THE RELATIONSHIP BETWEEN CATION UPTAKE AND ORGANIC ACID ACCUMULATION IN APPLE LEAVES

ABDUL WAHID MOHAMMAD AL. HACHIM

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THE RELATIONSHIP BETWEEN CATION UPTAKE
AND ORGANIC ACID ACCUMULATION
IN APPLE LEAVES

by
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B.S. North Carolina State University, 1961
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ABSTRACT

THE RELATIONSHIP BETWEEN CATION UPTAKE
AND ORGANIC ACID ACCUMULATION
IN APPLE LEAVES

by

ABDUL WAHID MOHAMMAD AL HACHIM

A study was made to determine the relationship between calcium, magnesium and potassium and organic acid accumulation in apple leaves and the effects of these cations on the plant buffer system under controlled sand culture conditions. Leaf samples were taken twice a month from June 30 to September 30. Alcohol extracts of fresh leaf tissues were analyzed for organic acid content, utilizing ion exchange and thin layer chromatography. Total acids were determined by titration. A new fluorometric method was developed for malic acid determination by thin layer chromatography. The pH of the extract from each treatment was measured to determine the buffering effect of each element. Mineral analyses were done by atomic absorption spectrophotometry.

Malic acid and total acids increased slowly but steadily as the season advanced in the complete, minus calcium and minus magnesium treatments. The levels of both malic acid and total acids were much less in minus potassium than in the other treatments. It was concluded that both calcium and magnesium had no effect on organic acid accumulation in apple leaves. It was postulated that the increase
in potassium content of the leaves as the season advanced in all treatments except the minus potassium treatment, maintains the organic acids at about the same level. The leaves from the minus potassium treatment had a lower pH than those from the other treatments and it was concluded that potassium is the major element involved in the plant buffer system. The results also provide some evidence to indicate that ammonium nitrogen had no effect on acid accumulation in apple leaves.
SECTION I
INTRODUCTION

Calcium, potassium and magnesium are essential elements for plant growth and development. Several investigators have shown that calcium, in particular, and magnesium, to a certain extent, increase in apple leaves as the season advances. Potassium, on the other hand, usually decreases as the season advances. The continuous accumulation of calcium and magnesium in apple leaves late in the season appears to far exceed apparent requirements for growth and tissue development, because visible growth has stopped at that time. Thus, it has been suggested that this increase of calcium and magnesium may be responsible for neutralizing the organic acids that might otherwise accumulate in toxic amounts. It also has been suggested that both of these elements are acting as buffers in the leaves. The relationship between calcium, magnesium and potassium and organic acid accumulation in plants has been studied extensively by several workers. However, this relationship is affected by plant species, nitrogen source, and age of the tissue. The objectives of the present study were:

1. To determine the effect of Ca, Mg and K on organic acid accumulation in apple leaves under controlled greenhouse conditions.

2. To determine the role of Ca, Mg and K in plant buffer systems.
SECTION II
LITERATURE REVIEW

Organic acids play an important role in plant metabolism through their relationship to fats, carbohydrates and proteins (13). These acids, through the citric acid cycle, account for terminal oxidation of carbohydrates in addition to the biosynthesis of amino acids, fatty acids, purines, pyrimidines and porphyrins (49).

The relationship between ion absorption and organic acid production in plant tissue has been studied by several investigators. Working mostly with root tissues, several workers showed that organic acid content increased as cation absorption exceeded that of anions. A decrease was observed as anions were absorbed in excess of cations (34, 37, 39, 40, 47, 75). Dunne (25) working with buckwheat, also showed that cation over anion absorption resulted in high organic acid content, and Pucher et al. (62) found a correlation between excess positive ions and ether-soluble organic acids in tobacco leaves.

Earlier workers believed that cations were absorbed by the plant to prevent toxicity of the organic acids. This was based mainly on the detection of precipitated calcium oxalate, and thus the calcium was claimed to eliminate the toxic acid by converting it to the insoluble form (24). Truog and Meacham (77) stressed the importance of cation absorption by plants to overcome the toxicity of organic acids that form during metabolism. Presently, many investigators
(40,41,75) suggest that the organic acid increase during cation uptake is the result rather than the cause of excess cation absorption. Acids formed in the tissue maintain ion balance when cation absorption exceeds that of anions.

EFFECT OF INDIVIDUAL CATIONS ON ORGANIC ACID ACCUMULATION

The relationship between individual elements and organic acid accumulation has been studied extensively by many workers (19,32,33,42,63,76,84). However, the relationship is rather complex because it is affected by the species (32), tissue age (43,45,77) and the source of nitrogen in the growing medium (33,37,81). This relationship will be discussed later.

Effect of Calcium

Rasmussen and Smith (63) showed that malic acid and oxalic acid increased significantly as calcium increased in orange leaves, and indicated that calcium is the dominant cation regulating the production of organic acids, especially oxalic acid. In tung leaves only oxalic acid was related to calcium uptake (33). Ddungu and Eggert (23), studying the relationship between cations and the organic acid content in apple leaves, correlated the increase in titratable acidity with the increase of calcium in the leaves.

