INFLUENCES OF NUTRITIONAL ENVIRONMENT ON THE NON-VOLATILE ORGANIC ACID COMPOSITION OF POST-HARVEST APPLE FRUITS

Thesis for the Degree of Ph. D.

MICHIGAN STATE UNIVERSITY

Herbert Conrad Dostal

1963

This is to certify that the

thesis entitled

INFLUENCES OF NUTRITIONAL ENVIRONMENT ON THE NON-VOLATILE ORGANIC ACID COMPOSITION OF POST-HARVEST APPLE FRUITS

presented by

Herbert Conrad Dostal

has been accepted towards fulfillment of the requirements for

Ph.D. degree in Horticulture

R. L. Kenworthy
Major professor

Date July 22, 1963

O-169

INFLUENCES OF NUTRITIONAL ENVIRONMENT ON THE NON-VOLATILE ORGANIC ACID COMPOSITION OF POST-HARVEST APPLE FRUITS

By

Herbert Conrad Dostal

AN ABSTRACT OF A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Horticulture

1963

ABSTRACT

INFLUENCES OF NUTRITIONAL ENVIRONMENT ON THE NON-VOLATILE ORGANIC ACID COMPOSITION OF POST-HARVEST APPLE FRUITS

by Herbert Conrad Dostal

Apple trees of two varieties, Golden Delicious and Jonathan, grafted on Malling IX rootstocks, were grown for a period of three years (1960-1962) under intensive treatment with modified Hoagland nutrient solutions. These treatments consisted of high and low levels of nitrogen, phosphorus, potassium, calcium and magnesium, and a control treatment.

Leaf samples were removed and analyzed spectrographically each year in order to trace the nutritional status of the trees. Fruit samples were taken at horticultural maturity in 1961 and 1962, and were analyzed for total acidity and non-volatile organic acids, using ion exchange chromatography.

Four organic acids were isolated and identified. These acids were citric, malic, quinic and shikimic. Phosphoric acid was also detected in the tissue extracts in considerable amounts.

Greatest variations in shikimic acid content of peel tissue were noted in response to several treatments. Shikimic acid of peel tissue was greatly increased in response to low potassium, low calcium, low magnesium, and high nitrogen. Quinic acid content of apple fruit tissue was increased by high nitrogen, high phosphorus, low potassium, low calcium, and low and high magnesium treatments.

The greatest significant differences in malic acid content of apple tissue was noted in response to three treatments. High nitrogen decreased malic acid levels. Low potassium also decreased malic acid content, but high potassium effected a significant increase in malic acid levels. Low nitrogen increased malic acid levels greatly. Possible explanations are discussed.

Citric acid variations were found in response to potassium and phosphorus treatments. Low potassium increased citric acid content, as did low phosphorus.

Phosphoric acid variations were greatest in response to phosphorus treatments. Low phosphorus decreased levels of phosphate in the tissue, while high phosphorus treatments increased phosphate.

Leaf analysis data indicated differences in mineral composition associated with treatments, closely approximating the desired levels.

Variations were noted in total extractable acidity and in total titratable acidity.

INFLUENCES OF NUTRITIONAL ENVIRONMENT ON THE NON-VOLATILE ORGANIC ACID COMPOSITION OF POST-HARVEST APPLE FRUITS

By

Herbert Conrad Dostal

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Horticulture

1963

ACKNOWLEDGEMENTS

The author wishes to express his sincere appreciation to Dr. A. L. Kenworthy for his guidance and assistance in the conduct of the experiment, and for his suggestions in the preparation of the manuscript; to Dr. D. R. Dilley for his guidance and assistance in the research work and his suggestions and guidance in the manuscript preparation; to Drs. R. S. Bandurski, M. J. Bukovac and H. M. Sell, for their guidance and suggestions in editing the manuscript.

Most important, deep appreciation is expressed to the author's wife and children, for their sacrifice and courage throughout the period of graduate study.

TABLE OF CONTENTS

	Page
INTRODUCTION	1
REVIEW OF LITERATURE	2
EXPERIMENTAL PROCEDURE	14
RESULTS	21
Organic Acid Composition	22
Total Titratable Acidity	32
Total Extractable Acidity	34
Mineral Composition	36
DISCUSSION	52
SUMMARY	58
LITERATURE CITED	60
APPENDIX	67

INTRODUCTION

A living plant is a complex biological system. One of the more fundamental aspects of such a plant is the relationship between the quantities of essential nutrient elements absorbed by and translocated within the plant, and its metabolism. Some of these nutrient elements are synthesized into vital constituents, while others may serve as catalysts. Thus, a deficiency in any nutrient affects the normal metabolic system, and may disturb the balance of chemical constituents within the plant.

Abnormal accumulations of certain constituents may be associated with the development of a typical symptom, usually attributed to an abnormal supply of a specific nutrient.

Numerous earlier investigations have indicated definite and marked variations in the composition of apple fruits as affected by various environmental factors.

This study was undertaken to establish the relationship between varying levels of certain essential nutrients as applied to the growing medium of apple trees, and the non-volatile portion of the organic acid complement of the resulting fruits.

REVIEW OF LITERATURE

Since the inception of horticultural research, workers have recognized the importance of the various nutrient elements to be biochemical balance of fruit trees and other horticultural crops. However, the concept of a rigid nutrient balance is a relatively recent innovation. This concept, which included all of the essential elements, was stated by Shear et al. (75) in 1946. They proposed that as any element is increased or decreased from its normal concentration at optimum intensity, the maximum benefits possible as regards yield and quality, within the new limits of supply of that element, can result only when the concentrations of all of the elements have been redistributed in balance with the new level of intensity determined by that element.

When the essential elements are in optimum balance, any disruption of that balance, at any intensity, without concomitant redistribution of all of the elements, will result in an unbalanced nutrition which will be reflected in one or more non-conformities to the criteria used to evaluate plant yield and quality. Deleterious effects resulting from an unbalance may manifest themselves in the form of reduced yield, abnormal growth, or poor storage quality.

Applications of mineral elements to the soil may affect the content of these substances in the various parts of the plant. Several investigators

have studied the gross effects of these factors by observing the storage behavior of apple fruits in relation to the nutrient supply to the trees. Many such studies have indirectly indicated a relationship between nutritional environment and organic acid composition, but none have made a direct association between the two factors.

The investigations of Eave and Leefe (24) demonstrated a positive correlation between the level of leaf potassium and fruit acidity, as well as between fruit acidity and levels of potassium applied to the soil.

In a similar study, Wilkinson (85) showed that as potassium levels in the apple fruit increased, the total titratable acidity of the fruit likewise increased.

Further evidence of this relationship between potassium and fruit acidity was provided in the investigations by Bunemann (14), wherein he indicated that the two factors were positively correlated.

It has been suggested by Tomkins (82) that the quantity of acid formed from carbohydrates is determined by the amount of potassium which enters the fruit, and that under varying conditions, rates of various metabolic reactions may fluctuate accordingly. Thus, as a result of these disturbed endogenous conditions, some physiological disorders may occur.

It was the conclusion of Krotkov et al. (52), however, that the variations in sugar contents within the apple fruit were not a reflection of the variations in organic acid levels within the fruits. Contemplating these

factors, Hulme (44) concluded that there must be a unique mechanism whereby the apple fruit has the capacity to accumulate acids, and to maintain a pH which is considerably lower than that of any other portion of the organism.

Until recently the most commonly employed method of expressing acidity in apples, and in other plant material, was in terms of equivalents of malic acid (3, 25, 27, 30, 34, 51). It has been estimated by Kidd and West (50) that up to 98 percent of the total acidity in a mature apple fruit may be in the form of malic acid. Thus, errors in calculations of total acid as malic acid in mature fruits tended to be very small, although a large portion of the acid may be combined with cations as a salt (44).

A number of organic acids have been found to occur in the various tissues of the apple fruit, and in apple fruit products. These observations are noted in Table 1.

An extremely comprehensive review, concerning research on organic acids of apple fruits prior to 1958, was provided by Hulme (44).

Since the acid fraction is almost certainly involved in a series of metabolically coupled reactions (44), any differences or changes in acids which may be present, even in trace amounts, are likely to be of great importance to the metabolism of the organism as a whole.

It has been suggested by Buchloh (11) that the organic acids have an active role in the induction of bitter pit.

Investigations by Hilkenbaumer et al. (32) have shown that apple fruit

TABLE 1. --Observations Concerning Organic Acids of Apple Tissues and Apple Products.

Acid	Apple Fruit	Apple Juice	Apple Coating
Chlorogenic	36, 45, 70, 86	69	-
Citramalic	37, 38	10	-
Citric	46, 47, 49, 52	-	-
p-Coumarylquinic	87	-	-
Galacturonic	-	10, 71, 88	-
Glyceric	44	-	-
Glycolic	76	88	-
Glyoxylic	7, 39, 44, 49	-	-
Isocitric	8, 9, 49	-	-
alpha-Ketoglutaric	39, 44	-	-
Lactic	26	10, 33, 58, 59 71, 78, 79	-
Malic	46, 47, 49, 74	79	-
Oxalacetic	39, 44, 49	-	-
Oxalic	26, 39, 42	2	-
Pyruvic	39	-	-
Quinic	35, 39, 43, 46 47, 70, 71	10, 69, 78, 79	-
Shikimic	40, 42, 43, 46, 47	-	-
Succinic	5, 8, 9, 26, 41	69, 79	-
Tartaric	49, 60, 61, 77	-	-
Ursolic	-	-	31, 55, 73

tissues affected by bitter pit are characterized by an increase in soluble calcium and citric acid. These workers presumed that calcium was probably dissolved by citric acid and translocated to other regions of the parenchyma. Perhaps the calcium and citrate within the vacuole of the cell formed a complex similar to a chelate, and was thus more readily translocated in this manner.

In a study of the changes in organic acid composition of apple fruits which occurred during growth on the tree, Hulme and Wooltorton (46) found that at maturity quinic acid in both the peel and the pulp tissues had fallen to less than one-tenth of its concentration in the immature fruit. Malic acid increased in concentration in June and, at maturity, fell to slightly less than its original concentration at 25 days after petal fall. Citric acid concentration increased during June and July, but at maturity, had returned to its original concentration at 25 days after petal fall.

In a subsequent study (47) these workers found that during storage the content of malic acid decreased, with a concomitant increase in citramalic acid levels. The authors presume a possible pathway for citramalic acid synthesis via methylation of malic acid. It was also found that as quinic acid content of the peel decreased the shikimic acid concentration of the peel increased, thus implying a direct relationship between these compounds. Citric acid concentration increased in the pulp tissue during storage, but remained constant in the peel tissue during the same period.

Kenworthy and Harris (49) observed gross differences in organic acid

levels of apple fruits of two varieties from three locations. The most striking variation in this study was the absence of malic acid in the tissue of Red Delicious apples grown in Michigan. It was presumed that this difference was due to the advanced post-climacteric stage of respiration of the fruit.

Malic and citric acids are intrinsic parts of a cycle. However, malic acid is the main substrate in respiration during storage (47). Citric acid content would not increase, but rather tended to remain constant throughout the storage of the fruit.

Studies have been carried out on the effects of the various forms of nitrogen on the organic acid fraction of tomato plants (17, 20, 53). Similar studies on the organic acid composition of oat plants in response to varying levels of nitrate supplied have been conducted by Pepkowitz et al. (68). Clark (20), working with tomato plants, found that tomatoes grown in sand culture and supplied with the ammonium form of nitrogen, may contain less than one-tenth as much of each individual organic acid as compared with tomato plants supplied with the nitrate form of nitrogen. MacVicar and Burris (53) found that in the tomato plant there was rapid absorption, translocation and assimilation of the ammonium form of nitrogen, with considerable amide production and accumulation.

Carangal et al. (17), however, found that the organic acid content of tomato fruits was not influenced primarily by any single element, nor was it always a function of the form of nitrogen supplied. Rather, there appeared

to be inter-effects and similar effects of entirely different combinations of ions which are essential to plant growth.

It has been hitherto difficult to demonstrate the Krebs cycle in either cytoplasmic particles or tissue slices obtained from apple fruit. However, in a recent investigation Hatch et al. (29) obtained evidence for the operation of the classical Krebs cycle-cytochrome oxidase respiratory system in cut tissue and mitochondria from Granny Smith apples. The respiration of cut tissue increased when either citrate, alpha-ketoglutarate, succinate, malate, fumarate or pyruvate were added. Both the endogenous and acid-stimulated respiration were inhibited by malonate, cyanide and azide. The rapid oxidation of Krebs cycle acids by cytoplasmic particles from apple flesh was also demonstrated. These particles showed cytochrome oxidase activity and contained a succinoxidase system dependent on cytochrome c.

