

RELATIONSHIP OF LEAF CALCIUM CONTENT  
TO FIRE BLIGHT ERWINIA AMYLOVORA  
IN SELECTED APPLE CULTIVARS

by

JAMES WILLIAM SISTRUNK

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## INTRODUCTION

Fire blight (Erwinia amylovora (Burr.) Winslow et al.) is well known to horticulturists and plant pathologists. It was the first bacterial plant pathogen to be discovered in the United States (1) and is considered to be one of the most destructive diseases of pomaceous fruits (5).

All known cultivars of apple, pear and quince are susceptible to fire blight in that inoculations of virulent culture into succulent tissue will produce blight (2). However, certain cultivars will resist the rapid advance of the disease into the tissue.

It has been observed by several investigators (5, 15, 18, 24, 29) that a relationship between tree vigor and fire blight susceptibility does exist. Lewis and Kenworthy (15), working with eleven elements at both high and low levels in quartz sand cultures, found that Bartlett pears showed a marked low susceptibility to fire blight when grown with a high supply of available calcium.

With this in mind, the writer set out to see if a correlation could be established between calcium content and natural resistance to fire blight in various apple cultivars. The possibility that rootstock-scion combinations might play a role in differential absorption of calcium was also considered. It has been suggested that if various apple cultivars do have "critical" nutrient levels these levels might vary with rootstock (30), and that clearcut differences in mineral composi-

tion parallel rootstock effect (22).

A general review of the voluminous literature on fire blight seems unwarranted. Such discussions of previous work which appear necessary and applicable will be found in the body of this paper under appropriate headings.

#### REVIEW OF LITERATURE

Calcium as a Factor in Fire Blight Resistance. The mode of action and the migration of the fire blight organism has been the subject of study for some time. It is generally agreed that the bacteria invade and migrate intercellularly (1, 3). It was believed that this migration and the subsequent cell destruction was due primarily to mass action and osmotic pressure (3). The opinion that a toxic product may have been secreted by the fire blight pathogen which then killed the cell was also entertained (28). It now is thought that the bacteria may secrete an adaptive enzyme or enzymes (pectases) which attack the pectic substances in the cell wall and cause their disintegration (9, 18, 20). The enzymes pectinmethylesterase (PME) and pectic depolymerase have been found to be secreted by a fungus disease of tomatoes, Fusarium oxysporum (7), and it seems possible that the fire blight organism, Erwinia amylovorus, could possess a similar ability (18). That the fire blight bacteria attacks the middle lamella, separating the cells into groups or individuals and eventually causing plasmolysis and death, is generally accepted (18).

Young tender tissue is more susceptible to fire blight

than older tissue even a few inches away (18, 20, 24). Pectin is present, but the middle lamella in the young meristematic areas is never of calcium pectate (17). As the cells attain maturity insoluble pectates are formed; commonly these are calcium pectates, which are considered the bulk of the middle lamella (12, 17). These older areas have been found to be more or less resistant, if not to the disease itself, at least to the advance and migration of the bacteria (18).

If calcium is a main constituent of the middle lamella and of the cell wall, then the supply of available calcium is important to tissue maturity and strength. Young tissue and growing organs must have a continuous abundant supply of soluble (mobile) calcium, which seemingly must be supplied from an external source. Calcium, unlike other nutrients, remains largely insoluble and unavailable to the young plant parts (4). Calcium pectate, which is the main constituent of the middle lamella, is maintained only when a sufficient quantity of calcium ions are present in the external medium and with it the normal retention of the contents in the cell. The calcium released from the middle lamella by enzymes is precipitated and removed as a source of soluble calcium (7). When the quantity of calcium ions falls below an equilibrium concentration, according to the laws of mass action, other cations replace calcium in the middle lamella. These cations could be polyvalent, such as magnesium ions, or could be monovalent, such as potassium ions (17). In the latter case the middle lamella would likely disintegrate. Accordingly, it seems likely the

more calcium ions available, the more pectate bonds are formed, making it difficult for enzymes to destroy the middle lamella.

There are two other possible functions of calcium in relation to fire blight resistance.

The fire blight bacteria need moisture to live and this moisture is derived partly from the cell sap (3) and partly from the intercellular atmosphere (18). In gooseberry, a deficiency of calcium resulted in a higher leaf water content (17). A higher leaf water content would be advantageous to the fire blight organism. If tissue can be kept from breaking down and releasing cell sap because of strong walls, and if intercellular moisture can be held down through an adequate supply of mobile calcium, then it would seem that the bacteria would have a difficult time in becoming established and in migrating.