Effect of Potassium

In orange leaves it has been found that an increase of potassium increased oxalic acid with no effect on malic acid, citric acid and total acids (63). Other workers showed
that potassium deficiency increased malic acid (19, 72),
citric acid (19, 42), and succinic and fumaric acids (19) in
different plant species. Jones (42), however, reported that
a decrease in potassium resulted in a decrease in malic acid
and oxalic acid in tomato leaves. Freeman (32) studied the
effect of potassium deficiency on organic acid levels in sev­
eral plant species, and found that a potassium deficiency re­
sulted in organic acid increase in cabbage, beet and potato
leaves, a decrease in lettuce, and no appreciable change in
ryegrass. He also reported varied relationships between cit­
ric acid, malic acid and potassium in these species. Freeman
(32) also suggested that plants can be divided into three
groups in relation to the effect of potassium on organic acid
production. Potassium deficiency may (a) increase organic
acids as in cabbage, beet and tomato, (b) decrease them as in
lettuce or (c) have no effect as in ryegrass.

**Effect of Magnesium**

Valikhanova (76) found that a magnesium deficiency in
bean leaves resulted in an increase in malic acid and a de­
crease in citric acid. Clark (19) reported a decrease in
trans-aconitic acid and an increase in malic and succinic
acids in magnesium deficient corn leaves. Rasmussen and Smith
(63), however, found no effect of magnesium on the organic
acid level of orange leaves. They indicated that the increase
of malic and oxalic acids in magnesium deficient leaves is
due to an increase of calcium and potassium rather than to a
decrease of magnesium.
Although the role of some organic acids in plant metabolism is well established, the relationship of these acids to the potassium, calcium and magnesium content of plant tissue is rather complex. These cations are essential for plant growth and development. A lack of any one of them will affect the normal metabolic processes and thus upset the balance of the plant constituents. As a result there is an inhibition of enzymatic steps either by a deficiency of substrate or a decrease in activity of the enzyme or enzymes (29). The variation of these and other elements in leaves is often accompanied by changes in organic acids. In plants the electrostatic balance of ions unincorporated into organic substances is maintained by the production of organic acids (2).

FACTORS AFFECTING ORGANIC ACID PRODUCTION

It has been mentioned previously that the relationship between cation uptake and organic acid production in plants is affected by the species, form of nitrogen and the age of tissue. Since the effect of species has been discussed for each individual cation, the other two factors will be considered below.

Form of Nitrogen

It has been found that the total and individual organic acids in plant tissue are influenced by the form of nitrogen supplied to the plant (18,44,75,80,81). Malic, citric, and oxalic acids decreased drastically in tomato plants supplied with ammonium nitrogen (18,44,81). Since
ammonium ions were found to reduce cation absorption by plants (33,44,79,80,81), it is possible that the reduction of organic acids is the result of the decrease in cation absorption. The low cation and high anion content of plants supplied with ammonium nitrogen can be attributed to inter-ionic effects (2). The absorption of \( \text{NH}_4^+ \) would decrease the absorption of cations. However, the absorption of \( \text{NO}_3^- \) would decrease the absorption of anions and increase the absorption of cations. Wadleigh and Shive (79) suggested that the decrease of cation uptake by plants supplied with ammonium nitrogen is the result of decreasing the pH of the solution film surrounding the roots, regardless of the solution pH.

Although ammonia and nitrate serve as the nitrogen source for plants, assimilation of these two forms in plants is different. The assimilation of nitrate produces hydroxyl groups while the assimilation of ammonium produces hydrogen ions (41), as illustrated in the following equations:

\[
\text{NO}_3^- + 8\text{H}^+ + 8\text{e} \rightleftharpoons \text{NH}_3 + 2\text{H}_2\text{O} + \text{OH}^-
\]

\[
\text{NH}_4^+ \rightleftharpoons \text{NH}_3 + \text{H}^+
\]

Kirkby and Mengel (44) indicated that nitrate uptake increased plant pH and might have led to the accumulation of organic acids. Ammonia, on the other hand, decreased the plant pH and reduced organic acid synthesis. Many plant species were found to have a lower pH when supplied with ammonium nitrogen (74,79).

**Age of Tissue**

Many investigators have reported that top (young)
leaves contain a lower level of organic acids than bottom (old) leaves (43, 45, 77). Generally, young leaves contain less organic acid than old leaves because the former are actively growing and derive their energy from organic acids and other metabolites. Older leaves as compared to younger leaves have larger vacuoles which store more organic acids (6). Older leaves also usually accumulate more total cations as the season advances which may contribute to the accumulation of organic acids.