Numerous investigations have been conducted on the effects of nutrient environment on the incidence of storage disorders of apple fruits, yielding a plethora of data and results, which, after interpretation and correlation, finally succeed in confusing the reader as to cause and effect. A number of these experiments are, however, considered to be pertinent to the present investigation, and are presented.

The experiments of Kidd and West (50) indicated that apple fruits from trees treated with potassium-carrying fertilizers were more susceptible to low temperature breakdown than were fruits from the control trees.

Wilkinson (85) reported that those apples in which potassium content was high were more resistant to low temperature breakdown than were fruits in which the potassium content was low.

Collins (21) found that when varying rates of nitrogen, phosphorus, and potassium were applied to McIntosh apple trees the most significant results were obtained with nitrogen. As the nitrogen levels increased in the tree, the keeping quality of the fruit decreased. Fruit firmness was likewise decreased.

Abe and More (1), in an investigation concerning the effects of nitrogen nutrition on mineral composition of leaves and other factors, found an inverse relationship between leaf nitrogen level and apple fruit quality.

Conversely, Baxter (6) indicated that he found no difference in the storage life of apples from trees which had received no nitrogen and those from trees which had received 2 pounds of ammonium sulfate per tree.

The results of deHaas (28) are a bit more conditional, in that he found that nitrogen had a most pronounced effect on quality and storage life, but always in relation to fruit set. Following poor fruit set, a high nitrogen level produced large fruits of low quality and reduced storage quality, whereas, following a full fruit set, the same amount of nitrogen applied resulted in fruit of good quality and near-optimum storage quality. In the same study, deHaas found a consistent effect of calcium and potassium on fruit quality.

In long-term nutrition experiments, Tiller et al. (80) found that the

application of nitrogen to the soil reduced the storage quality and increased storage disorder incidence with all the varieties investigated. Some of the physiological disorders studied were internal breakdown, fungal rots and superficial scald. The addition of phosphorus, or a combination of phosphorus and potassium, with the nitrogen applications improved storage quality. It was also found that, in the case of Jonathan variety, a balanced application of nitrogen, phosphorus and potassium gave better storage results than nitrogen plus phosphorus only.

Bunemann (13, 14) found a positive correlation between the nitrogen content of the leaf and fruit size, but not fruit quality. High leaf phosphorus was positively correlated with good storage quality of the fruit. Variations in leaf levels of potassium and calcium had greater effects on storage life and quality than the other elements. When leaf content of potassium or calcium was high, storage quality was improved.

O'Grady (65) found that the quality of McIntosh apple fruits was excellent after storage at 35° F., regardless of the leaf levels of nitrogen and potassium. Poorest fruit color resulted when the nitrogen content of the leaf rose above 2.5 percent dry weight.

Ostrowski et al. (66) studied the effect of mineral fertilization on storage quality and found the lowest percentage of rotted apple fruits came from unfertilized trees, while the highest percentage came from trees which received applications of nitrogen and phosphorus. These workers also found

that the firmest fruits were harvested from the high-nitrogen plus high-phosphorus trees, and the softest fruits resulted from the treatment high in nitrogen, phosphorus, potassium and calcium. They found no effects of fertilizer applications on any physiological disorders.

Oberly (64) concluded that intensive use of potassium and nitrogen fertilizers on apple trees, coupled with inadequate applications of lime may have been responsible for the high incidence of bitter pit in apples.

Chittenden (19) found that apples from trees which had received twice the normal annual applications of 3 pounds of ammonium sulfate showed a marked increase in bitter pit incidence after storage, as compared with fruit from trees which had received only the normal quantity of nitrogen.

It was shown by Martin et al. (56) that the incidence of bitter pit in the apple variety Cleopatra was increased by applications of magnesium nitrate and decreased by applications of calcium nitrate. These investigators indicated that calcium was the critical element in bitter pit incidence, and that magnesium may also play an important part.

Bunemann (15) found that Northern Spy apple fruits affected with bitter pit had a nitrogen content which was twice as high as that of apples free from bitter pit.

Nyhlen and Rootsi (63) discussed the importance of the balance between nutrients in relation to bitter pit in apples. A lower incidence of bitter pit occurred in the apple fruit when the nitrogen and phosphorus levels in fruits

were in balance. Potassium, phosphorus and magnesium levels were lower in healthy than in diseased fruits. They also found that the calyx end of the diseased fruit had a higher pH and a lower content of total acid than did the healthy fruit.

The results of Van Schreven et al. (84) confirmed those of Nyhlen and Rootsi (63). Higher fruit content of magnesium and potassium tended to increase occurrence of bitter pit, while higher levels of calcium tended to reduce it.

The results of Oberly (64) and Chittenden (19) concur with those of Conway (22). Conway recommended nitrogen fertilizers be used in moderation, or be omitted entirely if the trees were healthy.

Tomana (81) studied the effects of variations in nutritional conditions on the occurrence of 'Jonathan spot'. He found that the largest number of spots occurred on the fruits harvested from trees which had been heavily fertilized with nitrogen, but which had not received any phosphorus applications. Such spotting did not occur on any fruits which were harvested late in October, even from trees which were heavily fertilized with nitrogen. Inasmuch as Jonathan spot incidence is generally increased by late harvest, these observations may have been on lenticel spot rather than on Jonathan spot.

Quidet et al. (72), working with the Starking Delicious variety, have shown that the early and late storage quality of apples was highest when

nitrogen, phosphorus and potassium were applied, with potassium at twice its normal concentration. These workers concluded that potassium is indispensable for optimum maturation and prolonged storage.

Bunemann et al. (12) found that apple flesh firmness was affected solely by nitrogen, and that internal breakdown during storage was intimately associated with a low level of potassium.

The data of Nyhlen (62) showed that incidence of brown heart of apples increased when the fruit was held at 18 to 20° C. after removal from a controlled atmosphere storage. Later pickings also exhibited a greater degree of brown heart incidence. When the soil in which the apple trees were grown showed a high $K_2O:P_2O_5$ ratio, the incidence of brown heart was also increased.

EXPERIMENTAL PROCEDURE

Plant Material

One-year-old apple trees (Malus sylvestris, Miller) of the Golden Delicious and Jonathan varieties (Malling IX rootstocks), were planted in quartz sand (4-10 mesh) on May 15, 1960. Treatments consisted of high and low levels of nitrogen, phosphorus, potassium, calcium and magnesium, and a median level as a control, applied as solutions (Appendix Tables 1 and 2). Each treatment consisted of three randomly selected trees of each variety. Treatments were applied commencing May 16, 1960 and on succeeding alternate days, terminating on October 12, 1960. The trees remained out-of-doors over winter. Treatments were resumed the following spring, and again in 1962. Dates of full bloom are indicated in Tables 3 and 4 of the Appendix.

Leaf Sampling Procedure

Leaf samples were taken during the month of July in each season. The samples were collected from the outer perimeter of the trees, taking only those leaves which were about midway between the base and the extreme tip of the current season's growth, and were free from damage by insects or disease. A sample of 25 to 30 leaves was removed from each tree and was analyzed according to methods described later. These data were then employed to ascertain nutritional status of the trees as altered by treatments.

Fruit Sampling Procedure

Fruit samples were collected on the dates shown in Tables 5 and 6 of the Appendix. All samples were taken at mid-day on all sampling dates. Following harvest, the fruit samples were placed immediately into polyethylene bags, cooled over dry ice, and stored at 5° C. for 24 hours.

Sample Preparation

Sample preparations were carried out at 5° C. The fruits in each sample were separated into peel, pulp, and core tissues, using a peeler-corer machine (Goodell Company, Antrim, New Hampshire). The core tissue was discarded. The peel and pulp tissues were weighed, placed in polyethylene bags, quick frozen over dry ice, and placed in a -20° C storage room until extraction.

Organic Acid Extraction

The method for organic acid extraction as described by Markakis et al. (54), with minor modifications, proved eminently applicable to organic acid extraction and analysis of apple fruits, and is herein briefly outlined.

A 50-gram sample of apple tissue was dropped into 75 ml. boiling water and blended in a Multi-mixer (Lourdes Instrument Company, Brooklyn, New York) at ca. 7500 rpm for 2 minutes. The slurry was boiled, cooled and filtered. The aqueous extract was concentrated in vacuo, the pectins were removed by coagulation in ethanol, and lead sub-acetate was added to precipitate the organic acids. The precipitate was washed with ethanol and

centrifuged four times. The acids were regenerated from the lead salt form by bubbling H_2S gas through the suspension. The lead sulfide pellet was washed and discarded. The acid extract was concentrated and passed through a 0.7 x 20.0 cm. column of Dowex x 8 (H^+ form, 50-100 mesh), to remove all cations, brought to 50 ml. volume with water and stored at 5° C.

Organic Acid Analysis

Aliquots of the extract were added to the resin column and were removed by employing a concentration gradient elution system which was modified from that described by Markakis et al. (54). Two acid reservoirs were used, and were connected to the mixing vessel by means of a 3-way stopcock. Air pressure was applied at the top of the system sufficient to maintain a flow rate of ca. 60 ml. per hour.

The initial eluting solvent consisted of 50 ml. 1.5 N acetic acid, followed by 50 ml. 3.0 N acetic acid, 75 ml. 4.5 N acetic acid, 50 ml. 6.0 N acetic acid, 100 ml. 6.0 N formic acid, and finally 75 ml. 10.0 N formic acid was added. Eighty fractions of 5.0 ml. volume were collected per sample aliquot, using an automatic fraction collector (Gilson Medical Electronics, Madison, Wisconsin).

The fractions were evaporated to near dryness in vacuo at 40° C. using a Rotary Evapo-mix (Buchler Instruments, New York, New York), and transferred to a 40° C. vacuum oven to complete drying. After drying, the fractions were dissolved in ca. 1.5 ml. hot water and allowed to equilibrate for 15

minutes. An automatic-reloading tubercular syringe was employed to dispense 1.0 ml. dilute (0.001 N) NaOH solution containing 0.001% phenolphthalein into each tube, to indicate the titration "base line". Those fractions which indicated the presence of acid (lack of color reaction) were titrated to the phenolphthalein end-point using 0.010 N NaOH. After titration, the fractions were dried in vacuo at 40° C. for subsequent qualitative determination by paper chromatography.

The residues were dissolved in 0.5 ml. 50% ethanol, and 0.1 ml. of an aqueous slurry of Dowex 50 x 8 resin (H^+ form, 50-100 mesh) was added to each fraction to remove excess sodium ions. The dissolved fractions were spotted on Whatman No. 1 filter paper sheets 46 x 57 cm., 2.5 cm. apart and 7.5 cm. away from the long edge of the sheet. The papers were placed in a chromatography cabinet and the atmosphere was brought to equilibrium using the lower phase of a solvent system described later. After 4 hours equilibration, the papers were irrigated descendingly for a period of ca. 12 hours at ca. 20° C., using the upper phase of a mixture of 1-butanol, formic acid and water, combined in a ration of 4:1:5 (v/v), respectively. The papers were dried in an air stream for ca. 6 hours and sprayed with color-specific reagents (67).

The resin column was standardized for 15 authentic organic acids by passing the acids through the column in four groups of five acids each, and recording their effluent volumes, as well as their order of appearance in the

acid profile, as shown in Figure 1. Recovery of these acids was, in all cases, greater than 90%. The authentic organic acids were also chromatographed on paper, following elution from the column, to further confirm identity. These chromatograms, plus applications of solutions of known acids to the papers containing unknown acids, were used as references for identification of the unknowns.

Quinic and shikimic acids were further characterized on the chromatograms by applying a specific color reagent (18, 57). Phosphoric acid was characterized by elution from the chromatogram and subsequent treatment with Fisk-Subbarow reagent (83). Values of R_f both theoretical and observed, are shown in Table 7 of the Appendix.

Spectrographic Analysis

Analysis for mineral content (P, Ca, Mg, Mn, Fe, B, Zn, Cu, Mo, Al) of leaves and fruit was carried out in the Plant Analysis Laboratory using a 1.5 meter "Quantograph" (Applied Research Laboratories, Inc., Glendale, California) (48). Nitrogen was determined using the Kjeldahl-Gunning method. Potassium was determined by flame photometry, using a Beckman Model B Spectrophotometer (Beckman Instruments, Inc., South Pasadena, California).