The second function is also tied to the moisture requirement of the bacteria to some degree. The middle lamella is a part of the cork tissue which makes up phellem and phelloderm. Plants have the ability to lay down a phellogen layer in practically any part of the plant. This phellogen layer is put down in the layers of uninjured living parenchymatous tissue adjacent to a wound (8). The barrier prevents water from the healthy tissue from getting into the infected areas, while protecting this healthy tissue from the blight. The phellogen layer may eventually surround the bacteria and cause its death due to a lack of living tissue upon which to grow (18). The fire blight bacteria will not live in dead tissue (5).

Douglass pear, a fire blight resistant cultivar, has been found to be susceptible, but the tendency for the tissue to harden rapidly soon after it is formed impedes the blight and injury is less severe (28).

From the above discussion, it would appear that the hardening of plant tissue requires available forms of calcium and the calcium content of plants seems to be a factor in fire blight infection and migration.

Fire Blight Resistance in Apple Cultivars. In reviewing the literature the writer found a variety of descriptions for the degree of susceptibility of apple cultivars to fire blight. Literature sources seemed generally to concur that the Winesap cultivar was comparatively resistant (5, 23, 26, 27), but that Jonathan was "rather susceptible" (2), "moderately susceptible" (5), "very susceptible" (26), and "susceptible" (23). In turn, the Rome cultivar was rated as "slightly susceptible" (23) and "susceptible" (26). These somewhat confusing ratings do agree, at least, that Rome and Jonathan are susceptible.

#### MATERIALS AND METHODS

Location. The Doniphan Experimental Orchard, Doniphan County, Kansas, was the site of the orchard experiment. All trees used were planted in 1944 and were located on Knox silt loam (12) which was found to have a pH ranging from 6.0 in the first foot to 6.3 in the second foot.



Trial Selections. Three apple cultivars were chosen: Winesap, as a "resistant" cultivar, and Rome and Jonathan as "susceptible" cultivars. Two rootstocks, French Crab and Hibernial, were chosen for each cultivar.

Treatments. In addition to the naturally occurring calcium content of the various cultivar-rootstock-scion combinations, applications of calcium-containing materials were made to determine their effect on the calcium content of the trees.

Three treatments were administered, as follows:

1. Soil Liming. Applications were made May 2, 1964, to a 12 by 12 foot square beneath the trees. The equivalent of two and a half tons per acre of hydrated lime was broadcast directly on the surface of the soil. The lime was used as a nutrient source and not as a soil conditioner (4).
2. Calcium Nitrate. Three foliar applications were made to the same trees at the rate of six pounds per 100 gallons of water or the equivalent to a total of .378 pounds of actual calcium per tree. The spray mixture was applied at 10 gallons per tree. These applications were made at approximately two week intervals, June 6, June 22 and July 4, 1964.
3. Calcium Acetate. One application was made June 6, 1964, as a foliar spray at the rate of 8.33 pounds per 100 gallons of water or the equivalent to a total of .0021 pounds of actual calcium per tree.

Four separate test trees were used for each treatment and



check. Each of the four treatments was replicated four times for the six rootstock-scion combinations, making a total of 96 individual trees. The trials were randomized so far as was possible under the planting plan existing in the orchard.

Each cultivar-rootstock-scion combination was color coded for the individual treatments. Small colored plastic labels were attached to the tree trunks for easy recognition: blue for the check, green for the liming, red for calcium nitrate and yellow for calcium acetate treatments.

Plant Materials and Sampling. Leaf tissue was used for the calcium determinations and samples were taken in the following manner, with modification, as suggested by Smith (25).

Fifty leaves per tree were taken at random from the approximate middles of non-fruiting shoots distributed around the outside of the tree. Leaves higher than six feet from the ground level were not sampled. An effort was made to see that the leaves were the same general size and maturity. Samples were taken June 3, July 1, August 1 and September 1, 1964.

Sample Preparation. The leaves which had received foliar treatments were washed as described by Hammer (10) and Mason (14), with modification as indicated below.

Leaves were placed loosely into a heavy-gauge wire (hardware cloth) basket. The sample was then agitated vigorously for approximately 30 seconds in a one percent hydrochloric acid (by volume) solution, rinsed once in warm tap water, twice more in distilled water and then allowed to dry in the open air. Leaves were not immersed in the solution or water

for more than a total of one minute to lessen the chance of leaching soluble calcium.