EFFECT OF CATIONS ON PLANT BUFFER SYSTEM

Organic acids exist as free acids and salts of cations, and thus constitute an important buffer system in the plant tissue (7). According to Pucher et al. (62), the important function of organic acids in the leaf tissue is to maintain the pH of the cell solution at the proper level. Burstrom (12) indicated that the synthesis or breakdown of organic acids in tissue occurs as a direct response to the excess uptake of cations or anions in order to maintain optimum pH.

Dunne (25) reviewed the literature concerning the plant components that are responsible for the buffering effect in plants, and found that the more important components are salts of organic acids, amino acids and their amides, phosphates, carbonates and soluble proteins. Hoagland (35) indicated that organic acids in association with cations and phosphates, amides, amino acids and other compounds, constitute a buffer system to maintain a fairly
constant pH in plants.

**Effect of Calcium**

Newton (54) studied the relationship between calcium and the plant buffer system. Barley, peas and beans were grown in culture solutions with low and high calcium. No appreciable increase in hydrogen ion concentration was associated with low calcium supply. Dunne (25) working with buckwheat in sand culture with low and high calcium, concluded that calcium is not an indispensible part of the plant buffer system. Ddungu and Eggert (23) correlated the increase in titratable acidity with the increase of calcium and magnesium in apple leaves and suggested that both cations may be responsible for neutralizing the acidity of the leaves.

**Effect of Magnesium**

According to Pierce and Appleman (61), magnesium neutralizes and precipitates oxalic acid in certain plant species. They found that insoluble oxalate was higher than the insoluble calcium of beet, spinach and buckwheat, and concluded that the extra oxalate was precipitated as magnesium oxalate. This was supported by Wilkinson (83) who investigated the relationship between cations and titratable acidity in apple fruits. It was suggested that magnesium may be involved in the buffer system. Miller (51) indicated that magnesium and potassium are the principal cations involved in the plant buffer system.

**Effect of Potassium**

Many workers have reported on the importance of
potassium in plant buffer systems (21,35,55). Hoagland (35) stated that potassium is the chief cation in equilibrium with organic acid systems, and indicated that potassium deficiency increases acidity even though there is a large content of calcium and magnesium. He reported that sap from potassium-deficient prune leaves has a lower pH than normal leaves. Low pH was also found in leaves of plants low in potassium such as corn (79) and tomato (56). Wilkinson (83) measured the titratable acidity of apple fruits and suggested that potassium and magnesium were involved in the buffer system.

THE INTERACTIONS BETWEEN CALCIUM MAGNESIUM AND POTASSIUM

Elements which are essential for the growth and development of plants have been found to serve specific functions in plant processes. These elements should be in proper proportion in order for these processes to proceed at an optimum rate. It has been known for a long time that the presence of any element in the nutrient medium in a large concentration results in depression of plant growth either by the so-called toxic effect of that element or by reduction in the absorption of other elements.

The term "antagonism" has been used by many investigators for the reciprocal relationship between any two nutrient elements. Jacob (38), defined antagonism as "the frequently observed phenomenon that the uptake of an ion by the plant is inhibited by the increased supply of other ions, whether as a result of soil processes or the influence of the plant itself."
Effect of Calcium on Potassium and Magnesium

The effect of calcium on potassium and magnesium has been demonstrated by many workers. Swanback (71) investigating the relationship between calcium and tobacco growth indicated that the liming of soil resulted in a decrease in the potassium content of plants. This effect was also demonstrated with other crops (64). Cain (13) working with McIntosh apple seedlings in sand culture showed that increasing either calcium or magnesium in the nutrient medium under greenhouse conditions tended to decrease potassium in the leaves. Calcium also was found to depress potassium uptake in olive (30) and tung leaves (66,67). Collander (20), however, reported that ion antagonism in the plant was between closely allied cations and showed that the uptake of potassium was independent of the calcium concentration of the culture solution. Viets (78) presented data to show that the presence of calcium in the nutrient solution appreciably increased the absorption of potassium by barley roots. Viets' finding has been confirmed by Epstein (28). Overstreet et al. (59), however, indicated that the effect of calcium on potassium uptake is dependent upon the potassium concentration in the medium, and that calcium exerts both a depressive and stimulatory effect. They indicated that calcium depresses potassium uptake when the potassium concentration is low, and exerts a stimulatory effect when the potassium concentration increases. However, when potassium concentration is high, calcium has no effect on potassium uptake. They suggested that calcium acts as a co-
factor in the utilization of a potassium complex produced during absorption. The interaction between calcium and potassium is also affected by species. Khan and Hanson (46) reported that calcium promoted potassium uptake in corn, and inhibited it in soybean. They reported that calcium increased the affinity between the potassium ion and its carrier, which is greater in corn than in soybean, and thus corn has more potassium than soybean.

Moore, et al. (52,53) showed that increase in calcium in the nutrient solution decreased magnesium uptake by excised barley roots. Shear (67), however, working with tung seedlings found that increasing calcium supply decreased magnesium in the whole plant and in storage and feeding roots, but did not significantly affect magnesium in the leaves, petioles or stem.