Total Titratable Acidity

A 50-gram sample of apple tissue was blended with 100 ml. of distilled water in a Multi-Mixer (Lourdes Instrument Company, Brooklyn, New York) for 2 minutes at 7500 rpm. The slurry was filtered through eight

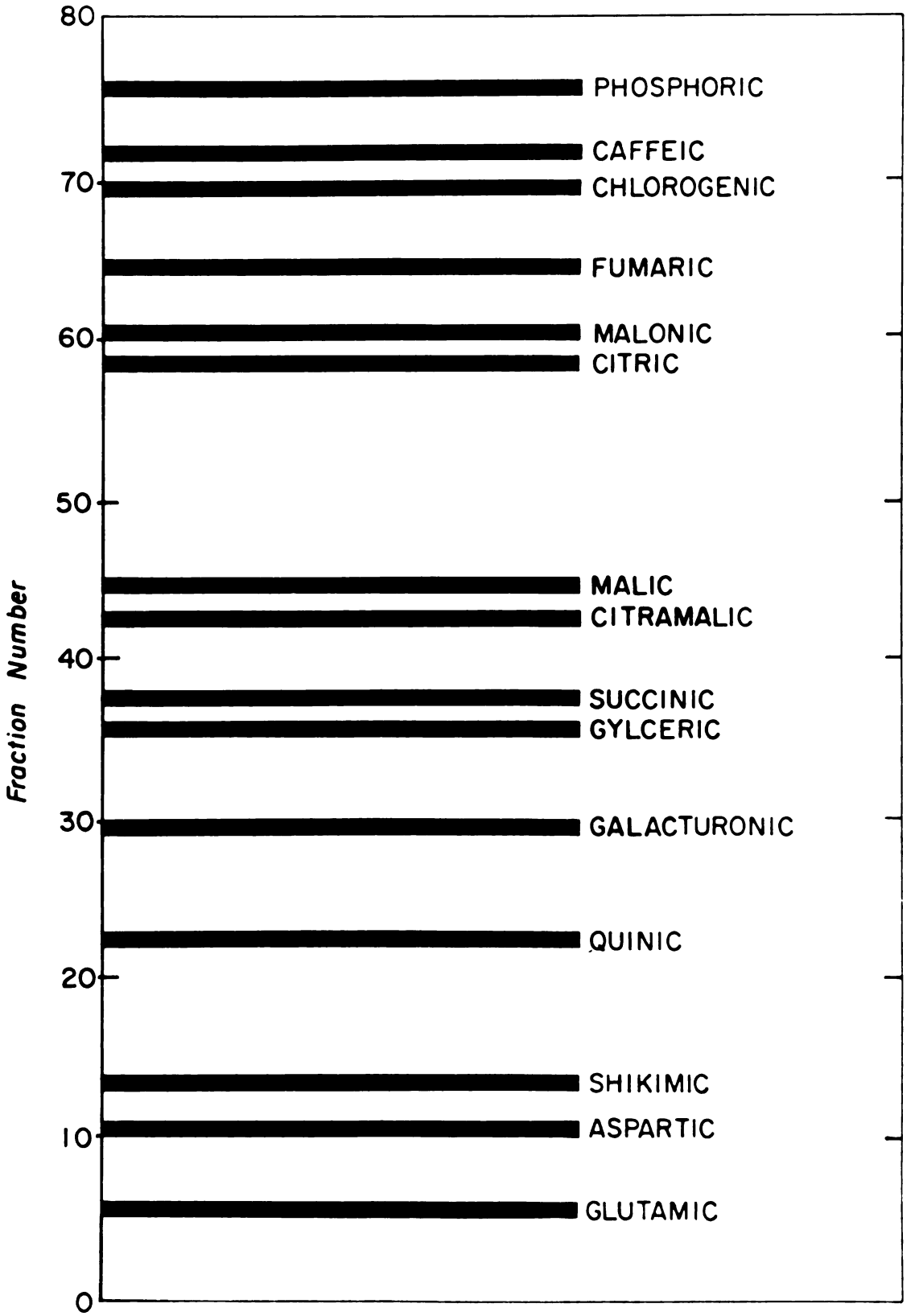

Figure 1. Sequence of Elution of Organic Acids from Dowex 1 Resin Column.

layers of cheese cloth, and duplicate 25 ml. aliquots of the filtrate were titrated with 0.010 N NaOH to pH 8.1, using a pH meter Model Zeromatic (Beckman Instruments, Inc., South Pasadena, California).

Total Extractable Acidity

The concentrated organic acid extract, the preparation of which is described in a previous section, was removed from cold storage, and three 1.0 ml. aliquots were removed and titrated to the phenolphthalein end-point with 0.010 N NaOH, to determine total extractable acidity per given quantity of tissue. From these values the total quantities of each individual acid per given quantity of tissue was computed.

RESULTS

Statistical Analysis

Analyses of variance were made on mineral composition, organic acid content and total acidity. The Duncan Multiple Range Test (23) was used to determine statistical significance.

In all tables, the mean values in a group which are followed by a different subscript are significantly different at odds of 19:1.

Organic Acid Composition

All mean values for acid composition in Tables 2 through 6 are expressed as mg. per 100 g. fresh weight.

In Tables 2 through 6, all mean values expressed as 0.0 are indicative that the organic acid was not detected.

All mean values for acid content in Tables 7 and 8 are expressed as milli-equivalents per 100 g. fresh weight.

Mineral Composition

All mean values for mineral content in Tables 9 through 13 are expressed as percent dry weight.

All mean values for mineral content in Tables 14 through 20 are expressed as parts per million dry weight.

Organic Acid Composition

Shikimic Acid (Table 2)

Average shikimic acid contents were 21.7 and 6.3 mg. per 100 g. fresh weight in the peel tissue of Golden Delicious and Jonathan with the control treatment. No shikimic acid was detected in the pulp tissue of either variety with the control treatment.

High and low nitrogen treatments had no effect on the shikimic acid content of pulp in either variety. Low nitrogen decreased shikimic acid in peel of Golden Delicious but increased that acid in the peel tissue of Jonathan. High nitrogen decreased shikimic acid in peel of Golden Delicious and increased shikimic acid in peel of Jonathan.

There were no significant differences noted in the content of shikimic acid in either peel or pulp tissue in either variety in response to either high or low phosphorus treatments.

Significant variations in shikimic acid levels in response to varying levels of potassium were noted only in the peel tissue of Golden Delicious. As the supply of potassium to the trees was increased, the level of shikimic acid in the peel tissue of Golden Delicious was decreased. No other significant differences were noted in either high or low potassium treatments.

High and low treatments of calcium had no significant effect on shikimic acid levels in pulp tissue of either variety. However, the effect of calcium on peel tissue content of shikimic acid was similar to that of potassium.

TABLE 2. --Shikimic Acid Content of Apple Fruit Tissue as Influenced by Nutrient Environment - 1961.

Treatment	Golden Delicious		Jonathan	
	Pulp	Peel	Pulp	Peel
- N	0.0	12.7 a, b	0.0 a	48.3 b
Ck	0.0	21.7 b	0.0 a	6.3 a
+ N	0.0	0.0 a	0.0 a	10.0 a
- P	0.0	6.0 a	0.0 a	5.3 a
Ck	0.0	21.7 a	0.0 a	6.3 a
+ P	0.0	14.7 a	10.0 b	5.7 a
- K	0.0	134.7 c	0.0 a	5.0 a
Ck	0.0	21.7 b	0.0 a	6.3 a
+ K	0.0	2.7 a	0.0 a	9.3 a
- Ca	0.0	50.0 c	0.0 a	8.3 b
Ck	0.0	21.7 b	0.0 a	6.3 a, b
+ Ca	0.0	0.0 a	0.0 a	0.0 a
- Mg	0.0	57.0 c	1.7 a	16.0 b
Ck	0.0	21.7 b	0.0 a	6.3 a
+ Mg	0.0	0.0 a	2.7 a	0.0 a

As the calcium supply was increased, shikimic acid content was decreased in the peel tissue of both varieties.

High and low magnesium treatments had effects which were similar to potassium and calcium. As the supply of magnesium to the trees was increased, the level of shikimic acid in the peel tissue was reduced. No significant differences in shikimic acid content of pulp tissue were noted in either variety in response to high and low magnesium treatments. However, shikimic acid was detected in the pulp of Jonathan in response to high and low magnesium.

Quinic Acid (Table 3)

Contents of quinic acid of pulp tissue in response to the control treatment were 11.7 and 10.7 mg. per 100 g. fresh weight in Golden Delicious and Jonathan. Peel tissue contents were 23.0 and 9.7 mg. per 100 g. fresh weight in Golden Delicious and Jonathan with the control treatment.

Low nitrogen treatment effected a significant increase in quinic acid content only in the peel tissue of Jonathan. Significant increases were noted in the peel tissue of both varieties in response to high nitrogen treatment.

Significant decreases in quinic acid content were noted in peel tissue of Golden Delicious in response to both high and low level treatments with phosphorus. The level of quinic acid was increased by high phosphorus treatment in peel tissue of Jonathan.

Low potassium treatments effected a significant increase in quinic acid content in pulp tissue of Golden Delicious and in peel tissue of Jonathan.

TABLE 3. --Quinic Acid Content of Apple Fruit Tissue as Influenced by Nutrient Environment - 1961.

Treatment	Golden Delicious		Jonathan	
	Pulp	Peel	Pulp	Peel
- N	5.7 a	18.0 a	34.3 a	43.7 b
Ck	11.7 a	23.0 a	10.7 a	9.7 a
+ N	7.3 a	46.0 b	25.3 a	161.7 c
- P	2.0 a	5.7 a	17.3 a	9.7 a
Ck	11.7 a, b	23.0 b	10.7 a	9.7 a
+ P	21.3 b	11.0 a	20.0 a	20.7 b
- K	37.3 b	15.3 a	80.3 b	173.3 c
Ck	11.7 a	23.0 a	10.7 a	9.7 a
+ K	9.3 a	18.7 a	14.3 a	18.7 b
- Ca	10.7 a	12.0 a	11.3 a	100.3 b
Ck	11.7 a	23.0 b	10.7 a	9.7 a
+ Ca	8.0 a	6.0 a	22.3 a	7.3 a
- Mg	12.7 a	7.0 a	6.0 a	55.7 b
Ck	11.7 a	23.0 b	10.7 a	9.7 a
+ Mg	119.0 b	21.7 b	14.7 a	15.3 a

An increase in quinic acid was also noted in peel tissue of Jonathan in response to high potassium supply.

Quinic acid was reduced significantly in peel tissue of Golden Delicious in response to both high and low levels of calcium. Low calcium effected a significant increase in quinic acid content of peel tissue of the Jonathan variety.

Low magnesium caused an increase in quinic acid content of peel tissue of Jonathan and a significant decrease in quinic acid content in peel tissue of Golden Delicious. A marked increase in quinic acid in pulp tissue of Golden Delicious was noted in response to high magnesium treatment.

Malic Acid (Table 4)

Average malic acid contents were 536 and 743 mg. per 100 g. fresh weight in the pulp of Golden Delicious and Jonathan. The peel of Golden Delicious and Jonathan contained 598 and 649 mg. per 100 g. fresh weight.

High nitrogen resulted in a reduction in malic acid content of pulp from both varieties and an increase in the peel of Jonathan. Low nitrogen resulted in an increase in malic acid in both pulp and peel of Jonathan, but no effect on either tissue of Golden Delicious.

Low phosphorus increased the malic acid in both the pulp and peel of Jonathan, but had no effect on either tissue of Golden Delicious. High phosphorus appeared to result in a decrease in malic acid in all instances except the peel of Jonathan, where an increase was observed.

Low potassium reduced malic acid in all tissues except the peel of

TABLE 4.--Malic Acid Content of Apple Fruit Tissue as Influenced by Nutrient Environment - 1961.

Treatment	Golden Delicious		Jonathan	
	Pulp	Peel	Pulp	Peel
- N	547 b	607 a	950 c	3030 c
Ck	536 b	598 a	743 b	649 a
+ N	318 a	600 a	589 a	1422 b
- P	588 c	688 b	847 c	1291 c
Ck	536 b	598 b	743 b	649 a
+ P	278 a	386 a	656 a	919 b
- K	285 a	412 a	476 a	960 b
Ck	536 b	598 b	743 b	649 a
+ K	584 c	1068 c	874 c	1132 c
- Ca	512 a	492 a	616 a	928 b
Ck	536 a	598 a, b	743 b	649 a
+ Ca	547 a	664 b	595 a	852 b
- Mg	588 c	534 a	753 b	1078 b
Ck	536 b	598 a	743 b	649 a
+ Mg	220 a	504 a	351 a	1054 b

Jonathan where an increase occurred. High potassium resulted in an increase in malic acid in all tissues.