Extraction and Analyses. A modified dry ashing procedure (10, 13, 19) was used to determine the calcium content of the leaf samples:

1. Samples were dried at  $80^{\circ}$  C for 36 hours. Twenty-four hours would be sufficient at this temperature, but the 36-hour time length fit into the schedule of analyses.
2. Samples were ground in a Wiley mill through a 40-mesh screen into airtight glass bottles.
3. One gram samples were weighed out and put into numbered 50 ml beakers.
4. Two milliliters of five percent sulfuric acid in ethyl alcohol (50 ml concentrated sulfuric acid added to 950 ml 95 percent ethanol) was added to the plant material to prevent the material from sticking to the beaker when heated.
5. The excess alcohol vapors were burned off and the beakers were placed in a cool muffle furnace. The temperature was slowly increased to  $525^{\circ}$  C and maintained for six hours to insure that all the carbon was burned off.
6. The ash was removed and allowed to cool.
7. Ten milliliters of three normal (3N) hydrochloric acid were added to the ash.
8. The acidified ash was warmed on a hot plate until all

soluble salts were in solutions (silica will not go into solution).

9. The solution was then filtered through #2 Whatman filter paper into 100 ml volumetric flasks. The beaker and filter paper were then washed three or four times with hot distilled water. It is generally considered unnecessary to remove silica from the ash except by filtration prior to analysis for potassium, sodium, magnesium or calcium.
10. Samples were allowed to cool and then brought to volume (100 ml) with distilled water, leaving the final extract with a .3N HCl acidity.

A Beckman flame spectrophotometer, model DU, was used to determine the calcium content of the extracts.

The extracts were read at random at a wave length setting of 556 mu and compared with the readings from a .3N HCl standard solution of known concentration in parts per million (ppm). The four replicated samples were then averaged and this average was used in the final statistical analyses.

## RESULTS

Statistical Analyses. All possible combinations of main effects and interactions were evaluated by the F-test to find which were significant. Of these interactions only two, cultivar by month (C x M) and rootstock by treatment (R x T), were significantly different (Table 1).

Table 1. Table of variance for possible combinations in the determination of calcium content.

Source of Variation	Degrees of Freedom	Mean Square	F
Cultivars (C)	2	4905.70	86.89***
Rootstocks (R)	1	8673.50	153.63***
Treatments (T)	3	314.03	5.56**
Months (M)	3	24851.28	625.28***
C x R	2	153.38	2.72
C x T	6	108.96	1.93
C x M	6	2807.35	70.71***
R x T	3	273.29	4.84**
R x M	3	69.11	1.74
T x M	9	34.05	.86
C x R x T	6	25.35	.45
C x R x M	6	35.06	.88
C x T x M	18	38.79	.98
R x T x M	9	31.38	.79
C x R x T x M	18	30.48	.77
Trees: C x R x T	72	56.46	1.42*
M x Trees: C x R x T	216	39.70	
Total	383		

\* - Significant @ 5%  
 \*\* - " @ 1%  
 \*\*\* - " @ .1%

The significance for "Trees: C x R x T" in Table 1 shows only the differences within individual tree classes, that is, the four replicated trees receiving the same treatment, and is of little value in the evaluation of results.

Since all the main effects were involved in significant interaction (that is, the main effects must be evaluated by considering a second factor), their means were not tabled and evaluated for significance. Their over-all significance is illustrated by an F-test, however, indicating some consistent

effects when averaged over the remaining three factors.

Differences in Calcium Content by the Month by Cultivar.

The differences in calcium content presented in this portion of the study were not concerned with treatment or rootstock variations, but only with the particular cultivars by the month (Table 2, Fig. 1). All trees, including check trees, were sampled.

June. All differences were significant, as can be seen in Table 2. The Winesap cultivar leaves showed the highest calcium content with 62.1 ppm; Jonathan leaves were intermediate with 52.6 ppm, and the leaves from the Rome cultivars were the lowest with 35.1 ppm.

July. A sharp change in order of concentration occurred in July. The Jonathan cultivar leaves were highest in calcium content with 71.7 ppm. Rome was next with 68.7 ppm and Winesap was the lowest with 59.3 ppm. The Rome and Jonathan cultivar leaves showed a significantly higher calcium content than did the Winesap. There were no significant differences in calcium content between the Jonathan and Rome cultivar leaves.

August. Jonathan leaf tissue was highest in calcium content with 81.1 ppm, Winesap intermediate with 71.5 ppm, and the Rome cultivar leaves were the lowest with 69.3 ppm. The Jonathan cultivar leaves showed a significantly higher calcium content than did the Winesap or Rome leaves. There were no significant differences in calcium content between the Winesap and Rome cultivar leaves.

September. All differences were significant. The Jonathan cultivar leaves were comparatively high at 101.7 ppm. Rome was next with 86.7 ppm and the Winesap cultivar leaves were lowest with 77.5 ppm.

Table 2. Calcium content by the month by cultivar.

Cultivar	PPM			
	June	July	August	September
Winesap	62.1	59.3	71.5	77.5
Rome	35.1	68.7	69.3	86.7
Jonathan	52.6	71.7	81.1	101.7

L.S.D @ 5% : 3.087

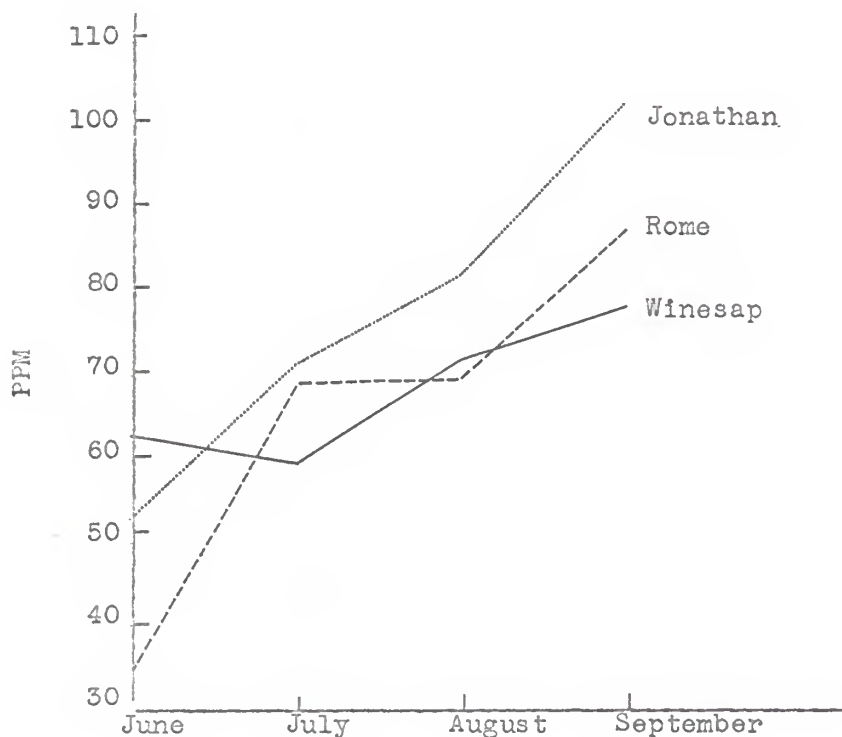


Fig. 1. A graphic representation of Table 2.

Differences in Calcium Content Within the Same Cultivar:  
Treatment by the Month. The differences in calcium content reported here reflect the treatments to the cultivars by the month regardless of rootstock effect.

The treated trees of each cultivar were compared with the check trees of that cultivar. There were no significant differences observed in this trial according to the F-test (Table 1), but the mean comparisons are included here as a matter of interest (Table 3). Essentially, these comparisons show the same thing as Table 2, except the calcium content of each cultivar is broken down by treatment.

Table 3. Differences in Calcium Content Within the Same Cultivar: Treatment by the Month.

Month	Cultivar	PPM			
		Check	Liming	Ca Nitrate	Ca Acetate
June	Winesap	59.625	60.750	66.500#	61.375#
	Rome	36.625	35.000	31.750#	37.125#
	Jonathan	49.625	57.000	48.750#	55.000#
July	Winesap	55.750	58.500	63.375	59.625
	Rome	65.125	67.500	69.750	72.375
	Jonathan	68.625	71.750	71.375	75.000
August	Winesap	69.125	72.000	75.375	69.375
	Rome	66.250	68.375	70.750	71.875
	Jonathan	79.750	78.750	81.375	83.500
Sept.	Winesap	72.250	80.500	80.125	77.000
	Rome	84.875	84.250	87.250	90.500
	Jonathan	99.625	98.750	105.125	102.875

# Untreated at this sampling  
F-test shows interaction to be non-significant



There were no foliar treatments made prior to June 6, but samples were taken June 3 from all the trees to compare any differences that might show up within the various replicated tree groupings that were to be foliarly treated later.

At the rates used in these trials, no phytotoxicity was observed in any of the trees as a result of the foliar applications.

Differences in Calcium Content by Rootstock. Trees on Hibernial rootstocks contained significantly more calcium than did those on French crab in all cases regardless of treatment, cultivar or month sampled (Tables 4, 5 and 6, Fig. 2). The Hibernial rootstocks responded to all treatments. More calcium was absorbed by the treated trees than by the check trees on the Hibernial rootstocks, regardless of cultivar.

The cultivars on French Crab rootstocks did not significantly respond to any of the treatments when compared to the check trees.

Table 4. Calcium content by rootstock by treatment.