Effect of Magnesium on Potassium and Calcium

Cain (13) found that the effect of increased magnesium in the nutrient medium on the decrease of potassium in apple leaves is greater than the effect of increased calcium. He also showed that increase of magnesium in the medium decreased calcium in the leaves. Mason (50) showed that the reduction of magnesium/potassium ratios decreased both magnesium and calcium, and increased potassium in leaves of McIntosh seedlings. He also reported that reduction of calcium/magnesium ratios increased magnesium, but decreased calcium and potassium. Ford (31) working with one-year Malling VII rootstock found that increasing magnesium supply decreased
potassium concentration of the leaves. Shear (67) using tung
seedlings showed that increasing external magnesium decreased
potassium and calcium uptake, with the effect being greater
at the low calcium level.

**Effect of Potassium on Calcium and Magnesium**

Extensive studies also have been made on the effect
of potassium on calcium and magnesium. Increasing potassium
supply decreased calcium content in leaves of fruit trees (26, 40, 69). Batjer et al. (5), however, reported that heavy
orchard application of potassium for ten years did not affect
potassium, magnesium or calcium levels in all parts of mature
Delicious apple trees. Ashby and Steward (3) studied the
effect of calcium and potassium on magnesium in apple leaves
in sand culture and reported that magnesium content decreased
with high potassium-calcium supply and no effect resulted
from high magnesium or calcium alone. In field experiments
they found that increasing potassium supply decreased calcium,
but had no effect on magnesium. Boynton and Burrell (8)
studied the effect of potassium on magnesium deficiency in
apple, and reported that high potassium in the nutrient medium
decreased magnesium in the leaves. They indicated that mag­
nesium deficiency was induced after 3-4 years of potassium
fertilization in the field and after six weeks in the green­
house. Cain (15), using four levels of nitrogen and potas­
sium on McIntosh apple trees found that potassium fertiliza­
tion decreased total leaf magnesium without adverse effect
on the growth. In other experiments with Golden Delicious,
Cain (16) reported that potassium and magnesium had no effect on the absorption of the other at regular level, and increasing the supply of either of them reduced the tissue concentrations of the other. He showed that the effect of potassium on reducing magnesium concentration is greater than magnesium on potassium under equivalent concentrations. Omar and ElKobbia (58), however, working with alfalfa in sand culture with different concentrations of potassium and magnesium found that high magnesium had no effect on potassium absorption, while high potassium decreased magnesium uptake.

The complexity of the interactions that occur between the essential nutrient elements with respect to absorption and accumulation is indicated by contradictions in the evidence cited above. Carolus (17), investigating magnesium deficiency in potato, observed that plants with deficiency symptoms sometimes contained more magnesium than the control. He remarked that a condition which had been designated as "magnesium deficiency" was probably not always associated with an extremely low magnesium content in the plant, but might be a result of disproportionate absorption of other cations in relation to magnesium. Concerning deficiency symptoms in apple, Cain (13) found that magnesium or potassium deficiency was induced by a high concentration of the other cation in the leaves and medium. However, when both potassium and magnesium were low in the medium, no deficiency symptoms appeared. He concluded that a deficiency symptom attributed to a lack of one element might not be actually
a deficiency but a toxic effect produced by an excess content of other elements.

The vital plant processes proceed at an optimum rate only when the cationic elements are present in a balanced state. Shear et al. (65) indicated that maximum growth and yield cannot be accomplished by a high level of nutritional intensity unless a proper balance exists between all of the elements. Discussing cation balance, Hoagland (35) indicated that the quantitative relationships between calcium, magnesium, potassium and sodium are of considerable physiological importance. He reported that it is not unusual to find that the decrease in absorption of one base is roughly compensated for by an increase in the absorption of other bases so that the total equivalents of bases present in the plant may remain approximately constant.
SECTION III
MATERIALS AND METHODS

One-year-old liner Robusta #5 rootstock trees were used for this experiment. Tops and roots were pruned at the time of planting to improve uniformity of the plants. The roots were washed thoroughly with water. The trees were planted April 22, 1968 in nine-inch plastic pots filled with washed silica sand, grade No. 8 (Am. Graded Sand Co., Paterson, N.J.).

The pots containing the trees were placed in the greenhouse on wood racks. Before growth started, all pots were watered with distilled water once every day. This also served to rewash the sand each time water was applied. When the trees started to grow, they were watered twice a day. No nutrient solution was applied until shoot growth reached 6-8 inches, in order for the trees to utilize their reserve nutrients. All of the trees were then given a complete nutrient solution to produce sufficient vegetative growth for future sampling.

The differential treatments were started June 9. The experimental design consisted of six replicates of four treatments, each containing three trees. All of the trees received the same amount of nutrient solution and distilled water. Trees were fed twice a week, and watered twice a day. The pots were not flushed because watering twice daily and good drainage served the purpose of flushing the pot to prevent salt accumulation in the sand.