High and low calcium appeared to reduce malic acid in the pulp of Golden Delicious, and increase it in the pulp of Jonathan. Low calcium resulted in a lower malic acid content in the peel of Golden Delicious.

Citric Acid (Table 5)

Citric acid was found to be present in the pulp tissue of both varieties in response to the control treatment. Average citric acid contents of pulp tissue with the control treatment were 4.0 and 6.7 mg. per 100 g. fresh weight in Golden Delicious and Jonathan. No citric acid was detected in the peel tissue of either variety in response to the control treatment.

No significant differences in citric acid content of apple pulp tissue in relation to low nitrogen level were noted in either variety in this study. There was, however, a noteworthy increase in peel content of citric acid as a result of high nitrogen treatments in both varieties.

When phosphorus treatment levels were reduced, citric acid content increased in the peel of the fruit of both varieties, while levels of citric acid in the pulp of Jonathan fruits was increased by high phosphorus treatments.

High level potassium treatments increased citric acid in the pulp of Golden Delicious, but not in Jonathan pulp tissue. The greatest significant difference lay in the peel tissues, where high and low potassium treatments

TABLE 5. --Citric Acid Content of Apple Fruit Tissue as Influenced by Nutrient Environment - 1961.

Treatment	Golden Delicious		Jonathan	
	Pulp	Peel	Pulp	Peel
- N	3.7 a	3.0 a	5.0 a	0.0 a
Ck	4.0 a	0.0 a	6.7 a	0.0 a
+ N	4.7 a	10.0 b	1.7 a	12.3 b
- P	0.0 a	11.0 b	3.0 a	5.0 b
Ck	4.0 a	0.0 a	6.7 a	0.0 a
+ P	2.7 a	0.0 a	17.3 b	0.0 a
- K	4.7 a	15.0 c	7.3 a	24.7 c
Ck	4.0 a	0.0 a	6.7 a	0.0 a
+ K	10.0 b	5.0 b	8.0 a	11.0 b
- Ca	0.0 a	5.7 b	6.3 a	0.0 a
Ck	4.0 b	0.0 a	6.7 a	0.0 a
+ Ca	5.0 b	0.0 a	6.3 a	0.0 a
- Mg	0.0 a	0.0 a	0.0 a	0.0 a
Ck	4.0 b	0.0 a	6.7 b	0.0 a
+ Mg	0.0 a	0.0 a	0.0 a	0.0 a

increased the citric acid content over that of the control treatment.

Differences in citric acid levels in response to calcium treatments were observed only in the Golden Delicious variety. Pulp content was increased by high calcium treatments, and decreased by low calcium applications, while peel tissue content was increased by low level calcium applications.

No citric acid was detected in the tissues of either variety in response to either high or low magnesium applications.

Phosphoric Acid (Table 6)

While phosphoric acid is not classified as an organic acid, it is nonetheless a compound which is relatively ubiquitous in plant extracts, and should be included in consideration of analytical data.

Phosphoric acid was detected in the extract of pulp tissue of both varieties but not in the extract of peel tissue in response to the control treatment. Average phosphoric acid content of the extract of pulp tissue of Golden Delicious was 10.7 mg. per 100 g. fresh weight, and 11.7 mg. per 100 g. fresh weight in the extract of pulp tissue of Jonathan in response to control treatment.

High nitrogen effected a considerable increase in phosphate levels within the peel tissues, while levels in the pulp tissues were not of this magnitude. However, pulp tissue contents of phosphate were increased to a large

TABLE 6. --Phosphoric Acid Content of Apple Fruit Tissue as Influenced by Nutrient Environment - 1961.

Treatment	Golden Delicious		Jonathan	
	Pulp	Peel	Pulp	Peel
- N	8.3 a	6.0 b	10.0 a	0.0 a
Ck	10.7 b	0.0 a	11.7 a	0.0 a
+ N	15.0 c	34.3 c	7.7 a	83.0 b
- P	2.0 a	0.0 a	0.0 a	0.0 a
Ck	10.7 b	0.0 a	11.7 b	0.0 a
+ P	28.0 c	5.7 b	32.3 c	19.0 b
- K	8.3 a	37.3 b	1.7 a	1.7 a
Ck	10.7 b	0.0 a	11.7 b	0.0 a
+ K	14.7 c	1.3 a	14.3 b	0.0 a
- Ca	0.0 a	0.0 a	1.7 a	34.7 b
Ck	10.7 c	0.0 a	11.7 c	0.0 a
+ Ca	3.7 b	0.0 a	5.7 b	0.0 a
- Mg	2.3 b	3.7 b	1.7 a	3.0 b
Ck	10.7 c	0.0 a	11.7 b	0.0 a
+ Mg	0.0 a	0.0 a	0.0 a	0.0 a

degree by high phosphorus treatments, and were reduced by low phosphorus treatments. High potassium treatments increased pulp contents of phosphate, while low potassium supply appeared to effect an increase in phosphate in the peel tissue of the Golden Delicious variety.

Low calcium treatments were effective in altering the level of phosphate in all tissues except the peel tissue of Golden Delicious, with a large increase in the peel tissue of the Jonathan variety.

High and low magnesium treatments effected a significant reduction in the phosphate content of pulp tissue of both varieties. Low magnesium supply appeared to effect an increase in phosphate in the peel tissue of both varieties.

Total Titratable Acidity (TTA) (Table 7)

Mean values for total titratable acidity (TTA) in the control treatments were 3.44 and 5.82 milli-equivalents per 100 g. fresh weight of pulp tissue and 3.82 and 7.23 milli-equivalents per 100 g. fresh weight of peel tissue in Golden Delicious and Jonathan varieties, respectively.

Low and high nitrogen treatments were effective in increasing TTA in the peel tissue of both varieties. High nitrogen increased TTA in peel tissue, as did high potassium in both varieties. Total titratable acidity was increased in Jonathan pulp tissue by high calcium and high magnesium. High potassium decreased TTA in pulp tissue of Jonathan variety, but increased TTA in the

TABLE 7. --Total Titratable Acidity of Apple Fruit Tissue as Influenced by Nutrient Environment - 1961.

Treatment	Golden Delicious		Jonathan	
	Pulp	Peel	Pulp	Peel
- N	4.42 a, b	5.01 c	5.73 a	12.76 b
Ck	3.44 a	3.82 a	5.82 a	7.23 a
+ N	4.52 b	4.24 b	7.45 b	8.34 a
- P	3.87 a	3.48 a	4.73 a	3.70 a
Ck	3.44 a	3.82 b	5.82 b	7.23 b
+ P	3.29 a	3.49 a	5.52 b	8.31 b
- K	2.05 a	2.99 a	3.76 a	3.48 a
Ck	3.44 b	3.82 b	5.82 c	7.23 b
+ K	4.79 c	8.60 c	4.55 b	10.17 c
- Ca	3.80 a	2.82 a	5.66 a	8.04 b
Ck	3.44 a	3.82 b	5.82 a	7.23 b
+ Ca	3.57 a	6.68 c	6.59 b	3.91 a
- Mg	3.64 a	2.98 a	4.76 a	7.02 a
Ck	3.44 a	3.82 b	5.82 b	7.23 a
+ Mg	3.79 a	5.25 c	7.64 c	11.89 b

pulp of Golden Delicious. High magnesium increased the TTA of peel tissue of both varieties.

Total Extractable Acidity (TEA) (Table 8)

Low nitrogen treatment resulted in an increase in total extractable acidity (TEA) of both peel and pulp of Jonathan, but no significant differences in TEA of either tissue of Golden Delicious. High nitrogen increased TEA in peel tissue of both varieties, but decreased TEA in pulp tissue of both varieties.

Low phosphorus treatment had a significant influence only upon the peel tissue of Jonathan, resulting in an increase. High phosphorus increased TEA in the peel of Jonathan, but significantly reduced TEA in both tissues of Golden Delicious.

There appeared to be an increase in TEA as potassium supply was increased. However, low potassium increased TEA in peel tissue of Jonathan.

Low and high calcium reduced TEA in pulp tissue of both varieties. Peel tissue TEA of Jonathan was increased by high and low calcium, while high calcium increased TEA of peel of Golden Delicious.

Low magnesium increased TEA only in peel of Jonathan. High magnesium decreased TEA in pulp tissue of both varieties and in peel tissue of Golden Delicious, but increased TEA in peel tissue of Jonathan.

TABLE 8. --Total Extractable Acidity of Apple Fruit Tissue as Influenced by Nutrient Environment - 1961.

Treatment	Golden Delicious		Jonathan	
	Pulp	Peel	Pulp	Peel
- N	8.50 b	9.43 a	14.74 c	45.92 c
Ck	8.45 b	9.46 a	11.60 b	9.76 a
+ N	5.31 a	10.36 a	9.19 a	24.26 b
- P	8.89 b	10.51 b	12.78 b	19.47 c
Ck	8.45 b	9.46 b	11.60 a, b	9.76 a
+ P	5.16 a	6.00 a	11.30 a	14.36 b
- K	4.71 a	8.41 a	7.63 a	16.71 b
Ck	8.45 b	9.46 a	11.60 b	9.76 a
+ K	9.49 c	16.17 b	13.68 c	17.25 b
- Ca	8.42 a	7.78 a	9.40 a	14.50 b
Ck	8.45 a	9.46 b	11.60 b	9.76 a
+ Ca	7.70 a	10.03 b	9.37 a	12.76 b
- Mg	8.89 b	8.41 a, b	11.84 b	16.57 b
Ck	8.45 b	9.46 b	11.60 b	9.76 a
+ Mg	3.97 a	7.63 a	5.32 a	15.82 b

Mineral Composition

Nitrogen (Table 9)

Leaf content of nitrogen was altered only by the high and low nitrogen treatments with the exception of high potassium, where it was increased. The desired effect was achieved even in the first year of growth. Nitrogen content of the leaves of Jonathan trees was increased during the second year, but the low level treatment was still effectively reduced. The same was true for the leaves of the Golden Delicious variety. However, nitrogen values in the low nitrogen treatments were reduced.

Phosphorus (Table 10)

Variations in leaf content of phosphorus were not pronounced. From 1960 to 1961 there was a general increase in the levels of phosphorus. This could be due to phosphorus accumulations on the surface of the quartz sand medium, with a residue remaining from year to year. Phosphorus content varied considerably under high and low nitrogen and potassium treatments, as well as in those treatments consisting of high and low phosphorus, suggesting strong inter-relationships between those elements.

Potassium (Table 11)

Significant differences were noted in potassium content of apple leaves in response to all the treatments applied. Nitrogen treatments had a strong effect on potassium content during the first season (1960), but these differ-

TABLE 9. --Nitrogen Content of Apple Leaf Tissue as Influenced by Nutrient Environment.

Treatment	Golden Delicious		Jonathan	
	1960	1961	1960	1961
- N	1.91 a	1.83 a	1.87 a	1.97 a
Ck	2.29 b	2.39 b	2.20 b	2.33 b
+ N	3.07 c	3.14 c	2.85 c	2.91 c
- P	2.25 a	2.31 a	2.14 a	2.19 a
Ck	2.29 a	2.39 a	2.20 a	2.33 a
+ P	2.32 a	2.37 a	2.03 a	2.05 a
- K	2.17 a	2.27 a	2.06 a	1.98 a
Ck	2.29 a	2.39 a	2.20 a	2.33 a
+ K	2.79 b	2.40 a	2.23 a	2.23 a
- Ca	2.45 a	2.51 a	2.19 a	2.25 a
Ck	2.29 a	2.39 a	2.20 a	2.33 a
+ Ca	2.37 a	2.39 a	2.03 a	2.25 a
- Mg	2.34 a	2.41 a	2.25 a	2.32 a
Ck	2.29 a	2.39 a	2.20 a	2.33 a
+ Mg	2.30 a	2.38 a	2.23 a	2.37 a

TABLE 10. --Phosphorus Content of Apple Leaf Tissue as Influenced by Nutrient Environment.