Rootstock	PPM			
	Check	Liming	Ca Nitrate	Ca Acetate
Hibernial	70.0	75.6	75.1	77.3
French Crab	64.7	63.2	66.9	65.3

L.S.D. @ 5% : 3.061

Table 5. Calcium content by rootstock by the month.

Rootstock	PPM				Mean
	June	July	Aug.	Sept.	
Hibernal	53.44	71.59	79.00	94.04	74.52
French Crab	46.46	61.56	68.76	83.24	65.01

F-test for rootstocks has one degree of freedom; therefore over-all rootstock means are significantly different.

Table 6. Calcium content by rootstock by cultivar.

Rootstock	PPM			Mean
	Winesap	Rome	Jonathan	
Hibernal	71.66	69.12	82.76	74.52
French Crab	63.50	60.80	70.74	65.01

F-test for rootstocks has one degree of freedom; therefore over-all rootstock means are significantly different.

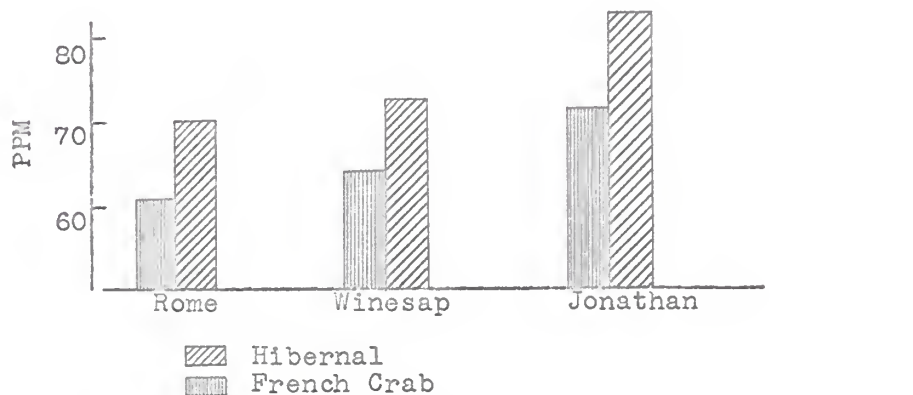


Fig. 2. A graphic representation of Table 6.

It can be seen from Tables 5 and 6 that the differences in calcium content of the leaves between the cultivars on Hibernial and French Crab rootstocks are approximately 10 ppm, both by the month and by cultivar.

Relationships Between Rootstock, Tree Size, Calcium Content and Yield. The mean average relationships of earlier work reported on tree size and yields over a ten-year period can be compared to the calcium content found in the leaves of the various cultivar-rootstock combinations in this experiment (Table 7).

Table 7. Relationships between rootstock, tree size, calcium content and yield.

Cultivar/ Rootstock	Trunk Circumference# inches	Total# Yield# pounds	Total Calcium ppm	June Calcium ppm
Winesap/ French Crab	31.92	1,654	63.50	58.69
Winesap/ Hibernial	26.65	2,200	71.66	65.44
Rome/ French Crab	29.70	1,387	60.80	32.31
Rome/ Hibernial	24.45	1,200	69.12	37.94
Jonathan/ French Crab	32.27	1,417	70.74	48.25
<u>Jonathan/ Hibernial</u>	<u>29.70</u>	<u>1,755</u>	<u>82.76</u>	<u>56.94</u>
Mean Average/ Rootstock				
French Crab	31.30	1,486	65.01	46.42
Hibernial	26.87	1,718	74.52	53.44

# Filingier, G. A. and Erwin Abmeyer. "The Doniphan Apple Orchard," Kansas Agr. Expt. Sta. Bul. 436. May, 1961.

## DISCUSSION

Differences in Calcium Content by the Month by Cultivar.

Calcium content varies greatly with species and with environmental conditions (17). In considering the calcium content of various apple cultivars in relation to fire blight resistance, the earlier (June) concentrations would seem to be the most important. Primary infection of the tissue by the fire blight organism takes place in late winter and early spring, and most of the migration occurs in the spring. A greater part of the bacteria die in the summer (2), so the major fire blight damage to the tissue occurs early, although it may not show up immediately. The calcium content in July, August and September is of interest in comparing cultivars, but would seem to have little influence on the migration of fire blight because there are comparatively few fire blight organisms present during the summer to migrate.

The calcium content in June (Table 2) was found to be highest in the Winesap cultivar. According to the fire blight resistance rating used in this experiment, this would indicate that high early calcium content does exist in the cultivar considered to be the most resistant to the fire blight organism. Continued investigations in successive years would be necessary to establish this more positively.

Better data could be obtained to show spring calcium content if the sampling were done earlier, in April and May, and this should be considered in future work of this nature.

The Winesap cultivar showed comparatively little increase in calcium content as the season progressed, when compared to Rome and Jonathan (Table 2). This may have been due to the elapsed time from bloom to picking maturity. Winesap requires approximately 160 days to mature, Rome 155 days, and Jonathan approximately 140 days (6). Calcium content in the leaves would be expected to increase sharply as the fruit approaches maturity (17, 25). This might explain the differing concentrations in calcium content as the season progressed.

It was stated earlier that it is the soluble (mobile) calcium that is required for the proper formation of the middle lamella. In these experiments only total calcium content was considered, but what percent of that total calcium content is soluble and what percent is insoluble? Do the various cultivars have differing ratios of soluble and insoluble calcium? What percentage of the total insoluble calcium content is in a pectate form in the middle lamella and what percentage is in other insoluble forms, such as calcium oxalate? If calcium in the form of insoluble calcium pectate in the middle lamella is a factor in fire blight resistance, then the answers to these questions would seem to be important.

The possibility of any calcium carry-over from year to year or what the variations of such a carry-over might be have not been investigated, but such an investigation may shed some light on what early spring calcium content might be in the various cultivars.

Differences in Calcium Content Within the Same Cultivar:  
Treatment by the Month. No changes of significance were noted in this phase of the experiment, but further investigations may show some differences.

The lime may not have had time to be properly leached into the root-zone. An increase in leaf calcium content may show up the year succeeding the treatment, after the calcium in the lime has had time to be carried into this zone and absorbed by the roots.

While non-significant in interaction when compared to the check trees, calcium from the calcium nitrate treatments did appear to be taken up to a greater degree than any of the other treatments applied to the same cultivar. This effect may be seen mostly in the Winesap cultivar trials (Table 3). In this experiment only one rate was applied, however, and work with varying concentrations of calcium nitrate would be desirable to determine their effect on leaf calcium content.

The applications of calcium acetate were much too low to tell anything definite about its ability to introduce calcium into the tree. If the nitrogen in the calcium nitrate were to stimulate growth to the point that the young succulent tissue would be more susceptible to fire blight, it would be desirable to have a calcium source without nitrogen. Calcium acetate might easily fill this role. It is quite soluble and appears to have no phytotoxicity, although work with greater concentrations is necessary before definite evaluations on phytotoxicity can be made.

Differences in Calcium Content by Rootstock. The leaves from cultivars grown on the two rootstocks showed consistent significant differences in calcium content. Without exception, leaves from cultivars grown on the Hibernial rootstocks showed higher levels of calcium than did those on French Crab stocks, regardless of cultivar, treatment, or month sampled (Tables 4, 5 and 6). The cultivars on Hibernial rootstocks showed a consistent leaf calcium increase of nearly 10 ppm over the cultivars on French Crab (Table 6).

The cultivars on Hibernial responded to all treatments, while those on French Crab responded to none. It appears that the Hibernial rootstocks affected the vegetative portions of the tree in such a way that they absorbed more calcium from external treatments than did those on French Crab, under the conditions of this experiment.

Whether a particular cultivar on a Hibernial rootstock shows a greater resistance to fire blight than the same cultivar on a French Crab has not been studied, as far as this writer can determine. There seems to be little work done on rootstock-cultivar relationships where fire blight resistance is concerned. There is little doubt that the rootstock (at least those studied here) exerts a great influence on the calcium content of the vegetative portion of the tree, and this is probably true for other nutrients as well.

If the nutrient (regardless of which nutrient) content of a cultivar has the effect on disease resistance that seems likely, then the kind of rootstock the cultivar is on also



affects the disease resistance. The effect of rootstocks surely reaches into many other aspects of fruit culture and orchard management as well. The rootstock-scion relationships would seem to offer the greatest potential for further study, of the trials run in this study, whether or not those studies are concerned with fire blight resistance.

Relationships Between Rootstock, Tree Size, Calcium Content and Yield. These observations may not have a direct relationship to fire blight resistance, but do offer some interesting comparisons (Table 6).

The cultivars on Hibernial rootstocks are all smaller in trunk circumference than those on French Crab, have the highest calcium content and, with the exception of the Rome cultivar, produced greater yields. The Winesap cultivar, considered the most resistant to fire blight, had the highest early (June) calcium content and the heaviest yield when compared to the other two cultivars on Hibernial. The mean averages of the various comparisons, in Table 7, show some definite differences by rootstock. The meaning of these relationships is not clear.

#### SUMMARY

Leaf tissue from selected apple cultivars was analyzed for calcium content via dry ashing and flame spectrophotometric techniques to see if a relationship could be found between known natural resistance to fire blight by these cultivars and their calcium content. The cultivars used were Winesap (resistant), and Rome and Jonathan (susceptible).

The effect of two different rootstocks -- Hibernial and French Crab -- on leaf calcium content was also investigated.

In addition to determining the natural occurring leaf calcium, three treatments were applied to observe the effect, if any, that they might have on the total leaf calcium content. These supplemental treatments were lime applied to the soil and calcium nitrate and calcium acetate applied as aqueous sprays to the foliage.

Leaf samples were analyzed for total calcium content June 3, July 1, August 1 and September 1, 1964.

Under the conditions in this experiment, the results may be summarized as follows:

1. The Winesap cultivar, rated the most resistant to fire blight of the cultivars studied in this experiment, had a higher early (June) calcium content with leaves than did either Jonathan or Rome, both of which are considered to be susceptible to the bacterial disease.
2. The calcium content of the leaves of Winesap, Rome and Jonathan varied from cultivar to cultivar at any given time of sampling and analysis.
3. It is possible to introduce calcium into apple cultivars via foliar applications of calcium nitrate.
4. Leaves of the three cultivars, Winesap, Rome and Jonathan, showed a higher calcium content on Hibernial rootstocks than they did on French Crab rootstocks.

5. The leaves of all cultivars on Hibernial rootstocks responded to all treatments as indicated by a higher leaf calcium content when compared to the check trees, where the same cultivars on French Crab rootstocks did not. Hibernial rootstocks seemed to impart some factor or condition to the vegetative portions of the tree which enabled them to absorb calcium from external treatments, whereas French Crab rootstocks did not with the cultivars used in this experiment.

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## LITERATURE CITED

1. Alexopoulos, Constantine John.  
Introductory mycology. New York: John Wiley and Sons,  
1960.
2. Anderson, Harry Warren.  
Diseases of fruit crops. New York: McGraw-Hill, 1956.
3. Bachmann, Freda M.  
The migration of Bacillus amylovorus in the host tissues. *Phytopath.* 3:3-13. 1913.
4. Blake, M. A., G. T. Nightingale and O. W. Davidson.  
Nutrition of apple trees. N. J. Agr. Expt. Sta. Bul.  
626. 1937.
5. Brooks, A. N.  
Studies of the epidemiology and control of fire blight  
of apples. *Phytopath.* 16:665-696. 1926.
6. Childers, Norman F.  
Modern fruit science. 2nd ed. New Brunswick: Horti-  
culture Publications, 1961.
7. Deese, Dawson C. and Mark A. Stahmann.  
Pectic enzyme formation by Fusarium oxysporum on  
susceptible and resistant tomato stems. *Jour. Agri. and  
Food Chem.* 10:145-150. Mar.-Apr. 1962.
8. Eames, Arthur J. and Laurence H. MacDaniels.  
An introduction to plant physiology. New York: McGraw-  
Hill, 1925.
9. Fulton, J. P., D. A. Slack, N. D. Fulton and H. J. Walters.  
Plant pathology laboratory manual. Minneapolis:  
Burgess Publishing, 1956.
10. Hagler, T. B.  
Advances in horticulture, lab ex. 4. Clemson: Dept.  
of Hort., Clemson College. 1958.
11. Hammer, Harold E.  
Effect of spray residues and other contaminants on leaf  
analysis. *Plant Physiol.* 31:256-257. 1956.
12. Hayward, Herman E.  
The structure of economic plants. New York: Macmillan,  
1948.

13. Johnson, Clarence M. and Albert Ulrich.  
Analytical methods for use in plant analysis. Calif.  
Agri. Expt. Sta. Bul. 766. 1959.
14. Khobel, E. W., R. H. Davis and H. W. Higbee.  
U.S.D.A. soil survey of Doniphan county, Kansas. No. 25,  
1927.
15. Lewis, L. N. and A. L. Kenworthy.  
Nutritional balance as related to leaf composition and  
fire blight susceptibility in the Bartlett pear. Proc.  
Amer. Soc. Hort. Sci. 81:108-115. 1962.
16. Mason, A. C.  
The cleaning of leaves prior to analysis. Ann. Report  
East Malling Research Sta. (England) 1951-52:105-108.  
1953.
17. Miller, Edwin C.  
Plant physiology. 2nd ed. New York: McGraw-Hill, 1938.
18. Miller, P. W.  
Studies of fire blight of apples in Wisconsin. Jour.  
Agr. Research. 39:579-621. 1929.
19. Nightingale, A. A.  
Some chemical constituents of apples associated with  
susceptibility to fire blight. N.J. Agr. Expt. Sta.  
Bul. 613. 1936.
20. Nixon, E. L.  
The migration of Bacillus amylovorus in apple tissue  
and its effect on the host cells. Penn. Agr. Expt.  
Sta. Bul. 212. 1927.
21. Piper, C. S.  
Soil and plant analysis. Adelaide (Australia): Uni-  
versity of Adelaide, 1942.
22. Roach, W. A.  
Biochemistry. Ann. Report East Malling Research Sta.  
(England) 1954-55:36. 1955.
23. Selby, A. D.  
Disease susceptibility of apple varieties in Ohio.  
Ohio Agr. Expt. Sta. Cir. 133. 1913.
24. Shaw, L.  
Studies on resistance of apple and other rosaceous  
plants to fire blight. Jour. Agr. Research. 49:283-313.  
1934.

25. Smith, Paul.  
Mineral analysis of plant tissues. *Ann. Rev. Pl. Phys.*  
13:81-108. 1962.
26. Smock, R. M. and A. M. Neubert.  
Apples and apple products. New York: Interscience Inc.,  
1950.
27. Stevenson, F. F. and Harry A. Jones.  
Some sources of resistance in crop plants. Washington,  
D. C.: Yearbook of Agriculture, 1953. p. 201.
28. Stewart, V. B.  
The fire blight disease in nursery stock. N. Y. Cornell  
Agr. Expt. Sta. Bul. 329. 1913.
29. Thomas, H. E.  
Some factors affecting the susceptibility of plants  
to fire blight. *Hilgardia*. 12:301-322. 1939.
30. Whitfield, A. B.  
The effects of stock and scion on the mineral compo-  
sition of apple leaves. *Ann. Report East Malling Re-  
search Sta. (England)* 1963:107-110. 1964.



RELATIONSHIP OF LEAF CALCIUM CONTENT  
TO FIRE BLIGHT ERWINIA AMYLOVORA  
IN SELECTED APPLE CULTIVARS

by

JAMES WILLIAM SISTRUNK

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AN ABSTRACT OF A MASTER'S THESIS

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A relationship between the tree vigor of apple cultivars and fire blight (Erwinia amylovora (Burr.) Winslow et al) susceptibility has been observed by many investigators. Lewis and Kenworthy (1962) reported a marked low susceptibility to fire blight by Bartlett pears when grown in sand cultures with a high supply of available calcium.

The purpose of this study was to see if a correlation could be established between calcium content and known natural resistance to fire blight in various selected apple cultivars.

Three cultivars were selected: Winesap as resistant and Rome and Jonathan as susceptible.

The effect of two different rootstocks -- Hibernial and French Crab -- on leaf calcium content was also investigated.

In addition to determining the natural occurring leaf calcium in these rootstock-cultivar combinations, three experimental treatments were also applied to observe the effect, if any, on total leaf calcium content. These supplemental treatments were lime applied to the soil and calcium nitrate and calcium acetate applied as aqueous sprays to the foliage.

Leaf samples receiving foliar sprays were washed for approximately 30 seconds in one percent hydrochloric acid solution, rinsed in distilled water and allowed to air dry. Leaves were oven-dried at 80° C, ground and weighed out into 1 gram samples. These samples were dry ashed at 525° C in a muffle furnace and the soluble salts were extracted with 3N HCl. The samples were brought to 100 ml with distilled water and read in a Beckman flame spectrophotometer, model DU, at 556 mu. The readings were

compared with a standard solution of known concentration in ppm and the average of four replicated sample readings were used in the final analyses.

Leaf samples from these various cultivar-rootstock-treatment combinations were taken June 3, July 1, August 1, and September 1, 1964.

Under the conditions in this experiment, the results may be summarized as follows:

1. The Winesap cultivar rated the most resistant to fire blight of the cultivars studied in this experiment, had a higher early (June) calcium content than did either Jonathan or Rome, both of which are considered to be susceptible to the bacterial disease. By September the Jonathan cultivar leaves showed the highest calcium content; Rome was next, and the Winesap cultivar showed the least.
2. The calcium content of the leaves of Winesap, Rome and Jonathan varied from cultivar to cultivar at any given time of sampling and analysis.
3. It is possible to introduce calcium into apple cultivars via foliar applications of calcium nitrate.
4. Leaves of the three cultivars, Winesap, Rome and Jonathan, showed a higher calcium content on Hibernial rootstocks than they did on French Crab rootstocks.
5. The leaves of all cultivars on Hibernial rootstocks responded to all treatments, as indicated by a higher leaf calcium content when compared to the check trees,

where the same cultivars on French Crab rootstocks did not. Hibernial rootstocks seemed to impart some factor or condition to the vegetative portions of the tree which enabled them to absorb calcium from external treatments. This was not true for the French Crab rootstocks combined with the various cultivars used in this experiment.