The nutrient solution for this experiment was a
modification of Hoagland solution No. 1 (36). Calcium nitrate and potassium nitrate were substituted by ammonium nitrate whenever needed. The nutrient solution was prepared from a stock solution at the time of feeding. The composition of the solution and the different treatments are listed in Table 1.

Table 1. Composition of the nutrient solution for all treatments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Nutrients or PPM</th>
<th>mg/l Nutrient Solution</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>KHPPO₄, KN₃, Ca(NO₃)₂, MgSO₄</td>
<td>N  39.1  K  70  Ca  14.0  Mg  200</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ca  195.5  Mg  48.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total  210  234.6  200  48.6</td>
</tr>
<tr>
<td>-K</td>
<td>NH₄H₂PO₄, NH₄NO₃, Ca(NO₃)₂, MgSO₄</td>
<td>N  14  K  56  Ca  14.0  Mg  200</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ca  195.5  Mg  48.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total  210  234.6  200  48.6</td>
</tr>
<tr>
<td>-Ca</td>
<td>KHPPO₄, KN₃, NH₄NO₃, MgSO₄</td>
<td>N  39.1  K  70  Ca  14.0  Mg  234</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ca  195.5  Mg  48.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total  210  234.6  200  48.6</td>
</tr>
<tr>
<td>-Mg</td>
<td>KHPPO₄, KN₃, Ca(NO₃)₂, NH₄NO₃, K₂SO₄</td>
<td>N  14  K  39.1  Ca  14.0  Mg  56</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ca  39.1  Mg  156.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total  210  234.6  200  48.6</td>
</tr>
</tbody>
</table>

Micro-Nutrients

H₃BO₃, MgCl₂·H₂O, ZnSO₄·7H₂O, CuSO₄·5H₂O, H₂MoO₄·H₂O, FeSO₄·7H₂O
Sampling Method

Three mid-shoot leaves were taken at random from each tree. The leaves from all replications of each treatment were combined to form one sample. A total of fifty-four leaves was collected from each treatment. The leaves were collected between 1 and 2 P.M. twice a month starting June 30 and continuing until September 30. The leaves were placed in sealed plastic bags, and were kept in an ice chest containing "Cold Pack" frozen cans to reduce the leaf temperature during transportation from the greenhouse to the laboratory. The bagged leaves were stored in the refrigerator at 4°C for a short time until further processing. The leaves of each treatment were placed on a large sheet of paper, mixed thoroughly, and twenty-five leaves were selected at random for acid extraction. The rest of the leaves were used for mineral analysis.

Organic Acid Extraction

Each twenty-five leaf sample was weighed, cut into small pieces with scissors, and placed in a 250 ml beaker containing 100 ml of boiling 80% ethanol for five minutes. After cooling, the tissue was homogenized in an Omnimixer for one minute at high speed. The slurry was filtered by suction and the residue re-extracted with 80% boiling alcohol as before and filtered. The residue on the filter paper was washed several times with 80% alcohol, the extracts combined, and diluted to 200 ml with 80% alcohol.

The pH of the extract from each treatment was measured to determine the buffering effect of each element. After pH
determinations were made, the solutions were stored in the freezer for later determinations of organic acids.

**Organic Acid Determination**

Organic acids were separated from other plant constituents by ion exchange chromatography according to the method of Palmer (60). Dowex 1 x 10 resin of 200-400 mesh size in the chloride form was used. The fine particles were removed by suspending the resin in distilled water, allowing the coarser particles to settle, and decanting the cloudy supernatant liquid. This procedure was repeated until most of the small particles were removed. The resin was then converted to the formate form by placing it in a glass column 2 x 15 cm and passing through it a 1M solution of sodium formate until the effluent gave a negative test with silver nitrate. After washing with distilled water several times, the resin was stored in a dark brown bottle.

A glass tube 1 x 10 cm was used for the chromatographic resin column. A piece of glass wool was placed above the constriction to support the resin. A small rubber tubing with an adjustable clamp was attached to the bottom of the column. A small amount of the resin slurry was poured into the column in several portions to obtain a uniform column 4 cm long. Care was taken to prevent entrapment of air bubbles in the column. This was done by keeping the water level above that of the resin at all times. A small piece of glass wool was placed on the top of the resin in the column before washing with distilled water.
Fifty ml of the alcohol extract was placed in a small leveling bulb connected to the resin column with a piece of tubing. An adjustable clamp was attached between the leveling bulb and the column to serve as a control valve. The sample was drawn through the column by suction at a rate of about 1 ml per minute. The resin then was washed with distilled water to remove any neutral substances.

The organic acids were eluted with 15 ml of 6N formic acid at the rate of about 1 ml per minute. The column was then washed with 4 ml of distilled water and the eluates combined.