Treatment	Golden Delicious		Jonathan	
	1960	1961	1960	1961
- N	0.307 a, b	0.310 a, b	0.359 c	0.312 a
Ck	0.171 a	0.246 a	0.170 a	0.237 a
+ N	0.326 b	0.334 b	0.277 b	0.331 a
- P	0.132 a	0.148 a	0.123 a	0.156 a
Ck	0.171 a	0.246 b	0.170 a	0.237 a
+ P	1.560 b	1.020 c	1.720 b	0.948 b
- K	0.514 b	0.601 b	0.413 b	0.535 b
Ck	0.171 a	0.246 a	0.170 a	0.237 a
+ K	0.210 a	0.264 a	0.183 a	0.270 a
- Ca	0.297 b	0.358 b	0.208 a	0.312 a
Ck	0.171 a, b	0.246 a	0.170 a	0.237 a
+ Ca	0.126 a	0.204 a	0.152 a	0.231 a
- Mg	0.223 a	0.325 b	0.193 a	0.293 a
Ck	0.171 a	0.246 a	0.170 a	0.237 a
+ Mg	0.182 a	0.241 a	0.187 a	0.275 a

TABLE 11. --Potassium Content of Apple Leaf Tissue as Influenced by Nutrient Environment.

Treatment	Golden Delicious		Jonathan	
	1960	1961	1960	1961
- N	1.78 a	1.76 a	1.61 a	1.62 a
Ck	2.50 b	1.59 a	2.26 c	1.65 a
+ N	1.68 a	1.63 a	1.90 b	1.86 a
- P	2.22 b	1.82 b	2.02 b	2.06 c
Ck	2.50 c	1.59 b	2.26 b	1.65 b
+ P	1.67 a	0.81 a	1.35 a	1.13 a
- K	0.41 a	1.04 a	0.41 a	0.31 a
Ck	2.50 b	1.59 b	2.26 b	1.65 b
+ K	4.25 c	3.83 c	3.73 c	3.34 c
- Ca	1.92 a	1.86 a	1.76 a	1.59 a
Ck	2.50 b	1.59 a	2.26 b	1.65 a
+ Ca	1.83 a	1.65 a	2.00 a, b	2.58 b
- Mg	2.55 b	2.24 c	1.95 b	2.01 b
Ck	2.50 b	1.59 b	2.26 c	1.65 a
+ Mg	1.66 a	1.27 a	1.57 a	1.67 a

ences were reduced to insignificance during 1961. Phosphorus treatment levels influenced potassium content of the leaves to a considerable extent, however such an influence of potassium on the phosphorus content was not observed. Effects of the levels of the calcium and magnesium treatments are also of significant magnitude.

Calcium (Table 12)

Calcium levels within the leaves of apple trees were not readily varied by treatment with various levels of any element other than potassium and calcium. Calcium contents were high in the high level calcium treatment, and low in the low level treatment, as compared with the control treatment. A low level of potassium increased calcium, while high potassium appeared to decrease calcium.

Magnesium (Table 13)

Magnesium levels within the leaf, like those of calcium, were influenced by potassium in both apple varieties, with low potassium increasing, and high potassium decreasing, magnesium. Magnesium content showed considerable variation in response to high and low calcium treatments. However, these variations were inconsistent.

Manganese (Table 14)

The manganese content of leaves of both apple varieties were interesting in that manganese levels were decreased in all the treatments from

TABLE 12. --Calcium Content of Apple Leaf Tissue as Influenced by Nutrient Environment.

Treatment	Golden Delicious		Jonathan	
	1960	1961	1960	1961
- N	1.03 a	1.09 a	1.50 b	1.04 a
Ck	0.97 a	1.43 a	1.06 a	1.49 b
+ N	1.34 a	1.18 a	1.31 a, b	1.28 a, b
- P	0.94 a	0.96 a	1.15 a	1.14 a
Ck	0.97 a	1.43 a	1.06 a	1.49 a
+ P	1.34 a	1.18 a	2.26 a	1.49 a
- K	1.73 b	2.19 c	2.13 b	2.12 c
Ck	0.97 a	1.43 b	1.06 a	1.49 b
+ K	0.64 a	0.61 a	0.94 a	0.59 a
- Ca	0.86 a	0.61 a	1.09 a	0.66 a
Ck	0.97 a	1.43 b	1.06 a	1.49 b
+ Ca	2.11 b	2.18 c	1.85 b	1.45 b
- Mg	1.08 a	1.93 b	1.44 b	1.59 b
Ck	0.97 a	1.43 a	1.06 a	1.49 b
+ Mg	1.16 a	1.11 a	1.35 a, b	1.04 a

TABLE 13. --Magnesium Content of Apple Leaf Tissue as Influenced by Nutrient Environment.

Treatment	Golden Delicious		Jonathan	
	1960	1961	1960	1961
- N	0.26 a	0.21 a	0.29 a	0.27 a
Ck	0.38 a	0.31 a	0.40 a	0.37 a
+ N	0.49 b	0.33 a	0.45 a	0.35 a
- P	0.35 a	0.36 a	0.35 a	0.35 a
Ck	0.38 a	0.31 a	0.40 a	0.37 a
+ P	0.44 a	0.41 a	0.66 b	0.48 a
- K	0.80 c	0.86 c	0.72 b	0.80 c
Ck	0.38 b	0.31 b	0.40 a	0.37 b
+ K	0.15 a	0.13 a	0.35 a	0.18 a
- Ca	0.48 a	0.46 b	0.73 b	0.51 a
Ck	0.38 a	0.31 a, b	0.40 a	0.37 a
+ Ca	0.33 a	0.23 a	0.30 a	0.36 a
- Mg	0.15 a	0.11 a	0.26 a	0.15 a
Ck	0.38 b	0.31 b	0.40 a	0.37 b
+ Mg	0.85 c	0.80 c	0.91 b	0.66 c

TABLE 14. --Manganese Content of Apple Leaf Tissue as Influenced by Nutrient Environment.

Treatment	Golden Delicious		Jonathan	
	1960	1961	1960	1961
- N	213 b	152 a, b	249 b	160 b
Ck	96 a	88 a	93 a	87 a
+ N	181 b	217 b	256 b	285 b
- P	141 a, b	107 a	131 a	82 a
Ck	96 a	88 a	93 a	87 a
+ P	202 b	194 b	245 b	191 b
- K	317 b	267 b	313 b	226 b
Ck	96 a	88 a	93 a	87 a
+ K	98 a	67 a	87 a	77 a
- Ca	346 b	188 b	294 b	216 b
Ck	96 a	88 a	93 a	87 a
+ Ca	152 a	82 a	128 a	54 a
- Mg	210 b	135 a	179 b	112 a
Ck	96 a	88 a	93 a	87 a
+ Mg	118 a	111 a	132 a, b	119 a

1960 to 1961, except in the instance of the high level treatment with nitrogen in the Jonathan variety. In this case, manganese content was increased slightly.

Iron (Table 15)

Contents of iron in apple leaves were significantly different only in relation to three other elements. A low level calcium treatment and the high level nitrogen treatment increased iron levels. Iron content was also increased under treatment with a low level of potassium, except in Jonathan variety in 1960.

Copper (Table 16)

Leaf contents of copper were influenced significantly by all treatments except calcium and magnesium treatments in the Jonathan variety in the second year. No significant differences were noted during 1960. Copper sprays were not applied to the experimental trees within these treatments.

Boron (Table 17)

Boron levels in apple leaves were increased by low levels of nitrogen, phosphorus, potassium, calcium and magnesium, and by the high level treatments of phosphorus in the Jonathan variety. Low level treatments of all the elements effected an increase in 1961 in boron content in the Jonathan variety, and in all but the high magnesium treatment in the Golden Delicious.

TABLE 15. --Iron Content of Apple Leaf Tissue as Influenced by Nutrient Environment.

Treatment	Golden Delicious		Jonathan	
	1960	1961	1960	1961
- N	409 a, b	492 b	489 a, b	435 a
Ck	252 a	262 a	360 a	415 a
+ N	460 b	512 b	611 b	635 b
- P	337 a	325 a	452 a	432 a
Ck	252 a	262 a	360 a	415 a
+ P	326 a	316 a	419 a	495 a
- K	389 a	410 b	356 a	389 a
Ck	252 a	262 a, b	360 a	415 a
+ K	282 a	229 a	414 a	504 a
- Ca	506 b	379 a	544 b	549 b
Ck	252 a	262 a	360 a	415 a, b
+ Ca	253 a	260 a	407 a, b	355 a
- Mg	404 a	298 a	445 a	467 a
Ck	252 a	262 a	360 a	415 a
+ Mg	241 a	293 a	358 a	398 a

TABLE 16. --Copper Content of Apple Leaf Tissue as Influenced by Nutrient Environment.

Treatment	Golden Delicious		Jonathan	
	1960	1961	1960	1961
- N	12.3 a	54.6 c	12.4 a	58.1 b
Ck	13.0 a	42.7 b	18.0 a	21.4 a
+ N	14.0 a	20.0 a	16.9 a	22.6 a
- P	11.5 a	56.9 c	12.6 a	55.8 b
Ck	13.0 a	42.7 b	18.0 a	21.4 a
+ P	10.5 a	21.2 a	10.5 a	29.0 a
- K	10.5 a	17.6 a	9.2 a	34.3 b
Ck	13.0 a	42.7 b	18.0 a	21.4 a
+ K	11.9 a	17.0 a	12.9 a	49.4 c
- Ca	11.2 a	19.5 a	13.9 a	22.3 a
Ck	13.0 a	42.7 b	18.0 a	21.4 a
+ Ca	10.5 a	16.1 a	14.0 a	17.6 a
- Mg	12.9 a	18.8 a	12.9 a	22.9 a
Ck	13.0 a	42.7 b	18.0 a	21.4 a
+ Mg	10.8 a	19.1 a	12.5 a	20.9 a

TABLE 17. --Boron Content of Apple Leaf Tissue as Influenced by Nutrient Environment.

Treatment	Golden Delicious		Jonathan	
	1960	1961	1960	1961
- N	35.2 a	54.6 b	35.5 a	58.1 b
Ck	32.5 a	42.7 a	31.8 a	41.1 a
+ N	33.9 a	47.1 a	38.6 a	50.4 b
- P	33.7 a	56.9 b	34.1 a	55.8 b
Ck	32.5 a	42.7 a	31.8 a	41.1 a
+ P	46.0 b	42.2 a	43.4 b	55.9 b
- K	30.6 a	60.8 b	34.5 a	65.5 b
Ck	32.5 a	42.7 a	31.8 a	41.1 a
+ K	36.0 a	47.1 a	38.4 a	40.8 a
- Ca	34.5 a	53.1 b	31.8 a	52.6 b
Ck	32.5 a	42.7 a	31.8 a	41.1 a
+ Ca	37.8 a	46.8 a, b	40.8 b	49.5 b
- Mg	33.1 a	44.4 a	30.6 a	51.7 b
Ck	32.5 a	42.7 a	31.8 a	41.1 a
+ Mg	35.2 a	41.2 a	36.1 a	42.4 a

Zinc (Table 18)

Variations in zinc content were quite similar to those of boron, with not a great deal of variability between treatments. Note that zinc levels increased in every instance from 1960 to 1961. This may have been due to a low zinc content of the soil in which the one-year-old trees were first grown, prior to transplanting in the quartz sand medium.

Molybdenum (Table 19)

Varying levels of treatments of potassium and calcium had the most profound effects on molybdenum levels within the leaves. The high level potassium treatment and the low level calcium treatment decreased molybdenum content in 1961, and low potassium treatment increased molybdenum levels in all instances.

Aluminum (Table 20)

Aluminum levels were only significantly influenced by nitrogen and potassium treatments, with a consistent pattern of increase from the control treatment to low nitrogen treatment to the high nitrogen treatment.

TABLE 18. --Zinc Content of Apple Leaf Tissue as Influenced by Nutrient Environment.

Treatment	Golden Delicious		Jonathan	
	1960	1961	1960	1961
- N	47 a	145 a	63 a	160 a
Ck	46 a	188 a	59 a	155 a
+ N	87 a	309 b	79 a	791 b
- P	109 a	261 a	44 a	171 a
Ck	46 a	188 a	59 a	155 a
+ P	51 a	185 a	59 a	231 a
- K	111 a	344 b	144 a	334 b
Ck	46 a	188 a	59 a	155 a
+ K	56 a	236 a, b	59 a	141 a
- Ca	193 b	420 b	118 a	352 b
Ck	46 a	188 a	59 a	155 a
+ Ca	57 a	98 a	64 a	73 a
- Mg	66 a	255 a	94 a	275 b
Ck	46 a	188 a	59 a	155 a
+ Mg	49 a	148 a	46 a	80 a

TABLE 19. --Molybdenum Content of Apple Leaf Tissue as Influenced by Nutrient Environment.

Treatment	Golden Delicious		Jonathan	
	1960	1961	1960	1961
- N	4.9 a	4.3 a	6.4 a	4.7 a
Ck	4.3 a	6.5 b	4.4 a	6.6 a
+ N	6.1 a	5.4 a, b	5.3 a	5.5 a
- P	4.2 a	4.4 a	5.1 a	4.2 a
Ck	4.3 a	6.5 b	4.4 a	6.6 b
+ P	5.4 a	7.0 b	7.7 b	6.6 b
- K	6.6 b	9.0 c	7.9 b	7.9 b
Ck	4.3 a	6.5 b	4.4 a	6.6 b
+ K	2.4 a	3.0 a	3.4 a	2.8 a
- Ca	4.0 a	2.9 a	5.2 a, b	3.2 a
Ck	4.3 a	6.5 b	4.4 a	6.6 b
+ Ca	7.3 b	8.8 c	6.7 b	5.8 b
- Mg	4.2 a	7.2 b	5.6 a	6.7 b
Ck	4.3 a	6.5 a, b	4.4 a	6.6 b
+ Mg	5.7 a	4.9 a	6.4 a	4.4 a

TABLE 20. --Aluminum Content of Apple Leaf Tissue as Influenced by Nutrient Environment.

Treatment	Golden Delicious		Jonathan	
	1960	1961	1960	1961
- N	196 a	176 a, b	271 a	348 a
Ck	257 a	153 a	440 b	314 a
+ N	283 a	287 b	459 b	548 b
- P	192 a	188 a	375 a, b	357 a
Ck	257 a	153 a	440 b	314 a
+ P	162 a	207 a	277 a	390 a
- K	174 a	206 a	369 a	314 a
Ck	257 a	153 a	440 b	314 a
+ K	263 a	167 a	351 a, b	448 b
- Ca	238 a	228 a	325 a	373 a
Ck	257 a	153 a	440 a	314 a
+ Ca	216 a	161 a	337 a	327 a
- Mg	297 a	174 a	339 a, b	374 a
Ck	257 a	153 a	440 b	314 a
+ Mg	226 a	182 a	299 a	384 a

DISCUSSION

The data presented in Tables 2 through 6 indicate a profound influence of nutrient environment on organic acid composition of apple fruits. The major variations were incurred in the malic acid content of both peel tissue and pulp tissue of apple fruit.

It was not within the scope of this experiment to attempt a physiological explanation for the variations in acid levels and mineral levels within the plant. Any such attempt to establish a direct relationship between the variations of any given mineral element found within the leaves, and the organic acid content of the fruit tissue would be presumptuous.

There are, however, some associations which bear the appearance of explicability, and it is proposed to herein discuss the possible explanations for such occurrences.

The peel tissue of apple fruits is the most active metabolic portion of the organ and more significant variations in response to treatment were observed in this tissue than in the pulp. The pulp tissue may be regarded as a storage for products from other more metabolically active portions of the organism. This is not to say, however, that the pulp is entirely dependent upon other tissues for the control of its various reactions and equilibria. This was best illustrated by the values obtained for total extractable acidity (TEA) and total titratable acidity (TTA).

High potassium effected an increase in TTA in all instances except in the pulp of Jonathan. A similar increase was noted in TEA in response to high potassium. In this instance even the TEA of the pulp of Jonathan was significantly increased. When trees were subjected to conditions of low potassium, TTA was significantly less than the TTA of the control, except in pulp of Golden Delicious. However, the TEA of Jonathan peel tissue was the only determination which was significantly increased over the control. In all other cases, TEA values were either nearly equal to, or less than, the TEA of the control.

As previously noted (85), TTA of apple fruit tissue was increased in response to increased Jonathan applications. A similar condition is indicated in Table 7 for both tissues of Golden Delicious, and for peel tissue of Jonathan. However, pulp tissue TTA of Jonathan is significantly lower than that of the control. Total extractable acidity of both tissues was increased by high potassium applications.

This net increase in TEA is reflected in contents of citric and malic acids (Tables 4 and 5). These general patterns did not hold true for any other element studied. None of the other acids was influenced by potassium in this manner.

Quidet et al. (72) showed that an ample supply of potassium was indispensable for optimum maturation and prolonged storage. Similar results by Tiller et al. (80) indicated that potassium is essential to good storage

quality. Tomkins (82) showed that the quantity of acids formed from carbohydrates is determined by the amount of potassium which enters the fruit. It has been shown that malic acid and citric acid accumulate in response to high level applications of potassium (16), and that increased potassium increases storage quality. Hence, it may also be assumed that a high level of malic acid is essential to good storage quality of apple fruits.

It has also been noted that excessive applications of nitrogen to the soil decreased the storage quality of apple fruits (1, 21, 28). The data in Table 4 indicate that, in the pulp tissue, as nitrogen applications are increased, malic acid content is decreased. This is further evidence that a high level of malic acid is essential to good storage quality of apple fruits.

High levels of nitrogen application also produced a marked increase in leaf nitrogen (Table 9). Such high levels of nitrogen may cause the equilibrium between organic acids and amino acids, via transamination, to be shifted toward amino acid synthesis, resulting in a lower level of organic acids within the cell vacuole.

Conversely, low levels of nitrogen application effected a dramatic increase in the organic acid content of the apple peel tissue, which may also be explained by the above series of reactions. Deprivation of the organism of sufficient nitrogen in any form would force the transamination equilibrium far toward organic acid synthesis, causing increases in acid levels over those of the control.

It was noted in the work of Hulme and Wooltorton (47) in organic acid changes in apple fruits during storage that malic acid decreased, citric acid increased, and there appeared to be an inverse relationship between quinic and shikimic acids. Presumably, quinic acid was converted to shikimic acid via 5-dehydroquinic acid, which is first converted to 5-dehydroshikimic acid, and then to shikimic acid.

Data presented in a previous section do not indicate any general trends in quinic or shikimic acid levels of apple fruits in response to alterations in nutrient environment, although significant differences were effected in many treatments.

The levels of phosphate in the various tissues follow the expected pattern. High phosphorus applications effected an increase in phosphate levels in both tissues, while low phosphorus treatments significantly reduced the level of phosphate in the pulp tissue. Inasmuch as the phosphate levels in peel tissue were below the level of detection in this experiment, significant responses to low phosphorus treatments in phosphate levels of peel tissue were not noted.

Potassium may function as a substitute for calcium and magnesium in ion balance, as is evidenced by the data presented in the tables on mineral composition (Tables 9 through 20). Calcium is thought to be present mainly in the cell walls. If potassium is to substitute for calcium, however, then calcium must also be present in the cell vacuole in considerable amounts,

inasmuch as the monovalent potassium ion could not effectively function in the capacity of the divalent calcium ion in the cell wall.

Potassium may interact with calcium in some other manner, thus causing the phenomenon noted.

The functions of magnesium are thought to be primarily as a cofactor in cyclic phosphorylation (4), and as a catalyst or co-enzyme in numerous phosphorylations, and as the metal of the chelate chlorophyll. Magnesium may also be highly functional in salt formation in the cell cytoplasm. Potassium and magnesium may be inter-related in that they may readily substitute for one another in cation balance of the organic acid fraction of apple fruits.

The results of this experiment show quite graphically that nutrient environment has a profound effect on the composition of the organic acid fraction of apple fruit tissues. Malic acid content shows the greatest variations, with similar effects appearing in the other acids of the complement. All the organic acids are important to the metabolism of the fruit, and may be intrinsic to good quality of fruit. However, further interpretation of the variations observed are not warranted on the basis of existent data.

Data are presented in Appendix Tables 8 through 13 which indicate that the noted variations were consistent for successive seasons. However, these data were incomplete and were not included in the statistical analyses. It is noted, however, that as mineral deficiencies become more acute, as is evidenced by leaf composition, the variations in acid content may fluctuate

more drastically from the control.

The possibility that fruit size may have an influence on TTA and TEA has been considered. However, data included in Appendix Table 16 compared with text Tables 7 and 8 indicate that, even though fruits were much smaller, TTA and TEA were much lower. Also, a similar condition existed even though there were fewer fruits per tree.

SUMMARY

An experiment was designed and performed to provide evidence that nutritional environment influences the total acid content of apple fruit tissue, as well as the stoichiometric balance within the fruit.

Apple trees of two varieties were grown for three seasons in quartz sand culture, and were treated with high and low levels of nitrogen, phosphorus, potassium, calcium and magnesium, and a control treatment, by means of modified Hoagland solutions.

Leaves of these trees were analyzed spectrographically to ascertain the degree of effectiveness of the treatments. Fruits were analyzed chemically by means of ion exchange chromatography to determine the magnitude of variations in organic acid composition in response to different nutritional environments.

Four organic acids were detected to be present in the apple fruit tissue. These were citric, malic, quinic and shikimic acids. Phosphoric acid was also detected to be present in the tissue extracts.

Greatest variations in shikimic acid content of peel tissue were noted in response to several treatments. Shikimic acid of peel tissue was greatly increased in response to low potassium, low calcium, low magnesium, and high nitrogen. Quinic acid content of apple fruit tissue was increased by high

nitrogen, high phosphorus, low potassium, low calcium, and low and high magnesium treatments.

The greatest significant differences in malic acid content of apple tissue was noted in response to three treatments. High nitrogen decreased malic acid levels. Low potassium also decreased malic acid content, but high potassium effected a significant increase in malic acid levels. Low nitrogen increased malic acid levels greatly. Possible explanations are discussed.

Citric acid variations were found in response to potassium and phosphorus treatments. Low potassium increased citric acid content, as did low phosphorus.

Phosphoric acid variations were greatest in response to phosphorus treatments. Low phosphorus decreased levels of phosphate in the tissue, while high phosphorus treatments increased phosphate.

Influences of the various elements on ion stoichiometry and the acid fraction are discussed. Alterations in nutrient supply to the trees may effect great differences in maturity stages, resulting in physiological disorders.

Variations of significance were also found in total titratable acidity and in total extractable acidity.

LITERATURE CITED

1. Abe, I., and More, H. Studies on the leaf analysis of apples. J. Hort. Assoc. Japan 27: 89-93. 1958.

2. Arbenz, E. Estimation of oxalic acid in food and in condiments. Mitt. aus dem Geb. der Lebensmtl. Untersuch. u. Hyg. 8: 98-104. 1917.

3. Archbold, H. K. Chemical studies in the physiology of apples. XII. Ripening processes in the apple and the relation of time of gathering to the chemical changes in cold storage. Ann. Bot. 46: 407-419. 1932.

4. Arnon, D. I., Whatley, F. R., and Allen, M. B. Assimilatory power in photosynthesis. Science 127: 1026-1034. 1958.

5. Barr, C. G. Investigations on the fluorometric determinations of malic acid and succinic acid in apple tissue. Plant Physiol. 23: 443-454. 1948.

6. Baxter, P. Fertilizers and apple storage. Agric. Vict. 56: 451. 1958.

7. Brunner, H., and Chuard, E. Phytochemical studies. Ber. 19: 595-622. 1886.

8. Bryant, F., and Overell, B. T. Displacement chromatography on ion exchange columns of the carboxylic acids of plant tissue extracts. Nature 167: 361-362. 1951.

9. _____. Quantitative chromatographic analysis of organic acids in plant tissue extracts. Biochem. et Biophys. Acta 10: 471-476. 1953.

10. Buch, M. L., Dryden, E. C., and Hills, C. H. Chromatographic comparison of non-volatile acids of fresh and stored apple juice concentrates. Food Chem. 3: 960-964. 1955.

11. Buchloh, G. The causes and prevention of bitter pit in apples. Erw Obstb. 2: 227-229. 1960.

12. Bunemann, G., Dewey, D. H., and Kenworthy, A. L. The storage quality of Jonathan apples in relation to the nutrient levels of the leaves and fruit. Quart. Bull. Mich. Agric. Exp. Sta. 41: 820-823. 1959.

13. Bunemann, O. The relationship between the harvest quality and the keeping quality of apples in relation to the mineral content of the soil and the leaves. Gartenbauwiss. 54: 457-471. 1959.

14. _____. The relationship between the harvest quality and the keeping quality of apples in relation to the mineral content of the soil and the leaves. Gartenbauwiss. 55: 53-66. 1960.

15. _____. The nitrogen content of apple tissue affected with bitter pit. Z. Pfl. Ernahr. Dung. 89: 188-190. 1960.

16. Burstrom, H. Mineral Nutrition of Plants. pp. 250-261. Univ. of Wisconsin Press. 1951.

17. Carangal, A. R. Jr., Alban, E. K., Varner, J. E., and Burrell, R. C. The influence of mineral nutrition on the organic acids of the tomato (Lycopersicon esculentum). Plant Physiol. 29: 355-360. 1954.

