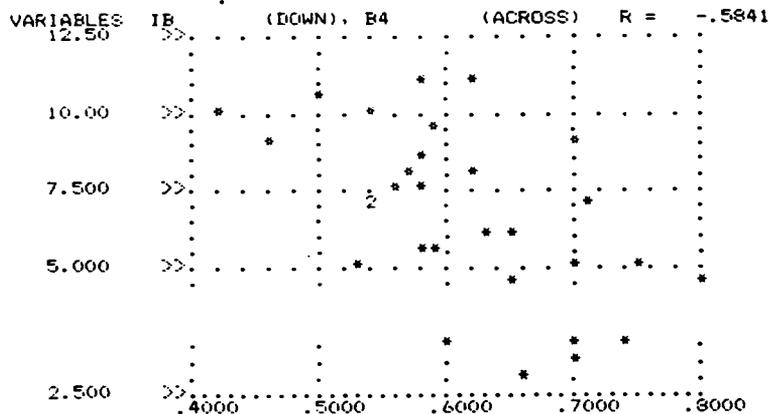


Fig. 126. Relationship between fruit K/Ca ratio and internal breakdown in Newtown apple at the sampling date July 20, 1983.

Fig. 127. Relationship between fruit K/Ca ratio and internal breakdown in Newtown apple at the sampling date August 10, 1983.

Fig. 128. Relationship between fruit K/Ca ratio and internal breakdown in Newtown apple at the sampling date August 29, 1983.

Fig. 129. Relationship between fruit K/Ca ratio and internal breakdown in Newtown apple at the sampling date September 19, 1983.

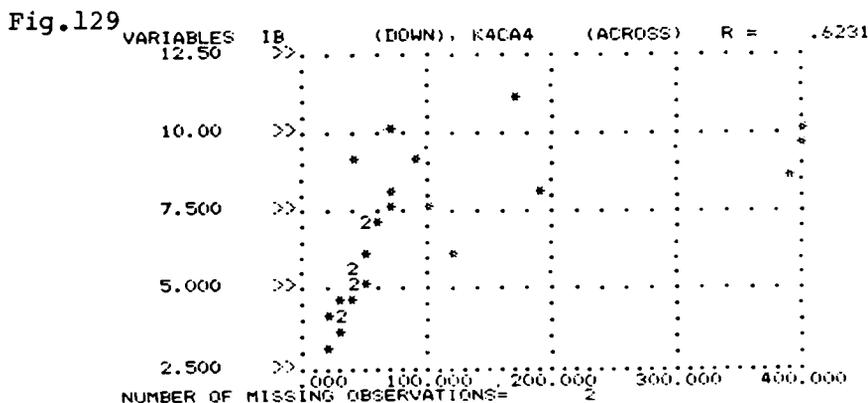
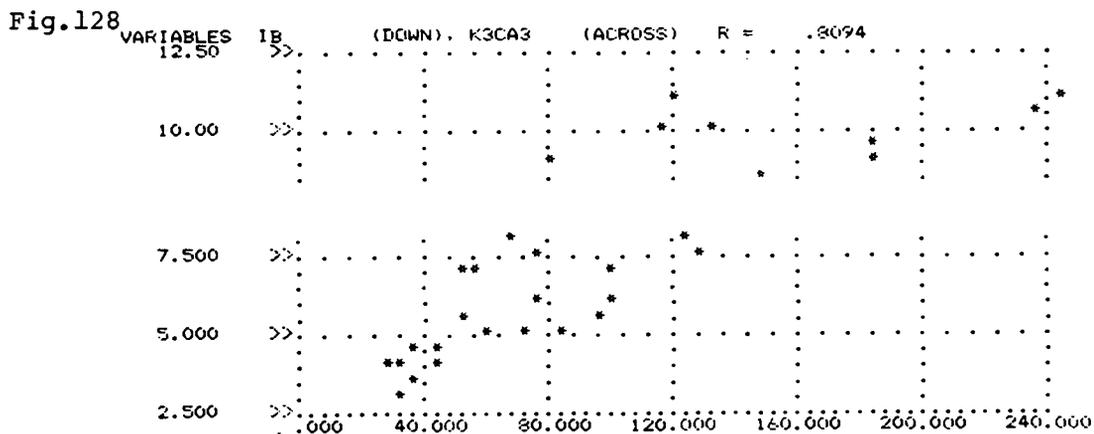
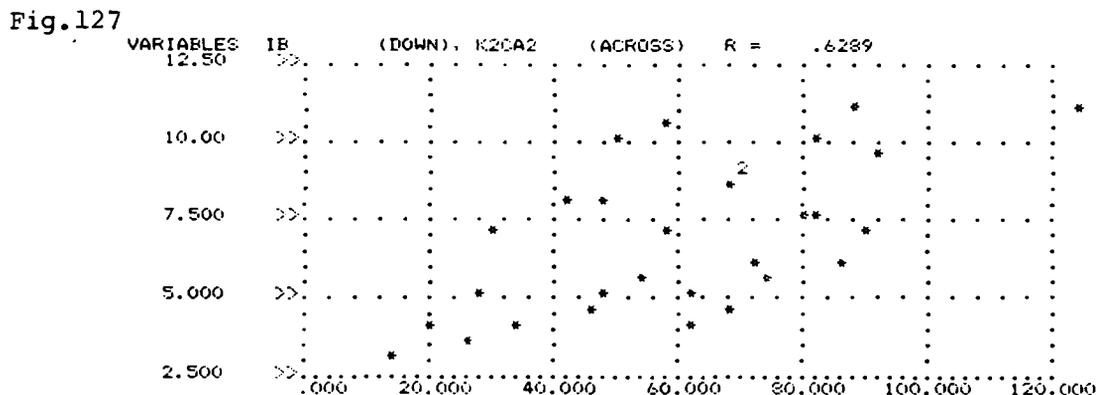
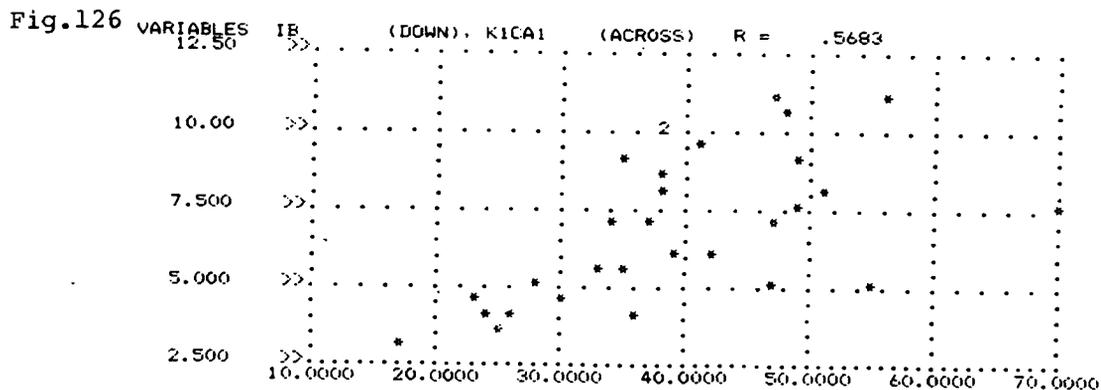


Fig. 130. Relationship between fruit N/Ca ratio and internal breakdown in Newtown apple at the sampling date July 20, 1983.

Fig. 131. Relationship between fruit N/Ca ratio and internal breakdown in Newtown apple at the sampling date August 10, 1983.

Fig. 132. Relationship between fruit N/Ca ratio and internal breakdown in Newtown apple at the sampling date August 29, 1983.

Fig. 133. Relationship between fruit N/Ca ratio and internal breakdown in Newtown apple at the sampling date September 19, 1983.

Fig.130

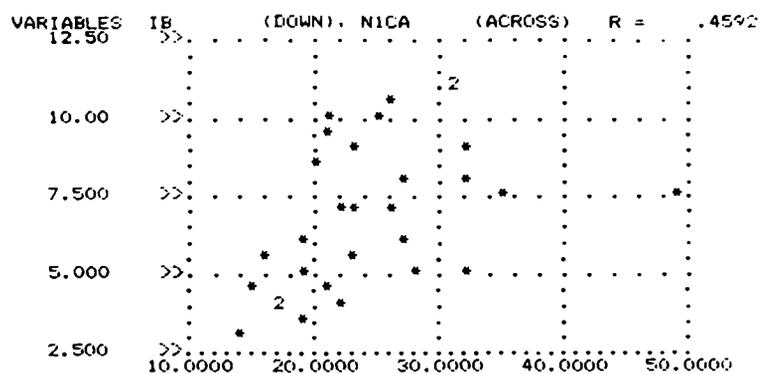


Fig.131

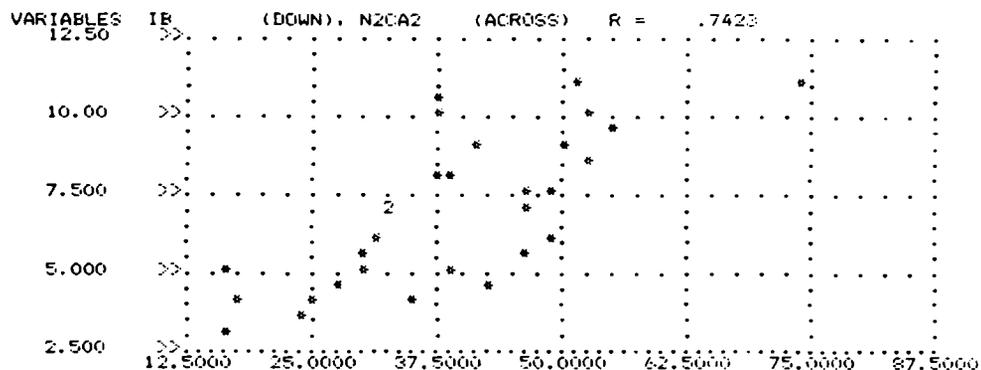


Fig.132

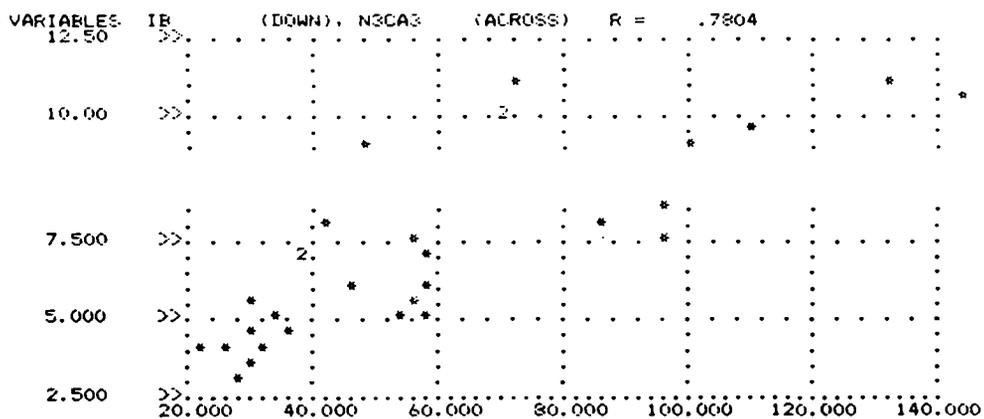
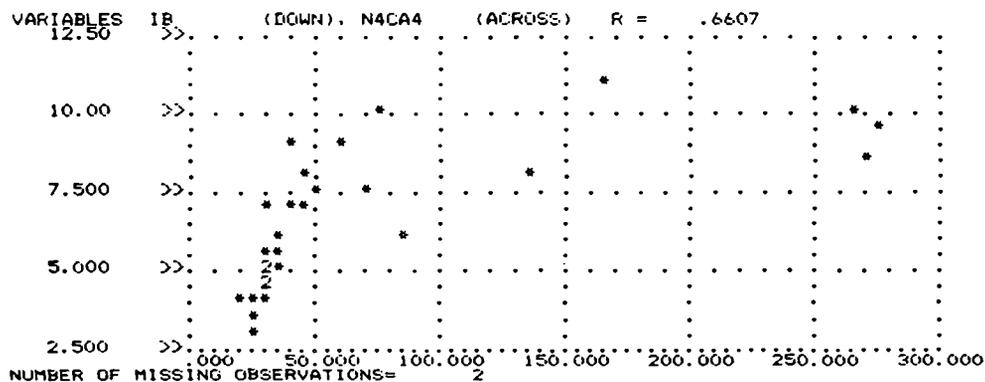


Fig.133



Relative prediction of BP, IB and CS incidence of apple and pear in 1984 based on equations developed in 1982 and 1983. All values are in percentage

Sampling date August 29	Sampling date July 19	Sampling date august 9
Predicted total BP	Predicted total IB	Predicted total CS
2.0	1.7	2.1
2.5	2.0	2.3
2.8	2.6	2.3
2.4	2.3	2.8
3.4	2.9	3.2
3.1	2.7	3.7
3.8	3.4	4.1
4.4	4.1	4.8
4.7	4.5	4.6
5.2	4.9	5.1

Average values per orchard. Samples of 10 orchards .