Preliminary investigation showed that some phenolic acids were eluted with the aliphatic acids. These fluorescent acids interfered with organic acid determination by the fluorometric method used in this study. In order to avoid this interference the phenolic compounds were removed from the solution according to the method of Anderson and Sowers (1). The procedure was modified slightly by passing the solution at approximately pH 3.5 through polyvinylpyrrolidone (PVP) (General Aniline & Film Corp., New York) in a small glass tube with suction. Most of the phenolic compounds were absorbed by the PVP. The column was then washed with 4 ml of distilled water and the final volume of the organic acid solution was made to 25 ml with distilled water.

A new fluorometric method based on the method of Buch et al. (11) was used for organic acid determinations. Ten and twenty microliters of the sample were spotted on a cellulose
thin layer plate. Ten and fifteen microliters of authentic samples of suspected acids also were spaced beside the unknowns. This was done in duplicate. The plate was developed in a solvent consisting of 80% formic acid: n-butanol (1:1) (22) until the solvent front reached a level about 2 cm below the top of the plate. The plate was then removed from the tank and dried in the hood at room temperature for 24 hours. The plate was sprayed with 0.1N ammonium hydroxide and 0.1N silver nitrate solution (1:1) mixed immediately before spraying. The plate was then hung in the hood away from direct sunlight. Acids appeared as white spots against a tan background. These spots were fluorescent under ultraviolet light, and the acids were estimated with a Turner fluorometer with scanner. A long wave (366 nm) light source was used with a 7-60 primary filter and #8 as the secondary filter. Sensitivity was set at 30X. The area of each acid under the curve was measured with a planimeter and the amount of each acid determined from a standard curve. Only malic acid was determined because previous work (22) and the preliminary investigation with this work showed that malic acid is the dominant acid in apple leaves. Citric acid occurs in small amounts and appears only toward the end of the growing season.

The total acids were determined by titrating duplicate 5 ml samples of the effluent from the resin column. The acid solution was first transferred to a 25 ml beaker and evaporated to dryness under a jet of cold air to remove the formic acid. The residue was dissolved in 5 ml distilled water and titrated with 0.01N sodium hydroxide to the bromthymol blue end point.
Mineral Analysis

Leaves were washed in distilled water with 0.1% detergent, rinsed with distilled water twice, and dried at 80°C for 48 hours. They were ground in a Wiley mill to pass a 40 mesh screen, and stored in glass bottles with plastic screw caps.

Analysis for the elements were run by Engineering Experiment Station personnel utilizing atomic absorption spectrophotometer methods. The amount of each element was calculated on a dry weight basis as meq/100 gm dry weight.

Statistical Analysis

When the experiment was planned, the analysis of variance was to be used for statistical analysis of the data. However, when the sampling period was started, it was discovered that there were insufficient leaves in each replication for mineral and organic acid analysis. Thus, it was decided that the leaves from all replications of each treatment would be combined to constitute a sample. Therefore, no analysis of variance could be carried out. Statistical analysis, therefore, involved only correlation coefficients, calculated according to Steel and Torrie (70).
SECTION IV
RESULTS AND DISCUSSION

The results of all treatments are presented in Table 2 and illustrated graphically in Fig. 1-4.

Complete Nutrient Treatment

As shown in Fig. 1, calcium increased as the season advanced. This is in agreement with the findings of other investigators working with apple trees (9,14,23,27,57,68). By September 30, calcium had increased by 91 per cent over the level of June 30. As seen in the graph, the increase in calcium after July 30 was affected by the potassium level in the leaves. Calcium increased when potassium decreased and remained unchanged when potassium increased. Many workers have reported that calcium decreased when potassium increased in leaves of apple trees (3) and other fruit trees (3,20,48, 69).

Potassium usually decreases in apple leaves as the season advances (4,9,14,23,27,57,68). Cain (16), however, using two levels of potassium (0.5 and 4.5 meq/l) in sand culture showed that the potassium content of apple leaves decreased with the low level and increased with the high level as the season advanced. He indicated that the growth and concurrent demand for potassium in the plant was greater than the supply and resulted in a decrease of leaf potassium at the low level of nutrient potassium. In the present study, the level of potassium in the nutrient solution was 6 meq/l. This high level and the reduction of the dilution effect account for the increase in potassium concentration in the
### Table 2. Seasonal Variation of Cations, Malic Acid and Total Acids in Robusta #5 Rootstock.

<table>
<thead>
<tr>
<th>Treatments</th>
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<th>Mg</th>
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<td>155.11</td>
<td>60.28</td>
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Fig. 1. Seasonal variation of malic acid, total acids, Ca, Mg and K in apple leaves with complete nutrient treatment.
leaves as the season advanced. By September 30, potassium had increased by 42 percent over the level of June 30.

Magnesium in apple leaves may increase (9, 14, 23), decrease (68) or fluctuate slightly (4) as the season advances. In the present study, magnesium decreased slightly as the season advanced. By September 30, magnesium had decreased by 6 percent under the level of June 30. The decrease may be the result of the increase of potassium in the leaves. It has been found that an increase in nutrient potassium decreased the magnesium content of plants (15, 58).