18. Cartwright, R., and Roberts, E. A. H. Theogallin as a galloyl ester of quinic acid. Chem. and Ind. pp. 230. 1955.

19. Chittenden, E. T. Bitter pit investigations. A. R. Cawthron Inst., 1960-1961, pp. 36-37. 1961.

20. Clark, H. E. Effect of ammonium and nitrate nitrogen on the composition of the tomato plant. Plant Physiol. 11: 5-14. 1936.

21. Collins, W. B. Influence of nutrition on apple storage quality. Abstract in Rept. Canad. Comm. Fruit Veg. Preserv. for 1956, pp. 2. 1957.

22. Conway, T. Reducing incidence of bitter pit in apples. N. Z. Jour. Agric. 100: 28. 1960.

23. Duncan, D. B. Multiple range and multiple F tests. Biometrics 11: 1-42. 1955.

24. Eaves, C. A., and Leefe, J. S. The influence of orchard nutrition upon the acidity relationship in Cortland apples. J. Hort. Sci. 30: 86-96. 1955.

25. Fidler, J. C. A comparison of the aerobic and anaerobic respiration of apples. J. Exp. Bot. 2: 41-64. 1951.

26. Franzen, H., and Helwert, F. Chemical constituents of green plants. XXV. Acids of the apple (Pyrus malus). Hoppe-Seylers Zeitschr. Physiol. Chem. 127: 14-38. 1923.

27. Griffiths, D. G., and Potter, N. A. Data for the study of the metabolism of apples during growth and storage. J. Hort. Sci. 25: 266-267. 1950.

28. de Haas, P.-G. The supply of mineral nutrients in relation to fruit quality in apples. ErwObstb. 1: 14-19. 1959.

29. Hatch, M. D., Pearson, A. J., Millerd, A., and Robertson, R. N. Oxidation of Krebs cycle acids in apple tissue. Austral. J. Biol. Sci. 12: 167-174. 1959.

30. Haynes, D. Chemical studies in the physiology of apples. I. Changes in the acid content of stored apples and its physiological significance. Ann. Bot. 39: 77-81. 1925.

31. Heulin, F. E., and Gallop, R. A. Studies in the natural coating of apples. I. Preparations and properties of fractions. Austral. J. Sci. Res. Ser. B, 4: 526-532. 1951.

32. Hilkenbaumer, F., Buchloh, G., and Zachariae, A. On the etiology of bitter pit in apple fruits. I. First communication. Angew. Bot. 24: 38-45. 1960.

33. Hillig, F., and Ramsey, L. L. Determination of lactic and volatile acids in fruits and fruit products. J. Assoc. Off. Agric. Chem. 28: 525-530. 1945.

34. Hulme, A. C. Data for the study of the metabolism of apples during growth and storage. J. Hort. Sci. 25: 277-281. 1950.

35. _____. The isolation of l-quinic acid from the apple fruit. J. Exp. Bot. 2: 298-315. 1951.

36. _____. The isolation of chlorogenic acid from the apple fruit. Biochem. J. 53: 337. 1953.

37. _____. Isolation of l-citramalic acid from the peel of the apple fruit. Biochem. et Biophys. Acta 14: 36-43. 1954.

38. Hulme, A. C. The oxidation of citramalic acid and beta-hydroxyglutaric acid to acetoacetic acid and the possible significance in plant metabolism. Biochem. et Biophys. Acta 14: 44-51. 1954.

39. _____. Organic acids of the apple fruit. Congr. Int. Bot. Paris, Pars. et Commun., Sec. 11 et 12, 8: 394-398. 1954.

40. _____. Current methods for the study of the free amino acids in fruits. Food Sci. 28: 345. 1956.

41. _____. Carbon dioxide injury and the presence of succinic acid in apples. Nature 178: 218. 1956.

42. _____. Shikimic acid in apple fruits. Nature 178: 991-992. 1956.

43. _____. Quinic and shikimic acids in fruits. Qual. Plant Maveg. 3/4: 468-473. 1958.

44. _____. Biochemistry of apple and pear. Adv. Food Res. 8: 297-413. 1958.

45. _____, and Williams, A. H. Chlorogenic acid in fruit trees. Nature 170: 168-169. 1952.

46. _____, and Wooltorton, L. S. C. The organic acid metabolism of apple fruits: Changes in individual acids during growth on the tree. J. Sci. Food Agric. 3: 117-122. 1957.

47. _____. Determination and isolation of pome fruits and a study of acid changes in apples during storage. J. Sci. Food Agric. 3: 150-158. 1958.

48. Kenworthy, A. L. Photoelectric spectrometer analysis of plant materials. Proc. XXXVI Ann. Meet. Council Fert. Appl. pp. 39-50. 1960.

49. _____, and Harris, N. Organic acids in the apple as related to variety and source. Food Technol. 14: 372-375. 1960.

50. Kidd, F. C., and West, C. The effect of fertilizer treatments on the keeping quality of Cox's Orange Pippin apples. Rept. Food Invest. Board, London. 1938. pp. 143-152. 1939.

51. _____, Griffiths, C. G., and Potter, N. A. Metabolism of malic acid in apples. J. Hort. Sci. 26: 169-174. 1951.

52. Krotkov, G., Wilson, D. G., and Street, R. W. Acid metabolism of the McIntosh apples during their development on the tree and in cold storage. Canad. J. Bot. 29: 79-87. 1951.

53. MacVicar, R., and Burris, R. H. Translocation studies in tomato using ammonium sulfate labeled with N^{15}. Am. J. Bot. 35: 567-572. 1948.

54. Markakis, P., Jarcyzk, A., and Krishna, S. P. Non-volatile acids of blueberries. J. Agric. Food Chem. 11: 8-11. 1963.

55. Markley, K. S., and Sando, C. E. Progressive changes in the cuticle of apples during growth and storage. J. Agric. Res. 46: 403-412. 1933.

56. Martin, D., Lewis, T. L., and Cervy, J. Bitter pit in the apple variety Cleopatra in Tasmania in relation to calcium and magnesium. Austral. J. Agric. Res. 11: 742-749. 1960.

57. Martin, S. M. A spray reagent for identification of organic acids. Chem. and Ind. pp. 427-428. 1955.

58. Mehlitz, A., and Ballschmieter, H. Contents of lactic and volatile acids in unfermented apple juices. Indus. Obst. -u. Gemussevert. 39: 222-231. 1954.

59. _____, and Matzik, B. The question of occurrence of lactic acid in unfermented apple juices. Indus. Obst. -u. Gemussevert. 40: 59-62. 1955.

60. Nuccorini, R. Early and late ripening fruits and their acid contents. Ann. di Chim. Appl. (Rome) 20: 302-309. 1930.

61. _____, and Zaccagnini, A. Chemical examination of fruits. III. The accelerated or retarded ripening in relation to the acid content of the fruit. (Pisa) R. Ist. Super. Agric. Bol. 4: 353-381. 1928.

62. Nyhlen, A. Brown heart of apples - a fertilizer problem? J. Roy. Swedish Acad. Agric. 98: 253-263. 1959.

63. _____, and Rootsi, N. Some observations on bitter pit. Sver. Pomol. Foren. Arsskr. 61: 39-62. 1961.

64. Oberly, G. H. The effect of mineral nutrition on the occurrence of bitter pit in Northern Spy apples. Ph. D. Diss., Michigan State University, 1959.

65. O'Grady, L. J. The N/K ratio in McIntosh leaves vs. color and storage of fruit. Fruit Growing Soc., Que., pp. 79-82. 1959.

66. Ostrowski, W., Guzewska, I., and Gdowski, J. The effect of mineral fertilization on storage quality of Antonovka apples. Prace Inst. Sadown., Skierniewice, 4: 365-375. 1959.

67. Paskova, J., and Munk, V. A combined detecting reagent for the identification of organic acids on paper chromatograms. J. Chromatography 4: 241-243. 1960.

68. Pepkowitz, L. P., Gilbert, S. G., and Shive, J. W. The importance of oxygen in the nutrient substrate for plant organic acids. Soil Sci. 58: 295-298. 1944.

69. Phillips, J. D. The use of selected yeasts in cider making. II B. The effects of fermentation treatment on the organic acid composition. Bristol Univ., Agric. Hort. Res. Sta. Ann. Rept. 1952: 137-141. 1953.

70. _____, and Pollard, A. Degradation of sugars on ion-exchange columns of Amberlite IRA-400 (OH). Nature 171: 41-42. 1953.

71. _____, and Whiting, C. E. Organic acid metabolism in cider and perry fermentations. I. A preliminary study. J. Sci. Food Agric. 7: 31-35. 1956.

72. Quidet, P., Richard, H., and Petit, P. Effect of fertilization on yield, conditions of maturation and cold storage quality of Starking Delicious apples. C. R. Acad. Agric. Fr. 47: 130-135. 1961.

73. Sando, C. E. Ursolic acid. J. Biol. Chem. 90: 477-495. 1931.

74. Scheele, C. W. Fruit and berry acid. Chem. Ann. f. Freunde der Natl. (Crell) v. 2: 291-303. 1785.

75. Snear, C. B., Crane, H. L., and Myers, A. T. Nutrient-element balance: A fundamental concept in plant nutrition. Proc. Am. Soc. Hort. Sci. 47: 239-248. 1946.

76. Stutz, R. E. The role of organic acids in photosynthesis. Brookhaven Conf. Rep., Carbon dioxide Assimilation Reactions in Biological Systems, pp. 77-96. Brookhaven Nat. Lab., Upton, N. Y. 1950.

77. Swaby, R. J. Extraction of citrates and tartrates from fruits. Agric. Gaz. N. S. Wales 54: 571-573. 1943.

78. Tanner, R., and Rentschler, H. Composition of fruit acids from Swiss fruit juices. II. Fruit acids from Swiss cider apple juices. Mitt. aus dem Geb. der Lebensmtl. Untersuch. u. Hyg. 45: 305-311. 1954.

79. Tavernier, J., and Jacquin, P. Organic acids of the apple must. (Paris) Acad. des Sci. Compt. Rend. 225: 1373-1374. 1947.

80. Tiller, L. W., Roberts, H. S., and Ballard, E. G. The Appleby experiments. A series of fertilizer and cool-storage trials with apples in the Nelson district, New Zealand. Bull. N. Z. Dept. Sci. Indust. Res. 129: 155-173. 1959.

81. Tomana, T. Histological and physiological studies on the cause of Jonathan spot in apples. II. Nitrogen fertilization and the time of harvest in relation to spot development. J. Jap. Soc. Hort. Sci. 30: 117-124. 1961.

82. Tomkins, R. G. Unsolved problems in the preservation of food. The influence of cultural conditions on the quality and preservation of fruits and vegetables. J. Sci. Food Agric. 5: 161-168. 1954.

83. Umbreit, W. W., Burris, R. H., and Stauffer, J. F. Manometric Techniques 3rd ed., pp. 272. Burgess Publishing Co., Minneapolis, Minn. 1957.

84. Van Schreven, A. C., Van der Boon, J., and Das, A. Bitter pit in apples. II. The relationship between bitter pit and the minerals calcium, magnesium, and potassium. Meded. Dir. Tuinb. 25: 181-191. 1962.

85. Wilkinson, B. G. The effect of orchard factors on the chemical composition of apples. II. The relationship between potassium and total titratable acidity, and between potassium and magnesium in the fruit. J. Hort. Sci. 33: 49-57. 1958.

86. Williams, A. H. Phenolic substances of pear-apple hybrids. Nature 175: 123. 1955.

87. _____. A p-coumarylquinic acid from apple fruits. Chem. and Ind. pp. 1200. 1958.

88. Winkler, W. O. Report on decomposition of fruits and fruit products. J. Assoc. Off. Agric. Chem. 34: 506-507. 1951.

APPENDIX

TABLE 1. --Composition of Stock Solutions.

Solution	Chemical Used[1/]	Amount
A.	KNO_3	1819 g/18 l.
B.	$Ca(NO_3)_2$-4 H_2O	4251 g/18 l.
C.	$MgSO_4$-7 H_2O	1775 g/18 l.
D.	KH_2PO_4	490 g/18 l.
E.	H_3PO_4	122.4 ml/3 l.
F.	$CaSO_4$	30.9 g/18 l.
G.	$CaCl_2$	147 g/ l.
H.	K_2SO_4	1411.4 g/ 18 l.
J.	$KHCO_3$	150.2 g/ 3 l.
K.	KOH	9.35 g/ l.
L.	$MgSO_4$	244.0 g/ l.
	$MgCl_2$	295.8 g/ l.
M.	NH_4NO_3	1440.9 g/18 l.
N.	Sequestrine	399.39 g.[2/]
	$ZnSO_4$-7 H_2O	1.584 g.
	H_3BO_3	20.592 g.
	$MnCl_2$-4 H_2O	13.032 g.
	$CuSO_4$-5 H_2O	0.576 g.
	H_2MoO_4-H_2O	0.144 g.

[1/] All chemicals were Technical Grade.

[2/] Solution N was also made up to 18 liters volume.

TABLE 2. --Composition of Individual Treatments.

Treatment	A	B	C	D	E	F	G	H	J	K	L	M	N
+ N	90	90	90	90	-	-	-	-	-	-	-	325	45
+ P	-	90	90	90	120	-	-	-	-	90	-	45	45
+ K	90	90	90	90	-	-	-	480	-	-	-	-	45
+ Ca	-	90	90	90	-	1/	90	-	180	-	-	-	45
+ Mg	90	90	90	90	-	-	-	-	-	-	180	-	45
Ck	90	90	90	90	-	-	-	-	-	-	-	-	45
- N	-	27	90	-	30	2700	72	120	-	-	-	-	45
- P	90	90	90	-	-	-	-	20	-	-	-	-	45
- K	-	90	90	-	30	-	-	-	-	-	-	-	45
- Ca	90	-	90	90	-	-	-	-	-	-	-	45	45
- Mg	90	90	-	-	-	-	-	-	-	-	-	90	45

1/ 47.55 g. $CaSO_4$.

2/ Solutions were made to 18 liters volume, using the above components.

3/ All above amounts are expressed as milliliters.

TABLE 3. --Dates of Full Bloom[1/] on Apple Trees Grown in Quartz Sand Culture - 1961.

Date	Golden Delicious		Jonathan	
	High	Low	High	Low
May 22	K	Ca, K, Mg, P	K	Ca, K, P
May 23	P	N, Ck	P	Mg
May 24	Ca, Mg	-	Ca	N, Ck
May 25	-	-	Mg	-
May 26	N	-	N	-

1/ Full bloom is defined as that stage of flowering when approximately 80% of the blossoms of a cluster were opened.

TABLE 4. --Dates of Full Bloom[1/] on Apple Trees Grown in Quartz Sand Culture - 1962.

Date	Golden Delicious		Jonathan	
	High	Low	High	Low
May 17	-	Ck	-	Ck
May 18	Ca, Mg	N, P	P	N
May 19	K, P	Mg	Ca, K, Mg	P
May 20	-	-	-	-
May 21	-	-	N	-

1/ Full bloom is defined as that stage of flowering when approximately 80% of the blossoms of a cluster were opened.

TABLE 5. --Dates of Sample Collection from Apple Trees Grown in Quartz Sand Culture - 1961.

Date	Golden Delicious		Jonathan	
	High	Low	High	Low
October 11	K	Ca, K, Mg, P	K	Ca, K, P
October 12	P	N, Ck	P	Mg
October 13	Ca, Mg	-	Ca	N, Ck
October 14	-	-	Mg	-
October 15	N	-	N	-

TABLE 6. --Dates of Sample Collection from Apple Trees Grown in Quartz Sand Culture - 1962.

Date	Golden Delicious		Jonathan	
	High	Low	High	Low
October 6	-	Ck	-	Ck
October 7	Ca, Mg	N, P	P	N
October 8	K, P	Mg	Ca, K, Mg	P
October 8	-	-	-	-
October 9	-	-	N	-

TABLE 7. --Values of R_f for Organic Acids of Apple Fruit Tissues Separated by Column Chromatography and Spotted on Chromatograms Beside Known Acids.

Acid	Value of R_f	
	Known	Unknown
Shikimic	0. 37	0. 38
Quinic	0. 22	0. 22
Malic	0. 52	0. 52
Citric	0. 47	0. 47
Phosphoric	0. 24-0. 26	0. 24-0. 26

TABLE 8. --Organic Acid Composition of Golden Delicious Apple Fruit Tissue as Influenced by Nutrient Environment - 1962.

Treatment	Pulp				
	Shikimic	Quinic	Malic	Citric	Phosphoric
+ N	No samples				
+ P	3	12	221	3	17
	7	23	253	1	27
+ K	No samples				
+ Ca	17	31	377	11	9
	0.0	4	523	3	3
+ Mg	9	81	174	2	0.0
Ck	23	4	568	4	11
	16	6	464	4	14
- N	8	21	561	5	12
- P	45	67	549	10	0.0
	24	0.0	607	9	4
- K	No samples				
- Ca	No samples				
- Mg	24	19	547	5	3
	9	33	531	4	6

TABLE 9. --Organic Acid Composition of Golden Delicious Apple Fruit Tissue as Influenced by Nutrient Environment - 1962.

Treatment	Peel				
	Shikimic	Quinic	Malic	Citric	Phosphoric
+ N	No samples				
+ P	24	17	376	0.0	7
	21	13	517	0.0	9
+ K	No samples				
+ Ca	5	12	554	2	1
	0.0	25	552	3	2
+ Mg	0.0	56	541	0.0	0.0
Ck	42	52	565	4	4
	26	33	531	6	0.0
- N	59	42	1241	10	13
- P	19	8	477	2	3
	7	46	559	0.0	5
- K	No samples				
- Ca	No samples				
- Mg	14	19	565	3	4
	13	19	451	4	2

TABLE 10. --Organic Acid Composition of Jonathan Apple Fruit Tissue as Influenced by Nutrient Environment - 1962.

Treatment	Pulp				
	Shikimic	Quinic	Malic	Citric	Phosphoric
+ N	0.0	12	689	28	6
+ P	26	29	601	19	43
+ K	0.0	12	870	34	19
	0.0	6	732	8	13
+ Ca	0.0	14	598	8	4
	0.0	58	612	11	14
+ Mg	0.0	17	360	0.0	0.0
	0.0	23	310	0.0	0.0
Ck	0.0	36	820	13	18
	0.0	4	770	6	10
- N	0.0	36	905	10	17
	0.0	29	1206	12	15
- P	0.0	35	882	6	0.0
- K	No samples				
- Ca	No samples				
- Mg	0.0	40	801	6	7
	0.0	13	709	3	1

TABLE 11. --Organic Acid Composition of Jonathan Apple Fruit Tissue as Influenced by Nutrient Environment - 1962.

Treatment	Peel				
	Shikimic	Quinic	Malic	Citric	Phosphoric
+ N	49	127	1692	17	76
+ P	7	17	1115	0.0	28
+ K	14	29	1158	15	7
	10	35	1071	10	2
+ Ca	19	67	854	3	0.0
	5	4	799	7	0.0
+ Mg	23	44	983	3	0.0
	5	6	963	10	0.0
Ck	9	35	797	2	7
	7	10	746	2	3
- N	78	60	3217	12	0.0
	125	286	2574	27	20
- P	10	36	951	8	1
- K	No samples				
- Ca	No samples				
- Mg	14	19	565	3	4
	73	19	451	4	2

TABLE 12. --Total Acidity of Golden Delicious Apple Fruit Tissue as Influenced by Nutrient Environment - 1962.

Treatment	Total Titratable Acidity		Total Extractable Acidity	
	Pulp	Peel	Pulp	Peel
+ N	No samples			
+ P	4.28	3.65	14.03	6.04
	3.61	3.11	11.48	8.19
+ K	No samples			
+ Ca	3.12	7.82	6.32	8.43
	4.06	7.03	7.96	8.47
+ Mg	4.39	5.00	3.12	8.36
Ck	4.13	5.32	9.11	9.14
	3.02	3.46	7.56	8.34
- N	3.82	4.78	8.96	19.63
- P	3.86	3.25	8.91	7.36
	4.69	3.78	9.42	8.76
- K	No samples			
- Ca	No samples			
- Mg	4.11	2.87	8.74	8.77
	3.48	3.64	8.53	7.36

TABLE 13. --Total Acidity of Jonathan Apple Fruit Tissue as Influenced by Nutrient Environment - 1962.

Treatment	Total Titratable Acidity		Total Extractable Acidity	
	Pulp	Peel	Pulp	Peel
+ N	7.23	2.32	10.61	28.74
+ P	5.84	8.31	10.31	18.02
+ K	4.79	9.78	14.03	17.95
	4.03	8.56	11.48	11.48
+ Ca	7.08	8.31	9.23	13.25
	6.78	8.11	10.04	12.06
+ Mg	7.13	10.92	5.46	15.09
	7.24	10.43	4.83	14.59
Ck	6.84	8.12	13.19	12.36
	5.63	7.64	11.90	11.36
- N	4.76	11.25	14.38	48.96
	5.98	10.63	18.86	41.64
- P	5.26	9.13	13.42	14.61
- K	No samples			
- Ca	No samples			
- Mg	4.11	2.87	8.74	17.34
	3.48	3.64	8.53	15.89

TABLE 14. --Mineral Composition of Golden Delicious Apple Fruits as Influenced by Nutrient Environment - 1961.

Treatment	N	K	P	Ca	Mg	Mn	Fe	Cu	B	Zn	Mo	Al
+ N	0.92	1.23	0.110	0.130	0.08	8	42	0.4	31.2	12	0.5	11
+ P	0.54	0.96	0.189	0.130	0.07	15	36	1.75	30.2	9	0.9	17
+ K	0.57	1.94	0.137	0.130	0.07	10	30	5.1	14.8	17	0.6	14
+ Ca	0.47	1.27	0.084	0.130	0.07	13	33	2.2	36.4	10	0.8	16
+ Mg	0.40	1.24	0.123	0.103	0.08	9	26	0.2	31.9	7	0.7	20
Ck	0.39	1.29	0.118	0.130	0.07	7	26	0.2	30.7	16	0.6	10
- N	0.30	1.25	0.67	0.120	0.06	7	23	0.1	54.3	3	0.5	14
- P	0.36	1.36	0.080	0.130	0.08	9	29	0.1	42.5	12	0.7	18
- K	0.33	0.34	0.134	0.130	0.07	8	24	0.5	38.3	9	0.6	13
- Ca	0.48	1.52	0.142	0.130	0.08	26	51	3.5	29.5	34	0.7	23
- Mg	0.66	1.65	0.175	0.130	0.07	9	33	0.5	51.0	14	0.6	14

1/ N, P, K, Ca and Mg are expressed as percent dry weight. Mn, Fe, Cu, B, Zn, Mo and Al are expressed as parts per million dry weight.

2/ Above values are average of three replications.

TABLE 15. --Mineral Composition of Jonathan Apple Fruits as Influenced by Nutrient Environment - 1961.

Treatment	N	K	P	Ca	Mg	Mn	Fe	Cu	B	Zn	Mo	Al
+ N	0.88	1.32	0.113	0.130	0.07	12	38	0.6	32.9	14	0.6	8
+ P	0.78	1.26	0.235	0.130	0.08	8	31	0.5	39.2	7	0.5	19
+ K	0.53	1.85	0.156	0.125	0.07	12	25	0.2	28.3	9	0.8	16
+ Ca	0.49	1.31	0.092	0.125	0.07	18	36	2.8	41.2	6	0.9	14
+ Mg	0.48	1.38	0.144	0.123	0.08	14	34	8.9	21.9	14	1.0	17
Ck	0.40	1.37	0.130	0.130	0.08	9	33	0.3	29.5	9	0.5	10
- N	0.42	1.36	0.136	0.130	0.07	12	20	0.0	28.3	5	0.5	15
- P	0.30	1.26	0.069	0.130	0.07	4	20	0.0	41.6	0	0.5	15
- K	0.34	0.52	0.111	0.130	0.08	7	32	0.8	40.1	7	0.8	16
- Ca	0.68	1.52	0.159	0.130	0.09	12	37	0.0	31.9	11	0.5	19
- Mg	0.57	1.63	0.165	0.130	0.07	11	24	0.8	36.8	11	0.6	12

1/ N, P, K, Ca and Mg are expressed as percent dry weight. Mn, Fe, Cu, B, Zn, Mo and Al are expressed as parts per million dry weight.

2/ Above values are averages of three replications.

TABLE 16. --Average Weight of Apple Fruits as Influenced by Nutrient Environment - 1961.

Treatment	Golden Delicious		Jonathan	
	g. /fruit	g. /tree	g. /fruit	g. /tree
+ N	103	172	92	92
+ P	84	672	76	912
+ K	128	896	110	1210
+ Ca	152	1368	121	968
+ Mg	148	1480	108	1296
Ck	135	1350	118	1298
- N	46	598	37	444
- P	63	441	58	638
- K	32	224	30	210
- Ca	48	432	42	252
- Mg	59	472	52	260

1/ Above values are averages of three replications.