Malic acid and total acids increased slowly but steadily as the season advanced. By September 30, malic acid and total acids had increased by 26 and 39 percent, respectively, over the levels of June 30. No significant correlation between cations and malic acid has been found, but there was a significant correlation between total acids and potassium, and total acids and calcium (Table 3). The importance of these correlations will be seen from the results of the other treatments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Malic Acid</th>
<th>Total Acids</th>
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</thead>
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<td></td>
<td>K</td>
<td>Ca</td>
</tr>
<tr>
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<td>- Ca</td>
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<td>0.561</td>
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<tr>
<td>- K</td>
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<td>0.388</td>
</tr>
<tr>
<td>- Mg</td>
<td>0.954***</td>
<td>0.944***</td>
</tr>
</tbody>
</table>

* 5% Significant level  
** 1% Significant level
Minus Calcium Treatment

As seen in Fig. 2, calcium increased slightly until July 30 and then remained at about the same level the rest of the sampling period. It did not decrease as the season advanced, which is an expected response for an element missing from the nutrient solution. Calcium is considered to be a relatively immobile element (10,82). Thus, it seems that the calcium, absorbed before the differential treatments started, accumulated in the leaves and resulted in the slight increase during the early period. It then remained at the same level when no additional calcium absorption occurred. By the end of the season, calcium had increased by 18 percent.

As the season advanced, potassium increased to higher levels than the complete treatment. This increase was likely the result of lower calcium levels than in the complete treatment. By September 30, it had increased by 67 percent over June 30.

Magnesium decreased dramatically as the season advanced due to the increase of potassium (8,15). By the end of the sampling period, it had decreased by 52% over June 30.

Malic acid and total acids levels were about the same as in the complete treatment even though the total amount of calcium was about half of the complete. Also, the total magnesium decreased more than 50 percent as the season advanced. By September 30, malic acid and total acids had increased by 57 and 73 percent respectively over June 30. This increase is more than double the increase in the complete treatment for the same period.
Fig. 2. Seasonal variation of malic acid, total acids, Ca, Mg and K in apple leaves with minus calcium treatment.
There was a highly positive correlation between malic acid and potassium and total acids and potassium at the 5% level of significance (Table 3). There was also a highly significant negative correlation between malic acid and magnesium and total acids and magnesium at the 1% level of significance (Table 3). It is also important to notice that both malic acid and total acids did not decrease even though 33 percent of the nitrogen of the nutrient was in the ammonium form which usually reduces malic acid (18, 44, 81).

Without examining the other treatments, it seems that calcium does not affect the accumulation of malic acid and total acids. It appears likely that the increase in potassium maintains the organic acid level at about that of the complete treatment. The result also suggests contrary to the other findings, that ammonium nitrogen does not affect the level of malic acid and total acids in apple leaves, especially in this type of rootstock.

**Minus Potassium Treatment**

Calcium and magnesium increased as the season advanced while potassium decreased as expected (Fig. 3). By September 30, potassium decreased by 56 percent while calcium and magnesium increased by 151 and 89 percent, respectively, over June 30. Malic acid and total acids were much lower than in the complete treatment even though total calcium and magnesium was higher than in the complete. There were a negative correlations between potassium and malic acid, and potassium and total acids at 5 percent and 1 percent levels of significance respectively (Table 3). However, there was a positive
Fig. 3. Seasonal variation of malic acid, total acids, Ca, Mg and K in apple leaves with minus potassium treatment.
correlation between calcium and total acids at the 1 percent level. No correlation was found between magnesium and malic acid or total acids. It is possible that the decrease in organic acids was due to the ammonium nitrogen (20%) used in the nutrient of this treatment. This is unlikely, however, because in the minus calcium treatment there was a 33 percent ammonium nitrogen, but no effect was observed. Thus, the decrease in both malic and total acids is likely due to the decrease in potassium level. This is another indication of the importance of this element to organic acid accumulation, possibly by controlling either synthesis, utilization, or both. Thus, it is expected that the reduction of the potassium content of the leaves below that required for normal growth would reduce or alter the normal metabolic processes related to organic acid production, but would not stop these processes completely. The leaves in this treatment would be expected to produce and accumulate organic acids at a low rate, due to the potassium deficiency, as long as the leaves were healthy and showed no deficiency symptoms.

Potassium deficiency symptoms usually show first on the lower (older) leaves then gradually move upward as the deficiency increases. The deficiency symptoms in this treatment first appeared in mid August on the lower leaves then showed on the midshoot leaves at the end of August. This would coincide with the maximum level of malic acid which reached a peak at the end of August then decreased thereafter due to the severity of the symptoms and the reduction of the metabolic processes. Total acids increased slightly after
the end of August, then declined by the end of September due also to the severity of the deficiency in the tissue. The slight increase in total acids on September 15 may be due to the increase of citric, fumaric and succinic acids because these acids are reported to appear at the end of the growing season and increase as the season ends (22).

**Minus Magnesium Treatment**

Both calcium and potassium increased at the same general rate as the season advanced while magnesium decreased (Fig. 4). The rate of increase of potassium was greater than in the control treatment. However, the rate of calcium increase was about the same as in the complete, but the total accumulation was less than in the complete. This is probably due to the increase in potassium level. By September 30, potassium and calcium increased by 177 and 76 percent, respectively, over June 30. Magnesium decreased by 70 percent over the same period.

Both malic acid and total acids also increased as the season advanced. Malic acid showed a slow but steady increase as the season advanced, similar to the increase in the complete treatment. The increase, however, was slightly higher then the control after July 30. Total acids showed a similar pattern, but the increase was slightly less than the complete treatment, except during September, where the increase was slightly above that of the complete treatment. By September 30, malic acid and total acids increased by 64 and 70 percent, respectively. There was a high positive
Fig. 4. Seasonal variation of malic acid, total acids, Ca, Mg and K in apple leaves with minus magnesium treatment.
correlation between calcium and both malic acid and total acids at the 1% level of significance. The same relationship existed for potassium. A high negative correlation was found between magnesium and malic and total acids at the 1% level. As with the other treatments, no effect by the ammonium nitrogen form (13%) on organic acid accumulation was found.

Considering the results of the other treatments, it seems that the high positive correlation (acids and calcium) or the high negative correlation (acids and magnesium) was a reflection of the experimental data rather than the actual relationship. In the minus calcium treatment no appreciable change was observed in the levels of malic acid and total acids in comparison with the complete treatment, even though total calcium decreased by about 50 percent of that of the complete. It is also important to notice that in the minus potassium treatment the levels of both malic and total acids decreased appreciably even though calcium and magnesium had increased above that of complete. This shows that the increase of both malic acid and total acids in the minus magnesium treatment was the result of potassium accumulation rather than the decrease or increase of magnesium or calcium respectively.

In order to have a better understanding of the effect of each element on the organic acid accumulation, the number of milliequivalents of acid per 100 milliequivalents of each element in the leaves for all treatments were calculated and presented in Table 4. One should notice that the values, which are underlined, for both malic acid and total acids are
high in each treatment when related to the element lacking from that treatment. This is natural since the amount of acids increased as the season advanced while the deficient element decreased. However, considering the other values, the data clearly show that the values for both malic acid and total acids per 100 milliequivalents of each element were very close to each other for potassium only, in all treatments. Calcium and magnesium had varied values due to the low values in the minus potassium treatment (Table 4). This is further evidence substantiating the important role of potassium in regulating organic acid production.

Table 4. Meq of acids per 100 Meq of elements in leaves.

<table>
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<tr>
<th>Treatments</th>
<th>Malic Acid</th>
<th>Total Acids</th>
</tr>
</thead>
<tbody>
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<td></td>
<td>Ca</td>
<td>K</td>
</tr>
<tr>
<td>Complete</td>
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</tr>
<tr>
<td>- Ca</td>
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</tr>
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<td>- K</td>
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<tr>
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<td>11.6</td>
<td>11.3</td>
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</table>

Ammonium nitrogen has been found to reduce malic acid appreciably (18,44,81), but in the present study, no such effect is evident. However, the increase of potassium in the leaves might balance the dramatic effect of ammonium nitrogen on malic acid, by increasing malic acid production. This concept needs further study.

Effect of each Element on the Plant Buffer System

The pH values of leaf extracts are shown in Table 5. The minus potassium treatment was lower in pH than the other
treatments, even though a high concentration of calcium and magnesium was present in the leaves. The decrease in pH of any system indicates either a high concentration of acid or a decrease in the substance that buffers the system. In the present study, total malic acid and total acids were lower in the minus potassium treatment than in the other treatments (Table 2). Thus, it is clearly evident that potassium is the element involved in the plant buffer system as indicated by other workers (21,35,55). The low pH in the minus potassium treatment of the present study confirmed the findings of other workers with prunes (35), corn (79) and tomatoes (56). The present study also shows that calcium and magnesium have no effect on the plant buffer system, especially in apple leaves, which is contrary to the suggestion of other workers (23). It is interesting to notice that minus calcium and magnesium treatments had a slightly higher average pH than the complete treatments, probably because they contain higher total potassium than the complete treatment.

Table 5. pH of leaf extracts.

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<td>July 30</td>
<td>Aug. 15</td>
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SECTION V

LITERATURE CITED


BIOGRAPHICAL DATA

Name In Full: Abdul Wahid Mohammad Al Hachim

Date of Birth: March 12, 1934

Place of Birth: Samarra, Iraq

Secondary Education:

Agricultural Secondary School, Abu Ghraib, Iraq

Collegiate Institutions Attended: Dates Degree

North Carolina State University 1956-1961 B.S.
Washington State University 1961-1964 M.S.

Positions Held:


