NON - VOLATILE ACIDS OF RED TART CHERRIES

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ABSTRACT

NON-VOLATILE ACIDS OF RED TART CHERRIES

By Seshumani Krishna Das

The non-volatile organic acids of red tart cherries, P.cerasus were identified and quantitatively determined in 1960, 1961 and 1962. The effect of degree of maturity and spray materials on the total titratable acidity and on the individual acids was also studied.

Acidified water extraction, lead precipitation, gradient elution column chromatography, paper chromatography and titration were used to identify and quantitatively determine the acids.

The acids identified in these cherries are: aspartic, chlorogenic, citramalic, citric, fumaric, galacturonic, glyceric, glycolic, glucuronic, glutamic, glutaric, isochlorogenic, lactic, malic, malonic, neochlorogenic, phosphoric, quinic, shikimic, succinic, and tartaric. Malic acid represented 75 to 95 percent of the total titratable acidity.

In 1961-1962, the total titratable acidity showed a marked decline as the fruit matured with a plateau at the time of the commercial harvest. In 1960, there was only a slight decline in acidity during maturation.

The changes in concentration of all acids, except for phosphoric, succinic, and the uronic acids, during fruit

maturation were similar in all three years for the fixed copper spray. The fungicidal sprays used in 1960 had no effect on the changes in individual acids during fruit maturation.

In 1961 and 1962 the Cyprex spray treatment had the same effect on the individual acids as the fixed Copper spray. Ferbam and glyodin, however, affected citric, malic and the uronic acids differently than copper and cyprex. There was a dip in the malic acid content in almost all treatments in 1960 and in all treatments in 1961 and 1962, either at the time of commercial harvest or a week prior to it. There is a possibility that this dip may be due to a malate-decarboxylation reaction.

NON-VOLATILE ACIDS OF RED TART CHERRIES

By

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INTRODUCTION

Organic acids are of great significance in plant and animal metabolism. In plants, these acids are early products of photosynthesis and thus serve as precursors for the synthesis of other compounds. Some acids also arise as products of degradation of certain chemical compounds in plants. The close metabolic relationship of organic acids to fats, carbohydrates, and proteins emphasizes their key role in plants. In animals it has been shown that the tricarboxylic acid cycle releases energy and interrelates fat, carbohydrate, and protein metabolism. A number of workers have shown the functioning of Kreb's cycle in certain seedlings (14), but the presence and importance of the cycle in mature plant tissues remains doubtful.

Early investigations have been primarily concerned with the organic acids present in significant amounts and the acids in minute quantities were only determined in case of special interest. However, the acids present in very small amounts, either free or in combined state, may play an important part in the overall metabolism of the tissues and, therefore, it would seem desirable to investigate the nature and amounts of the minor acids.

Academically this study would give us the knowledge of the acids present in the fruit tissue. From the practical

point of view, since it is known that acids participate actively in fruit metabolism, we may be able to determine their possible role in non-enzymatic browning reactions, other type of discolorations and flavor, and on the basis of this knowledge, suggest modifications in the handling or processing procedures which might minimize these undesirable changes.

The object of this study was to identify and quantitatively determine the non-volatile acids of red tart cherries (Prunus cerasus, Var. Montmorency) and to determine the effect of degree of maturity and fungicidal sprays on the total acidity and individual acids present.

This research was conducted on Montmorency cherries, not only because Michigan is the number one state in the production of this variety of cherries, but also because it is the only variety that is produced in substantial quantities in all regions that grow red tart cherries. The Montmorency variety is favored because of the excellent flavor, the color and size of the fruit, the long harvest season, and also because it is a dependable producer of good crops (82).

REVIEW OF LITERATURE

A. ACIDS OF RED TART CHERRIES

The acidity of red tart cherries has generally been reported either as total titratable acidity or as malic acid. The first known study on cherry acids was made by Keim in 1891 (69), who identified oxalic and succinic acids. In 1901, Desmouliere (24) detected salicylic acid in the fruit, and other workers (24, 25, 39, 64, 117) also reported the presence of salicylic acid; but, since 1905, no one has reported its presence. Truchon and Martin-Claude (118) found tartaric acid in the juice of cherries, and other workers (18, 86, 88, 89, 90) reported its presence in fruit tissues. Jorgensen, in 1907, (66) found succinic acid and Arbenz, in 1917)(2) determined the presence of oxalic acid. Citric (31), malic (9, 32), pyrrolidone carboxylic (79), and phosphoric (124) acids have been reported in the cherry fruit, glycolic acid in the peduncle (5) and lactic acid (85) in cherry juice. Kohman (70) lists the oxalic acid content of red tart cherries at 1.1 mg./100 gm of edible tissue. Bridges (11) reports the acid content of cherries (variety not specified) as follows: malic - 0.56 to 1.99%, citric - 0 to 0.01%, succinic - 0.07%, lactic - 0.13%, oxalic, trace.

B. NON-VOLATILE ACIDS IN PLANT METABOLISM

Terminal respiration in higher plants involves the oxidation of pyruvic acid to CO_2 and water with certain organic acids acting as intermediates in a series of reactions most commonly known as the Krebs or Citric Acid Cycle (72). In 1950, in a review of the metabolism of organic acids in plants, Thimann and Bonner (116) reported that the presence of citric and malic acids is quite common in leaves and fruits, oxalic fairly widespread in leaves and tartaric the main constituent of fruits of Vitaceae. Fumaric and succinic acids are also distributed widely in higher plants, while oxalosuccinic, isocitric, cis-aconitic, oxaloacetic and α-ketoglutaric acids are generally found only in traces, if detected at all. Isocitric is present in substantial quantities in Crassulaceae (110).

Organic acids have been shown to be involved both directly in a reducing sugar-organic acid reaction and synergistically in a reducing sugar-amino acid-organic acid type of reaction (49, 68). Others have also indicated (7, 62) that these non-nitrogenous organic acids, in addition to the amino acids, may play a part in browning reactions and in after-cooking discoloration reactions. The blackening of potatoes after cooking is one of the most undesirable qualities of potatoes. This colored product is considered to be a complex of ferric ion and chlorogenic acid (50). Hughes and Swain (51, 52) showed that citric acid was the most important

factor in preventing blackening of potato tubers. Citric acid chelated the iron and prevented its complexing with chlorogenic acid. Citric acid has been shown to be a good inhibitor of browning, not only as an inactivator of trace metals but also as a synergist for true antioxidants (3, 103, 114). Qureshi's work (103) indicates that oxalic acid was more effective than citric acid in inhibiting the browning of various tuber starches from Indian tubers. Malic acid in combination with ascorbic acid inhibited the browning of cherries (114).

Chlorogenic acid, composed of quinic and caffeic acids, has been shown to occur in many plants -- in leaves of red tart cherries, apricots, peaches, sweet cherry and grape-cherry (130); in fruits of sweet cherry (131) and sweet mountain-ash (75); in tea (113); in pear (121), in coffee (6) and many other plants. Its isomers, isochlorogenic acid and neochlorogenic have also been detected in many of the plants in which the presence of chlorogenic acid has been positively identified (6, 122).

Recent interest in chlorogenic acid has centered on its function as a substrate for polyphenolase. Joslyn and Ponting (67) suggested that the darkening of fruit on injury may be due to the oxidation (enzyme catalyzed) of chlorogenic rather than the true tannins. In the early part of 1963, Maier (80) identified the main substrate for enzymic browning in dates as a caffeoylshikimic acid. This was the first time

a crystalline caffeoylshikimic acid was isolated from natural material, and the first demonstration that such acids will undergo enzymic browning. He suggested that these acids may be widely distributed in plants but may not accumulate as much as chlorogenic acids, possibly because of their greater metabolic activity.

Caffeic acid has been reported to be present in the leaves of apricot, peach and grape (130), roots of Polygala senega (21), and in fruits of sweet mountain-ash (75) and sweet cherry (131). There has been no positive identification of this acid in either the leaves or fruit or any part of the tree of red tart cherry.

Quinic acid was first found in apple fruits (53). It was originally suggested that quinic acid might provide a link between aliphatic and aromatic metabolism in plants and that shikimic acid might be the first stage in the "desaturation" of quinic acid (53). Weinstein et al. (125), using labeled quinic acid to determine its role in aromatic biosynthesis in higher plants, found that the major labeled products were always tyrosine, phenylalanine, CO_2 and often shikimic acid.

In apples it was found that shikimic acid increases as the fruit ripens and becomes senescent (57). Weinstein and co-workers also studied the role of shikimic acid pathway in beans (126). They found that much of the C^{14} from shikimic acid was incorporated into phenylalanine and tyrosine and

only a negligible amount was found in free or bound tryptophan. They also noticed the irreversible conversion of quinic acid to shikimic acid.

Succinic acid was shown to inhibit oxidative processes (120), and it was indicated that it may be actually toxic to plant material (56). This latter assumption was based on the fact that on paper chromatograms of acid fractions from apples suffering from carbon dioxide injury, a spot corresponding in position to that of succinic acid appeared. While in normal healthy apples, succinic was found only occasionally and in trace amounts.

The widespread occurrence of malonate in plant tissues has been demonstrated and the pathways for its enzymatic utilization and synthesis have been described by Shannon et al. (108). Malonic acid was not found to be an inhibitor of the Kreb's cycle in soybean leaves (113). However, it has been shown otherwise in the mitochondria from cauliflower buds, where the organic acid concentration is small (14). Quinic and citric acids, on oxidation, have been shown to yield small amounts of malonic acid (55).

The appearance of uronic acids (especially galacturonic acid) is usually associated with pathological disturbances, physiological changes such as senescence, and various types of physical damage (cutting or crushing of tissue) (77). It is also believed that they are seldom found in a free state in intact plants. Loewus (77) states that the uronic acids

undergo the synthetic steps leading to ascorbic acid. This was shown in detached ripening strawberries where D-galacturonic acid-1-C^{14} was converted to L-ascorbic acid labeled in C-6.

Pyrrolidone carboxylic acid (PCA) has been shown to occur in a number of fruits and vegetables (79, 107), including red tart cherries, variety Montmorency (79), but only after the product has undergone some heat treatment. PCA, a product of glutamine decomposition, seems to be responsible for the off-flavor in certain products.

The presence of phosphoric (107, 131), pyruvic (12, 119, 131), α-ketoglutaric (12, 119), citramalic (121), oxalic (107, 123), glutaric and aspartic (107, 121), glyoxylic (12), oxalacetic (12), glyceric (16) and fumaric (12, 16), tartaric (16, 105), lactic (105), etc. have been shown to be present in plant tissues by many investigators.

Schwartz and co-workers (107) studied the relationship of organic acid concentrations to specific gravity and storage time in potatoes. Concentration of glutamic, aspartic, PCA, malic, oxalic, and phosphoric acids varied inversely with the specific gravity. During storage the citric acid content of potatoes increased while that of malic acid decreased, suggesting the conversion of one to the other. Such a conversion was also shown to occur in tobacco leaves (14).

Disks of Sweede root tissue treated with several acids separately (succinate, malate, citrate, pyruvate and α-ketoglutarate) showed an enhanced protein synthesis,

possibly due to increased concentration of C skeletons in the tissue (119).

C. METHODS OF QUALITATIVE AND QUANTITATIVE DETERMINATION OF ORGANIC ACIDS

Most of the work, conducted prior to the development of chromatographic techniques, on acidity in fruits was confined to the determination of total titratable acidity or total acidity as malic, citric or tartaric (major acids in certain fruits). But since the "acid fraction" is certainly involved in many reactions, measuring small changes in the concentration of a specific organic acid or acids is likely to be more valuable in elucidating metabolic pathways than would be measuring changes in total acidity. Hence, work on the fractionation or isolation of certain acids of the acid extract of fruits and vegetables was initiated.

i. Paper Chromatography

Following the original work on paper chromatography by Consden, Gorden and Martin (20), this method has become the most popular procedure for the separation of various substances in mixtures and biological fluids. The separation of acids on paper was first described by Lugg and Overell (78). They suppressed tail formation by adding formic or acetic acid to the solvent, thus keeping the acids un-ionized.

To identify a substance one usually measures the Rf value (the ratio of the distance traveled by the solute to

the distance traveled by the solvent front) with a number of suitable solvents. Also, Rf values of reference substances run on the same paper at the same time, are measured. However, it is not possible to identify an unknown substance by Rf value alone.

In view of the great biochemical interest of acids, a number of solvents and techniques have been developed for improved or specific separations. Whiting (127) examined aqueous extracts of acids by descending chromatography, using the following solvents:

a) Benzyl alcohol : isopropyl alcohol : tert. butanol : water (3:1:1:1) and 0.2% formic acid

b) n-propanol : conc. aqueous ammonia (7:3)

c) Phenol, 3 gm : water, 1 ml : formic acid, 1%

Some of the other solvents used in separating organic acids:

d) Iso-propanol : pyridine : acetic acid : water (80:80:10:40) (115)

e) Formic acid : butanol : benzyl alcohol : water (60:140:22:240) (91) (vol:vol)

f) Butanol : acetic acid : water (100:24:100) (4)

g) Butanol : formic acid : water (100:30:100) (4)

h) n-Butanol : 3N formic acid (V./V.) (81)

i) n-Butanol : water : diethylamine (100:15:1) (65)

j) Ether : acetic acid : water (15:3:1) (65)

k) 1-pentanol : 5 M aqueous formic acid (1:1) (65)

l) 2-ethyl-1-butanol : 5 M aqueous formic acid (2:3) (65)

m) 95% ethyl alcohol : water : conc. ammonium hydroxide (8:1:1) (65)

n) 95% ethanol : ammonium hydroxide : water (20:1:4) (10)

For the detection of the acids the volatile acid or alkali of the solvent is completely evaporated and the paper sprayed with an indicator, such as bromocresol green or bromophenol blue (0.04 g. in 100 ml. of 95% ethanol -- (74)). When acetic acid is included in the solvent, evaporation takes longer than with formic acid; however, in either case drying should be carried out slowly (for at least 2-3 hours) at room temperature.

One-dimensional paper chromatography has been used to determine mono- and dibasic acids (83), di- and tricarboxylic acids (111), mixture of organic acids (132), citric and malonic acids on $Ni(OH)_2$- impregnated paper (35), organic acids by use of papers containing ion-exchange resins and O-(carboxy-methyl)-cellulose (87), etc. There are a number of other techniques that have emerged from this simple one-dimensional paper chromatography. A two-dimensional paper chromatography has been developed for the separation of organic acids and used by several investigators (19, 29, 45).

A mixture of uronic acids was separated by circular chromatography (semi-circle technique) (115). Ascending chromatography has been used in combination with electrophoresis for a study of non-volatile organic acids of biological media. Badrinas et al. (4) used a fluorescent indicator for the chromatographic development of non-volatile mono-, di-, and tricarboxylic acids. The reaction is based on the inhibition of the formation of the fluorescent complex, of aluminum-8-hydroxyquinoline, by organic acids. Also, organic acids can be separated and identified on papers impregnated with ion exchange resins, O-(carboxy-methyl)-cellulose (87), and $Ni(OH)_2$ (35). A method has been developed for the separation of substances of very narrow Rf range. This is called "fractionated one-dimensional paper chromatography" (106). Fractionated chromatography is conducted by developing and drying the chromatogram, then submitting it again to the same solvent system. This produces a second migration and a new series of Rf values. The first is called the primary Rf value, the others secondary. This can be repeated up to ten times, thus extending the length of the chromatogram. Paper chromatography has also been used for quantitative determination of di- and tricarboxylic acids, with an accuracy up to 0.4-0.5% (111).

Electrometric contact method has been applied to paper chromatography of organic acids (133) using Kamienski's

microelectrode. The method allows detection of acids studied in quantities of the order of 10^{-12} mole on the active surface of the electrode (3.14 sq. m.m.). Both ascending paper chromatography (91) and descending paper chromatographic methods have been used (81, 127).

ii. Ion Exchange Column Chromatography

In ion exchange chromatography, an ion exchange resin serves as the chromatographic column. The solute mixture is first added to the resin in a vertical column and once the ions are exchanged on the resin, separation can be accomplished by displacement, elution, and frontal analysis (26).

In the past, anion exchangers were used for the separation of organic anions from cations and uncharged molecules present in biological extracts (74). The use of anion exchangers for the separation of organic acids from each other has only been reported recently. Wilson (128) has used ion exchange resins to determine fruit acids; Busch et al. (15) have used the formate form of Dowex-1 for the separation of the acids of the citric acid cycle. The organic acids were displaced with formic acid of constantly increasing concentration. Excellent separation of lactate, succinate, malate, fumerate, α-ketoglutarate, and cis-aconitate was achieved. Pyruvate, malonate, citrate, and isocitrate were not separated but could be resolved on a silica gel column. Reproducibility of the positions of the

peaks of known acids was fairly good. Following them, a number of investigators have used anion exchange resins for the separation of organic acids: Schenker and Rieman (105) have separated malic, tartaric, and citric acids from fruit extracts; Owens et al. (92) studied the organic acids in sugar beet liquors; Palmer studied the acids of tobacco leaves (93); Hulme and Wooltorton (59) investigated the acids in apples, strawberries, and sweet cherries (61). These investigations were followed by several others (16, 41, 129) who used either the Dowex-1 or Duolite A-3 or A-4, or Amberlite IR-4B columns for the fractionation and determination of organic acids in various plant materials.

Gradient elution chromatography was first described by Alm et al. (1). They showed that the successive zones into which the components of a mixture resolve could be narrowed considerably, and the elution peaks sharpened, by passing a gradually increasing concentration of eluting agent through the column. The separation of the various acids takes place primarily by elution chromatography on strongly basic anion exchange resins in the acetate or formate form (59, 81). The acetate form gives better separations of the weaker monobasic acids such as quinic and shikimic (59). The acids are displaced by "gradient elution." The principle of the method depends upon the fact that organic acid anions combine with positively charged groups on a synthetic organic resin of suitable composition

and remain fixed to it until displaced by an aqueous solution of an acid of increasing concentration which percolates through the column. Hulme and Wooltorton (59) believe that, in general, acids leave the column in the order of their pK values, but that there are exceptions. Also, unsaturated acids are more tightly bound to the resin than saturated acids of similar basicity and molecular weight. Most of the acids may be completely separated and a high percent recovery is obtained, but the yields of some acids are low, owing to decomposition or other unknown reasons. Acids with closely similar pK values cannot be separated quantitatively, especially if one of them is present in relatively large amounts. In such cases the mixed fractions may be resolved and the individual acids determined quantitiatively by other methods such as silica gel column chromatography or paper chromatography.

Peterson and Sober (97) have described a device for the production of a wide variety of concentration gradients using up to nine chambers.

iii. Other Methods

In the 1930's, Fidler investigated several methods for the determination of individual acids in plant material (60), but he found that they were not sufficiently accurate for the determination of acids which were present in apples in very small amounts.

Later, Pollard et al. (101) and Prigot and Pollard (102) converted acids into their piperazinium salts as a method of identification of organic acids.

Curl and Nelson (23) isolated citric, isocitric, and malic acids by distillation of their ethyl esters and characterized them as hydrazides. Oxalic acid was isolated and identified by crystallographic methods after precipitation as calcium oxalate.

Mixtures of dicarboxylic acids were also separated by preparing their amides or imides which were then distilled (8). Dunbar and Moore (27) studied carboxylic acids by preparing their p-toluidides and amides.

The uronic acids, especially galacturonic and glucuronic, have been separated on anion exchange columns (73) and determined on thin silica gel layers, with naphthoresorcinol solution as a specific detecting reagent (95). These acids have also been identified by lactonizing and chromatographing (36). Characteristic migration rates, the hydroxamic acid-ferric ion test for lactones, and the specific lead acetate test for galacturonic acid were used to identify these on paper chromatograms. D-galacturonic acid can also be determined colorimetrically with 2-thiobarbituric acid (134). The reaction in this case gives a yellow color which is measured at 400 mμ. Uronic acids have also been separated by circular chromatography (115), paper electrophoresis (44), and using anthrone (47).

Keto-acids can be separated in mixtures as the 2, 4-dinitrophenylhydrazones (12, 30) and quantitatively determined with the aid of a photometer (30). α-Ketoglutaric acid was determined fluorimetrically by Spinker and Towne (112) by treating it with o-phenylene diamine to give fluorescent quinoxaline.

Lactic acid has been determined spectrophotometrically by converting it to CHI_3, dissolving this in $CHCl_3$ and measuring at 347 mμ (34). Courtoisier (22) estimated lactic and tartaric acids by oxidation with permanganate and chromate in H_2SO_4 and malic acid by oxidation with ceric sulfate.

High-voltage paper electrophoresis has been applied to the study of organic acids (40). Also, electrochromatography has been used as a method of direct identification of non-volatile organic acids of biological media (91).

Following Isherwood's introduction (63) of column chromatography for the separation of organic acids on silica gel, the method has been adopted and somewhat modified by a number of investigators (13). Wagner and Isherwood (123) recently described the separation of some 25 different acids from peas, including lactic, succinic, oxalic, cis-aconitic, malic, and citric. Carles et al. (18) employed Celite columns for the separation of organic acids of various parts of grape vines by partition chromatography. Columns containing alumina (Al_2O_3) (38, 42) and cellulose (41, 99, 122)

have also been used for the separation of organic acids.

Thin layer electrophoresis has been applied to the separation of phenols and phenol carboxylic acids (96). Thin layer chromatography has also been used in the separation of phenolic carboxylic acids on layers treated with chelate-forming anions (43), and saturated aliphatic dicarboxylic acids (oxalic, malonic, succinic, glutaric, adipic, etc.) on silicic acid layers (98).

Shibazaki (109) simplified a gas-chromatographic apparatus for the determination of inorganic and organic acids.

Infra red spectroscopy as a method of organic acid determination is gaining popularity. Henshaw et al. very recently used it for the identification of organic acids of the rhizome of Iris pseudacorus (48).

MATERIALS AND METHODS

MATERIALS

Red tart cherries were collected during three seasons, 1960, 1961 and 1962. These cherry samples were obtained from the Horticultural Farm at Michigan State University. Cherries were picked at weekly intervals, starting with the 45th and 46th day after full-bloom. There were six such pickings in 1960, and eight each in 1961 and 1962. The trees were treated with eight different fungicidal sprays in 1960, three in 1961, and one in 1962, as shown below. The cherries were either washed with water, pitted, weighed, and extracted immediately or washed and stored at -10°F until extracted.

Fungicidal Sprays Used

1960

1. Fixed Copper, all season
2. Fixed Copper, early (2 applications); Ferbam[a] and Glyodin[b], late
3. Ferbam and Glyodin, all season
4. Ferbam and Glyodin, early (2 applications); fixed copper, late
5. Ferbam and Glyodin, early (2 applications); Nu-iron and glyodin, late

---

a Ferric dimethyldithiocarbamate
b 2-heptadecylglyoxalidine acetate

6. Cyprex[c], all season
7. Parathion[d], early (2 applications); actidione[e] and ferbam, late
8. Parathion, early (2 applications); actidione and Nu-iron, late (Sevin[f] was also used for cherry fruit-fly control)

Also, 1 through 7 were treated with lead arsenate all season.

1961

1. Ferbam and Glyodin
2. Copper (Fixed)
3. Cyprex (Organic)

1962

1. Fixed Copper

METHODS

A. Preparation of the Acid Concentrate

Two different methods were used in extracting and concentrating the acids before their chromatographic separation. These methods are described as the 1960 Method and the 1961-1962 Method, indicating the years in which each one was applied.

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c n-dodecylguanidine acetate
d 0,0 - diethyl O-p-nitrophenyl thiophosphate
e Beta - (2-(3, 5-dimethyl-2-oxocyclohexyl)-2-hydroxyethyl)-glutarimide
f 1-naphthyl-N-methyl carbamate

1. 1960 Method:

The procedure used was a modification of that described by Hulme and Wooltorton (59). Fifty grams of pitted fruit were blanched in approximately 75 ml. of boiling distilled water for one minute and then 400 ml. or sufficient 95% ethanol was added to give a 70% ethanol solution. This pulp-ethanol mixture was blended in a Waring blendor for three minutes at high speed. It was then cooled to room temperature, filtered through sharkskin paper, and the residue washed thoroughly with 50-60 ml. of 80% ethanol. Filtrate and washings were combined and concentrated to 40-50 ml. in vacuo in a flash evaporator, with the water bath at 40°C. The concentrate was then treated with 1.0 gm deactivated carbon for two hours on a shaker to remove the coloring matter and filtered.

The deactivated carbon was prepared by shaking 150 gm. of Darco, Grade G-60 activated carbon with 1 liter 5% acetic acid for an hour, filtering and washing with distilled water until acetic acid was removed, and drying at 40°C.

The cherry filtrate was applied on a water-washed Dowex-50 WX8 cation exchange resin column (H^+form 50-100 mesh, 15 cm long by 0.7 cm in diameter).

The organic acids were eluted by means of water. The column retained the amino acids and cations. The eluate from the column was concentrated in the flash evaporator and made to a volume of 25-40 ml., depending on the amount of washing required to remove the concentrate, both from the flash evaporator flask and the column. Representative aliquots were titrated with 0.1 N NaOH using phenolphthalein as an indicator to determine the acid content. Sample portions corresponding to a total acidity of 1.0 - 1.2 meq. were used for fractionation. The rest of the acid extract was stored at -10°F.

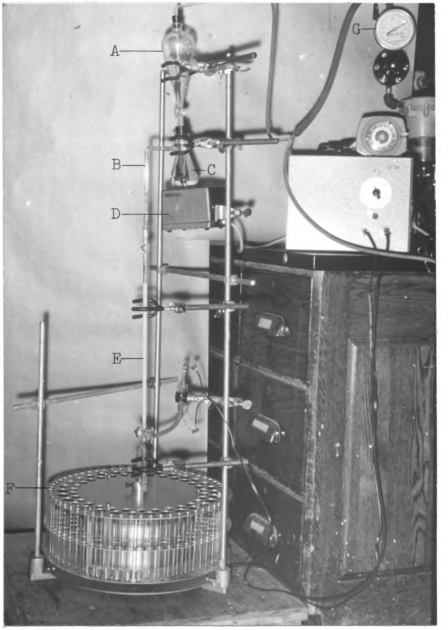
2. 1961-1962 Method

A modification of Hartman's lead precipitation procedure (46) for the preparation of the acid concentrate, was used. A weighed amount of pitted fruit (50-100 gm.) was blanched and homogenized in a Waring blendor, with 75 ml. distilled water, at high speed for three minutes as in 1960. The homogenate was boiled for ten minutes, then 2 ml. of 1 N HNO_3 were added and the acidified homogenate was cooled to room temperature. It was then made up to 250 ml. and filtered through a 6-1/2" single gauze milk filter disc. Two hundred ml. filtrate was concentrated to approximately 50 ml. in vacuo, and diluted

to 250 ml. with 95% ethanol. The precipitated pectins and other alcohol insoluble material were removed by a milk filter disc and 200 ml. of the filtrate was transferred into a 250 ml. centrifuge flask containing a Teflon-coated magnet. The pH was adjusted to 7.8 with 2N NH_4OH and lead subacetate, dissolved in a few ml. of water, was added. The amount of lead subacetate used was equivalent to about twice the titratable acidity of the filtrate before the pH adjustment. Hyflo supercel (0.2 gm) was added to the mixture, it was stirred for five minutes, and centrifuged at 2500 RPM for ten minutes. The supernatant liquid was decanted and 50 ml. of 80% ethanol were added to the sediment, the mixture was shaken for complete dispersion, the sides of the flask washed and the contents were centrifuged as previously described. A second decantation, redispersion in 50% ethanol and centrifugation followed. If the third supernatant was not clear, it was not decanted, instead, the sediment was again dispersed and the pH readjusted to 7.5 with 2N NH_4OH and recentrifuged. The sediment obtained after the third decantation was dispersed in 50 ml. of 50% ethanol and the suspension was saturated with H_2S, while constantly stirring with the aid of a magnetic stirrer (for five minutes).

The contents of the flask were centrifuged and the supernatant was checked for soluble lead with H_2S. If the precipitation of lead was complete, the supernatant containing the free acids was transferred to a flash evaporator flask. The precipitate (or sediment) of lead sulfide was dispersed in 50 ml. of 50% ethanol, centrifuged and the supernatant added to the previous one. This last step was again repeated to insure complete removal of the free acids from the centrifuge flask. The combined solution of free acids was concentrated to 15-20 ml. in a flash evaporator and then passed quantitatively through a column of Dowex-50 WX8 cation exchange resin, as described previously. During the passage of the solution of free acids through this column, pigments, cations, and amino acids (except part of aspartic and glutamic) were retained by the resin. The column was washed thoroughly and the eluate concentrated and made up to 25 ml. in every case. Representative aliquots were titrated to determine the acid content and sample portions corresponding to a total acidity of 1.0 - 1.2 meq. were used for fractionation. The rest of the acid extract was stored at -10°F.

FIGURE 1. CONCENTRATION GRADIENT ELUTION SYSTEM



A. Eluant Reservoir
B. Capillary Glass Tube
C. Mixing Flask
D. Magnetic Stirrer
E. Anion Exchange Resin Column
F. Automatic Fraction Cutter
G. Air Pressure Regulator

regulator (Moore Products Co., Philadelphia 24, Penna.) was connected to the separatory funnel by means of a rubber tubing to keep the pressure in the system at 150 inches of water. Mixing was accomplished by a Teflon-coated magnet in the flask and stirring by means of a magnetic stirrer beneath the flask. The level of the liquid in the mixing flask was kept just above the side arm. The first eluting solution consisted of 100 ml. 3N acetic acid, the second 50 ml. 6N acetic acid and the third 300 ml. 6N formic acid. Between 120 and 130 fractions of 4.0 ml. or slightly less volume were collected using an automatic fraction cutter (Rinco Instruments, Greenville, Illinois).

The fractions were dried in a vacuum oven at 40°C. For the quantitative determination the fractions were re-dissolved in hot water, kept in a hot water bath and titrated with 0.01 N NaOH, using phenolphthalein as indicator. The fractions for each peak were pooled, treated with Dowex-50 WX8 cation exchange resin to remove the sodium, filtered through cotton, and dried for qualitative determination. For the determination of the unknown peaks, 34 known acids were passed and eluted through the anion exchange column to determine the effluent volume of each acid and order of their emergence. To obtain sharper peaks only 3-4 acids were passed through the column at a time.

C. Paper Chromatography

The dried fractions were dissolved in 80% ethanol and paper chromatographed on Whatman No. 1 paper (46 cm x 57 cm). The spots were placed 2.5 cm apart and 7.0 cm away from the long edge of the paper. Sixty known acids were spotted in between the unknown fractions from cherry. Two different solvents, one acidic and one alkaline were used: (a) n-Butanol and 3N formic acid, 1:1 by volume; (b) Ethanol, ammonium hydroxide and water, 20:1:4 by volume (10). The lower phase of the mixture (a) or, the mixture (b) was used for vapor equilibration. The spotted papers were irrigated descendingly by the upper phase of the mixture (a) or, mixture (b) for 15 and 12 hours respectively, at a constant temperature of $22^{o} \pm 0.5^{o}$. At the end of this time, the solvent front was marked and the papers were dried in an air draft for at least three hours and sprayed with a 0.04% solution of Bromphenol blue (BPB - Na salt) in 95% ethanol. The acid spots showed as yellow spots against a blue background.

The Rf values were determined for all acids. The cherry acids were tentatively identified with the known acids on the basis of having similar Rf values. The presence and identity of chlorogenic, isochlorogenic and neochlorogenic acids were determined by examination under UV light for fluorescence. Spot tests or color reactions were used for the various acids to confirm the identity of these acids.

Pure acids were obtained from commercial sources for the identification of the acids from red cherries and to determine the quantitative recovery using column chromatography. Neochlorogenic acid was obtained from Dr. J. Corse of the Western Utilization Research and Development Division, U.S.D.A., Albany, California. Isochlorogenic acid was isolated from green coffee beans by the author, using the procedure given by Barnes (6).

D. Confirming Tests

1. Phosphoric acid test with molybdate (28): a mixture of 1 ml. of the test solution, 2 ml. of concentrated nitric acid, and 2 ml. of ammonium molybdate reagent was warmed and allowed to stand for at least five minutes. A fine yellow precipitate indicated the presence of phosphorus.

2. The fluorescent acids were further characterized by alkaline hydrolysis and subsequent paper chromatography of the hydrolysate. For the alkaline hydrolysis, 2 to 3 ml. of test solution were mixed with 2-3 drops of KOH solution (13% w/v) and left for 15 minutes at 20°C (54). The sodium ion of the hydrolysate was removed with the Dowex-50 WX8 cation exchange resin and the free acids were paper chromatographed on Whatman No. 1 filter paper with n-Butanol: 3N formic acid (1:1) as the solvent, along with pure solutions of caffeic and quinic acids.

3. Glyceric acid test with naphthoresorcinol : 0.5 - 1.0 ml. of test solution was heated with 0.75 ml. concentrated H_2SO_4 containing naphthoresorcinol (1 mg/10 ml), for 30-50 minutes in a water bath at 90°C. A blue color appeared in the presence of 10 γ or more of the acid (28).

4. Glycolic acid test with 2,7-dihydroxynaphthalene (2,7-naphthalenediol) and sulfuric acid : a mixture of test solution (0.5 - 1.0 ml.) and 2 ml. of concentrated H_2SO_4 containing 2,7-naphthalenediol (1 mg/10 ml) was heated for 10-15 minutes in a water bath. A red to violet red appeared according to the amount of glycolic acid present. At least 0.2 γ glycolic acid is necessary to give this color reaction (28).

5. Lactic acid test with p-hydroxydiphenyl : 0.5 - 1.0 ml. of test solution and 1 ml. concentrated H_2SO_4 were heated for two minutes in a water bath at 85°C and cooled to 28°C. A pinch of solid p-hydroxydiphenyl (phenylphenol) was added to it and swirled several times and allowed to stand for 10-30 minutes. A violet color was indicative of the presence of lactic and needs at least 1.5 γ for the reaction (28).

6. Galacturonic acid test : a drop of saturated basic lead acetate was placed on a test spot on a paper and heated for one minute on live steam. Brick red color appeared in the presence of galacturonic acid.

7. Quinic acid test with naphthoresorcinol : same test as for glyceric acid. A greenish color appeared in the presence of quinic acid (28).

8. Glucuronic acid test with naphthoresorcinol : same test as for glyceric acid. A yellow color with a greenish fluorescence indicated the presence of glucuronic acid in the test solution (28).

9. Tartaric acid test with dinaphthol : 0.5 - 1.0 ml. of the test solution was treated with a little solid β-β-dinaphthol in concentrated H_2SO_4 and heated for half an hour in a water bath at 85°C. When tartaric acid was present, a luminous green fluorescence gradually appeared during the heating and deepened on cooling. As little as 10 γ of this acid can be detected (28).

RESULTS AND DISCUSSION

The 1960 method, using carbon for the removal of color and sugars, was not used after the first year because it was found that quantitative recovery of the aromatic acids could not be obtained. The deactivated carbon, although it removed the pigments more efficiently than the cation exchange resin, absorbed and did not release readily the aromatic acids, particularly chlorogenic acid. Using pure chlorogenic acid solutions, the maximum recovery of acid obtained was 66% while with the lead precipitation method 99 to 100% recovery was obtained.

In the 1961-1962 procedure, the aqueous fruit homogenate was acidified with nitric acid, extracted with cold 95% ethanol and the acids precipitated as lead salts with lead subacetate. This method resulted in better recovery of the organic acids because (a) the free acids were more soluble in ethanol than the salt forms, such as tartarates, (b) the precipitation of the acids as the lead salts permitted their separation from the sugars, which in concentrated solutions were difficult to handle and also gave acidic degradation products when passed through the Dowex-1 column, (c) the use of cold ethanol instead of hot ethanol eliminated partial esterification of the acids (17, 104), and if no saponification was subsequently used, the quantitative determination would not be accurate. The pigment was

almost completely removed by the use of longer cation exchange resin columns (20 cm).

The modified ion exchange procedure used in this study was found to be more convenient and resulted in better fractionation and recovery of the organic acids of cherries than other methods. Palmer (93) has reported that the ion exchange chromatography has several advantages over silica gel methods for the determination of organic acids in plant materials.

A. QUALITATIVE ANALYSIS

The acids studied are listed in Tables 1 and 2 with their Rf values. The order of emergence of pure acids from the anion exchange column is shown in Figure 2. These positions were determined by passing through the column a mixture of 8 - 10 known acids at a time. The identity of the acids of each peak was established by the use of paper chromatography and chemical identification wherever possible. It was found that in a mixture of acids, generally the individual acids left the column in a specific order (mono-, di-, and tri-basic acids) and that these separations were quite clear. These results were in agreement with those reported by Hulme and Wooltorton (59). The exceptions were certain unsaturated acids (chlorogenic acids) and keto acids (pyruvic acid). Of all the acids studied, only glyceric, glycolic, and glutaric acids did not separate into distinct peaks and

FIGURE 2 FLOWING CHROMATOGRAM OF PURE ACIDS

lactic acid came out with shikimic acid.

The peaks obtained when the cherry acid extract was passed through the column are shown in Figure 3. Comparing the effluent volumes and the Rf values obtained with those of the pure acids, the following acids, in order of emergence from the column, have been identified in red tart cherry (variety Montmorency): glutamic, aspartic, lactic (occasionally), shikimic, quinic, galacturonic, glyceric, glycolic, glutaric, glucuronic, succinic, citramalic (occasionally), malic, tartaric (occasionally), malonic, citric, neochlorogenic, isochlorogenic, fumaric (occasionally), chlorogenic and phosphoric.

Most of the groups of acids which could not be separated by ion exchange were subsequently resolved by paper chromatography. Such groups were the glyceric, glycolic and glutaric, succinic and glucuronic, citric and malonic, and neochlorogenic and isochlorogenic. Reducing the size of fraction in column chromatography in the hope of separating these groups was not very successful. Glycolic, glyceric, glutaric, and malonic acids were detected only when the paper was heavily spotted. Since citric and isocitric acids cannot be separated by ion exchange and paper chromatographic methods, the fraction containing citric acid was chromatographed using silica gel chromatography (81). No isocitric acid was found to be present in the cherry extract. Glucuronic

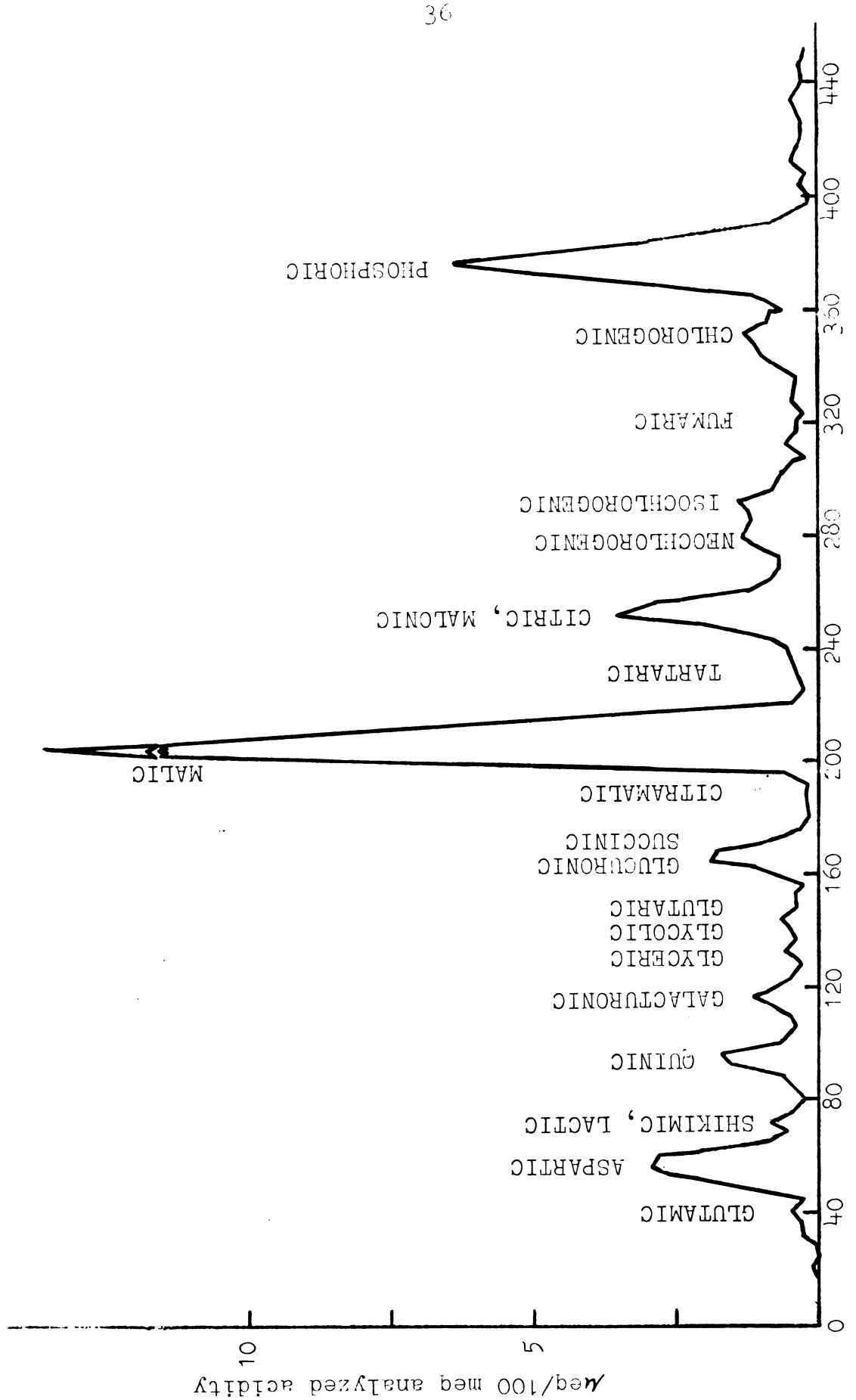


FIGURE 3. TITRATION OF COLUMN CHROMATOGRAPHIC FRACTIONS OF ACIDS OF MONTMORENCY CHERRIES 1962 - COPPER TREATMENT

acid usually gave double spots on the paper chromatograms. Partridge (94) has reported this due to the presence of glucuronolactone in the solutions.

Three polyphenolic acids, neochlorogenic, isochlorogenic, and chlorogenic acids were identified not only by paper chromatography (comparing the Rf values of knowns and unknowns), but also by their fluorescence under ultraviolet light and the products identified after alkaline hydrolysis. On alkaline hydrolysis, all three chlorogenic acids showed the presence of caffeic and quinic acids in the hydrolysates thereby excluding the possibility of being caffeoylshikimic acids. Maier (80) recently indicated that it is impossible to differentiate caffeoylshikimic acids from caffeoylquinic acids on the basis of Rf values and ultraviolet spectra.

No caffeic acid was ever detected in red tart cherries. Either caffeic acid is absent in red cherries, or is present in such trace amounts that it could not be detected by the methods used. One reason why caffeic acid does not accumulate as the other phenolic acids, is possibly because of its greater metabolic activity.

Phosphoric acid was the only inorganic acid and glutamic and aspartic acids were the only amino acids that were determined by this procedure.

B. QUANTITATIVE ANALYSIS

The various fractions were titrated for total acidity and paper chromatograms of the titrated fractions were run, to confirm the identity of the acids in each fraction.

Solutions of pure acids (of the acids identified in cherries) were prepared and divided into two equal aliquots. One aliquot was titrated directly and the other put through the column and then titrated. The recovery of each acid from the anion exchange column was then calculated from the titer values thus obtained. Recoveries were also determined with groups of two or three acids applied at a time on the column. There was no difference in the recovery figures obtained in the two ways. From these recovery figures correction factors were calculated to convert the acid concentration, obtained by titration, of some of the acids to actual concentrations. Table 1 gives the results of the recoveries for pure acids studied. From this table it is clear that this method was most precise for aspartic, shikimic, quinic, glyceric, glycolic, succinic, citramalic, malic, citric, malonic, fumaric, and phosphoric acids. It was fairly precise for glutamic, galacturonic and glutaric, but not for glucuronic, chlorogenic, and probably its isomers, in which case the necessary correction factor was applied. Isochlorogenic acid, isolated from green coffee beans, even after purification showed some contamination, both on the column and paper;

pure neochlorogenic acid was available only in very small amounts, so no recovery figures were obtained for them. Since isochlorogenic and neochlorogenic acids are isomers of chlorogenic acid, the same correction factor was applied to them as was applied to chlorogenic acid. A tentative structure for isochlorogenic acid has been given as 5-caffeoylquinic acid (6) (a position isomer of chlorogenic acid which is a 3-caffeoylquinic acid), while no structure has been yet proposed for neochlorogenic acid, except that it also is probably a position isomer of chlorogenic acid.

No correction was made for glutamic and aspartic acids because of poor and variable recoveries from the Dowex-50 resin. The recoveries ranged from 12 to 14% for glutamic and 2 to 4% for aspartic acid. No explanation can be given for these poor recoveries.

The use of Dowex-1 resin from different lots did not make any difference in the elution titration curve, as to the position of each acid peak and the recovery of the acid.

For the quantitative determination of the acids, the titer values (ml. base used), under each peak, were added together and the necessary correction from the recoveries was applied. The pK values of all cherry acids are tabulated in Table 1. Since the pK values of all acids were well below 8.5, all carboxyls were titrated with NaOH using phenolphthalein as an indicator. The phosphoric acid titration

values were multiplied by 3/2 because only two-thirds of this acid was titrated (81).

After the corrections were made for the recoveries, the ml. base used for the titration of each acid peak was then converted to milliequivalents of acidity. This gave the milliequivalents (meq.) of acidity of each acid in the total acidity applied on the column. From this, the meq. of each acid per 100 meq. of total acidity was calculated. Also, the meq. of each acid per 100 gm of fresh cherries was calculated from this since the meq. of total acidity per 100 gm of fresh fruit at each stage of maturity was known. Using this last step and the equivalent weights of the acids, mg of each acid per 100 gm of fresh fruit was obtained.

C. TOTAL TITRATABLE ACIDITY

The cherries harvested 53 days after full-bloom, in 1960, were less developed than those harvested in 1961 and 1962, 45 days after full-bloom. However, the total number of days required for fruit maturation for the 1960 fruit was less than those required in 1961 and 1962 because of slightly higher average daily temperatures.

The changes in total titratable acidity (free and bound) as milliequivalents per 100 gm of cherries during the development of the fruit are shown in Figure 4. The values are averages of 1961 and 1962 data for a similar treatment

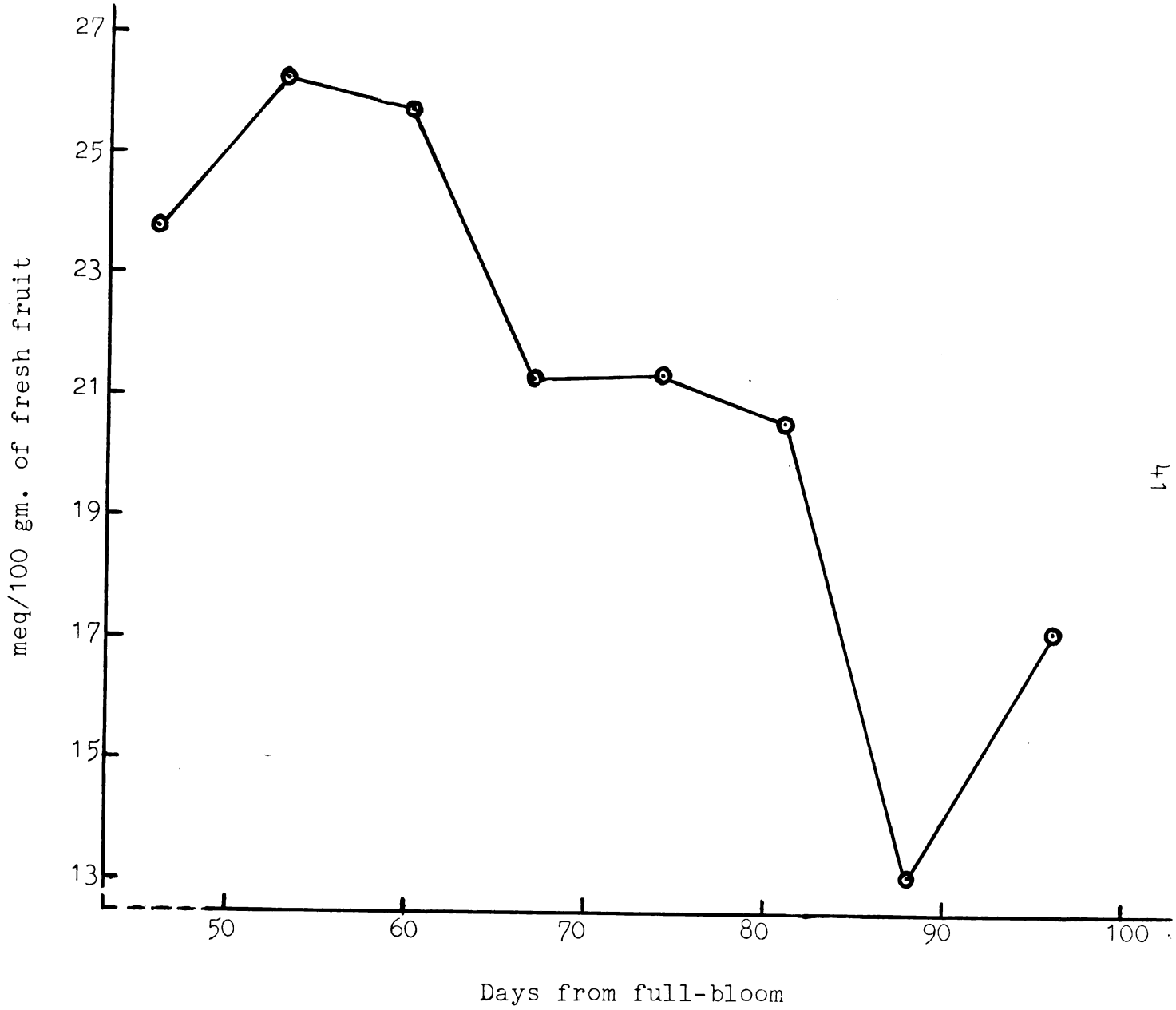


FIGURE 4. CHANGES IN TOTAL TITRATABLE ACIDITY DURING FRUIT MATURATION 1961-1962 - COPPER TREATMENT

(copper). The first analysis was made on fruit picked on the forty-fifth to forty-sixth day after full-bloom and continued once every week until the ninety-sixth day after full-bloom, when the fruit were overripe. The commercial harvest time for processing was between the seventy-fourth and eighty-first days after full-bloom in both years. The total titratable acidity rose during the first week after the forty-sixth day from full-bloom; it then dropped below the forty-sixth day level, passed through a plateau, decreased again, and finally went up when the fruit was overripe. The commercial harvesting coincided with the latter part of the acidity plateau. The terminal acidity increase was probably due to desiccation.

The changes in the total titratable acidity for all treatments in 1960 are tabulated in Table 3, and for all treatments in 1961 and 1962 in Table 4. The total titratable acidity in 1960 was similar for all spray treatments, and it remained relatively constant throughout the harvest period. The change in acidity in 1961 for Cyprex follows the same trend as the one for 1961-Copper, except that in the sixth analysis the former showed a sudden rise while there is plateauing in the latter. The change in the Ferbam-Glyodin treatment in 1961 parallels that in 1961-Cyprex, except that the third analysis showed a marked drop in the former, more than in the Cyprex treatment. This third

analysis in the Ferbam-Glyodin treatment also showed a very peculiar drop in phosphoric acid (Figure 24) to almost nothing, compared to the large amounts in other analyses. The whole extraction procedure and fractionation were repeated several times and it always showed this peculiarity.

D. CHANGES IN INDIVIDUAL ACIDS DURING FRUIT DEVELOPMENT

Changes in all individual acids were studied during fruit development, but the results are tabulated for only twelve of the acids. These acids were: aspartic, shikimic, quinic, galacturonic, glucuronic, succinic, malic, citric, neochlorogenic, isochlorogenic, chlorogenic and phosphoric. The other acids were sometimes present in trace amounts and sometimes absent. The results for 1960 will be discussed separately from those obtained for 1961 and 1962 because of the differences in time of fruit maturation and differences in methods of acid extraction.

Since fixed copper (proprietary copper) with lime was used as the control treatment in the fungicidal program, it was used as the basis to evaluate the changes in the acids during fruit development. The data for the 1960 copper-sprayed fruit is given in Table 5.

Aspartic Acid: There was no marked change in aspartic acid during the first three pickings; then it increased to a maximum and stayed relatively constant. The maximum level

of aspartic acid occurred at the time the cherries were considered mature for commercial harvesting (Figure 5).

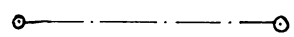
Shikimic Acid: There was a large amount of shikimic acid in the green fruits which gradually decreased to nothing in the ripe and overripe fruits (Figure 6). This was just the opposite of what was found in apples (57).

Quinic Acid: The changes in quinic acid content were similar to those of aspartic acid, where the acid dropped temporarily during the second picking, showed a slight rise at the third, and then reached a peak at the time of commercial harvest. It decreased slightly in the overripe fruits (Figure 7).

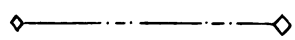
Galacturonic Acid: This acid, one of the pectin-degradation products, is usually associated with senescence (77, 84). It dropped during the first week, then leveled off and finally rose to a maximum in the overripe fruits (Figure 8). This maximum might be expected since there is a considerable amount of tissue breakdown in overripe fruits.

Glucuronic Acid: Another uronic acid, also believed to be associated with pathological disturbances, physiological changes, and physical damage (77), like galacturonic, is glucuronic acid. It was detected only in the second, third, fifth and sixth pickings, with a maximum in the sixth picking (Figure 9). This agreed very well with the previous finding of Loewus in strawberries (77).

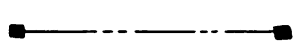
Spray Treatments



1. Fixed Copper, all season.



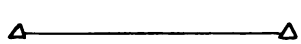
2. Fixed Copper, early (2 applications); Ferbam and Glyodin, late.



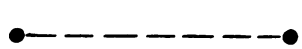
4. Ferbam and Glyodin, early (2 applications); Fixed Copper, late.

5. Ferbam and Glyodin, early (2 applications); Nu-iron and Glyodin, late.

6. Cyprex, all season.



7. Parathion, early (2 applications); Actidione and Ferbam, late.



8. Parathion, early (2 applications); Actidione and Nu-iron, late. (Sevin was also used for cherry fruit-fly control.)

Also, 1-7 were treated with lead arsenate all season.

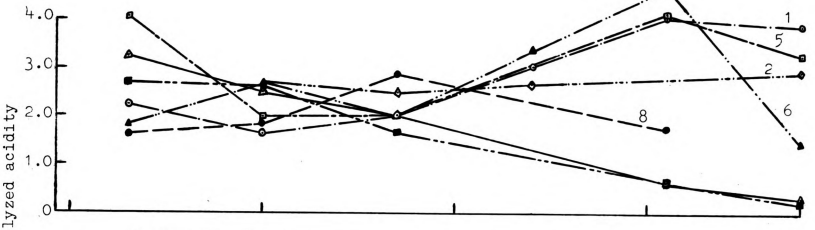


FIGURE 5. EFFECT OF SPRAY MATERIALS ON THE ASPARTIC ACID CONTENT DURING FRUIT MATURATION - 1960

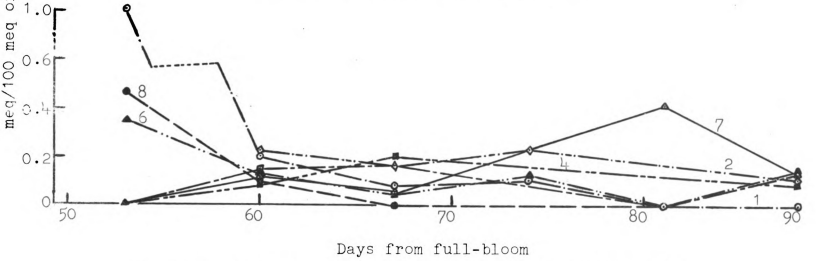
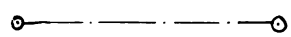
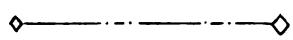


FIGURE 6. EFFECT OF SPRAY MATERIALS ON THE SHIKIMIC ACID CONTENT DURING FRUIT MATURATION - 1960

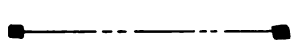
Spray Treatments



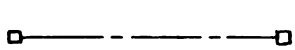
1. Fixed Copper, all season.



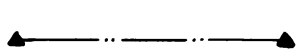
2. Fixed Copper, early (2 applications); Ferbam and Glyodin, late.



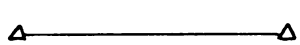
4. Ferbam and Glyodin, early (2 applications); Fixed Copper, late.



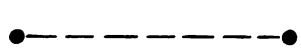
5. Ferbam and Glyodin, early (2 applications); Nu-iron and Glyodin, late.



6. Cyprex, all season.



7. Parathion, early (2 applications); Actidione and Ferbam, late.



8. Parathion, early (2 applications); Actidione and Nu-iron, late. (Sevin was also used for cherry fruit-fly control.)

Also, 1-7 were treated with lead arsenate all season.

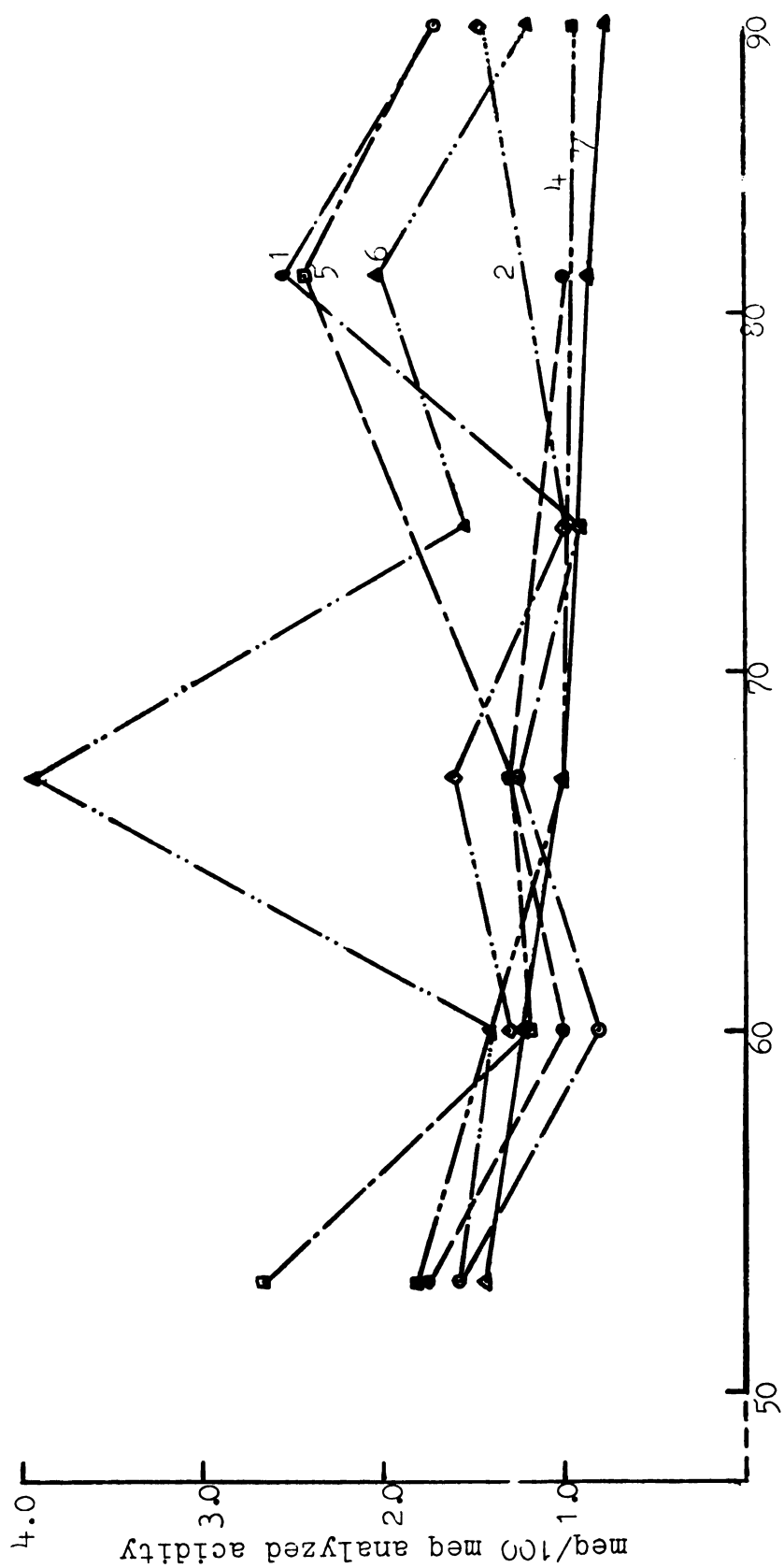
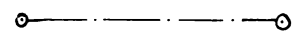
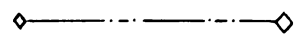


FIGURE 7. EFFECT OF SPRAY MATERIALS ON THE QUINIC ACID CONTENT DURING FRUIT MATURATION - 1960

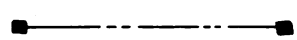
Spray Treatments



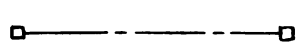
1. Fixed Copper, all season.



2. Fixed Copper, early (2 applications); Ferbam and Glyodin, late.

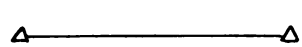


4. Ferbam and Glyodin, early (2 applications); Fixed Copper, late.

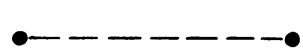


5. Ferbam and Glyodin, early (2 applications); Nu-iron and Glyodin, late.

6. Cyprex, all season.



7. Parathion, early (2 applications); Actidione and Ferbam, late.



8. Parathion, early (2 applications); Actidione and Nu-iron, late. (Sevin was also used for cherry fruit-fly control.)

Also, 1-7 were treated with lead arsenate all season.

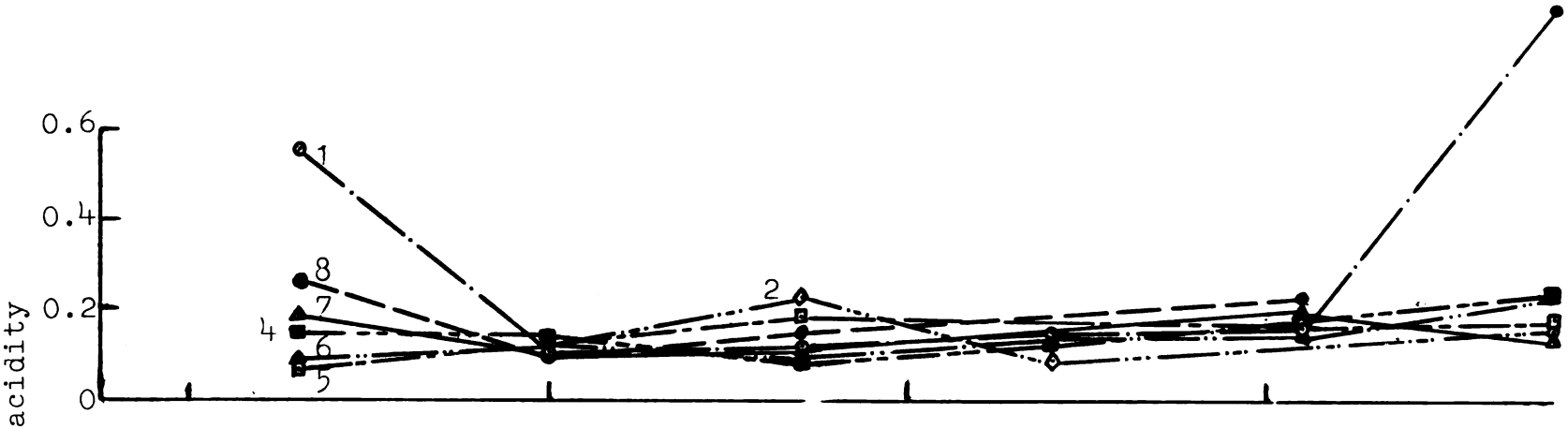


FIGURE 8. EFFECT OF SPRAY MATERIALS ON THE GALACTURONIC ACID CONTENT DURING FRUIT MATURATION - 1960

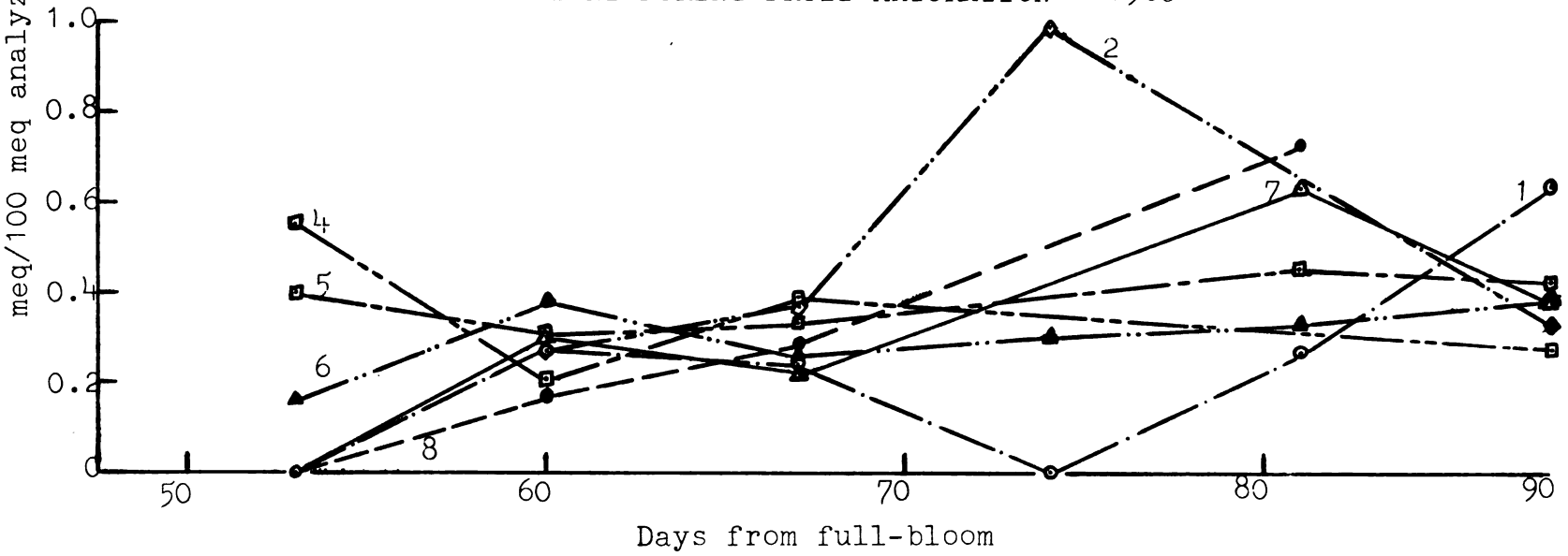


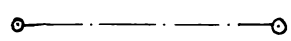
FIGURE 9. EFFECT OF SPRAY MATERIALS ON THE GLUCURONIC ACID CONTENT DURING FRUIT MATURATION - 1960

Succinic Acid: Succinic acid showed no marked change until the third picking, when it started to rise with a peak at the time of the fifth picking, corresponding to the commercial harvest; then it dropped slightly. (Figure 10).

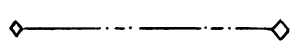
Malic Acid: Malic acid, the major acid in red tart cherries, increased in quantity in the early period of fruit development, then showed a dip and increased again. (Figure 11). Malate constituted about 75 - 88% of the total acidity in this particular treatment. Why it accumulates in such high concentrations is not very clear. It is believed that acid accumulation at any stage implies only higher production than withdrawal rates (76). If there is an active malate synthetase in cherries as there is in castor beans (71), then an explanation of the malic acid accumulation in red cherries is possible.

Citric Acid: There was a drop in the citric acid content from the first to the second picking, leveled off until the fourth picking and gradually increased up to the sixth, the last picking (Figure 12). The changes in citrate during the development of the fruit did not show an inverse relation to those in malate, as was found in potatoes (107) and tobacco leaves (14). There appeared to be some inverse relation between the two acids, citrate and malate, in the first three pickings, but this may not necessarily mean conversion of citric to malic.

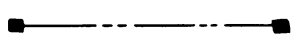
Spray Treatments



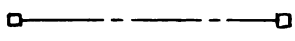
1. Fixed Copper, all season.



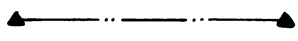
2. Fixed Copper, early (2 applications); Ferbam and Glyodin, late.



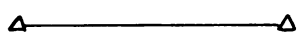
4. Ferbam and Glyodin, early (2 applications); Fixed Copper, late.



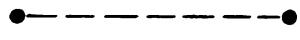
5. Ferbam and Glyodin, early (2 applications); Nu-iron and Glyodin, late.



6. Cyprex, all season.



7. Parathion, early (2 applications); Actidione and Ferbam, late.



8. Parathion, early (2 applications); Actidione and Nu-iron, late. (Sevin was also used for cherry fruit-fly control.)

Also, 1-7 were treated with lead arsenate all season.

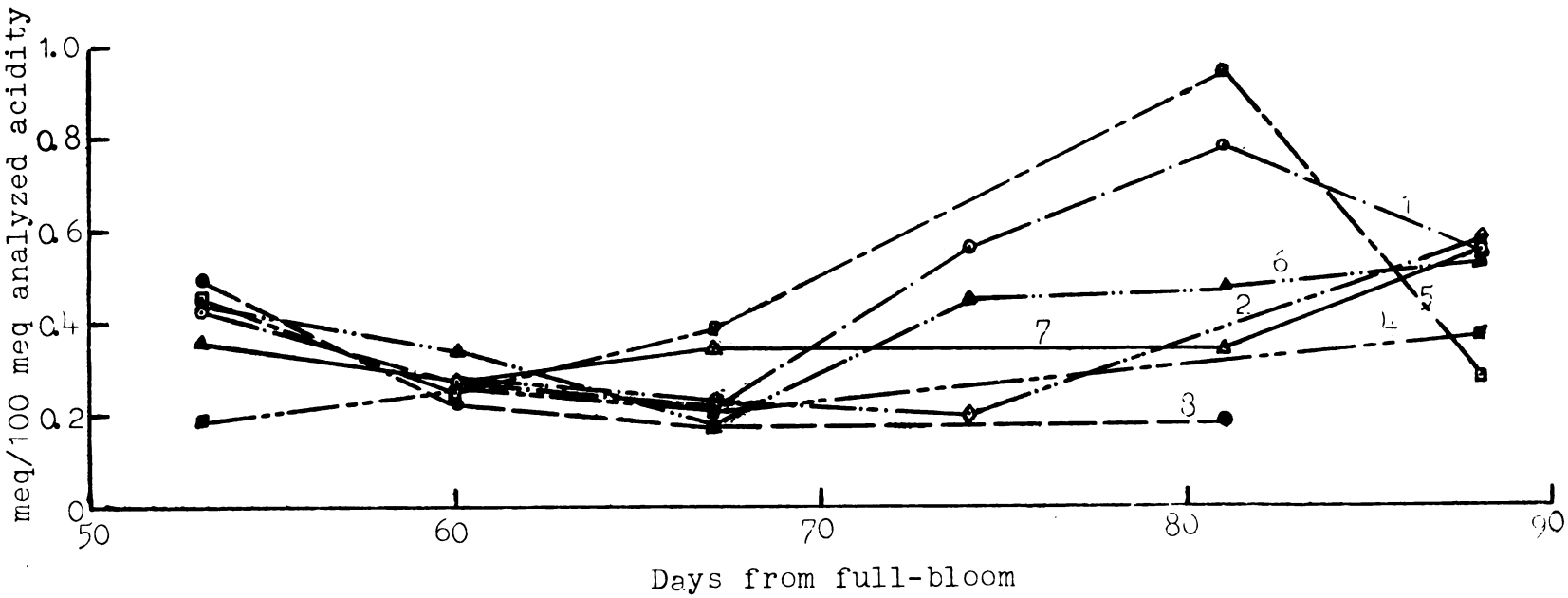
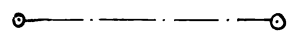
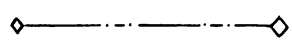


FIGURE 10. EFFECT OF SPRAY MATERIALS ON THE SUCCINIC ACID CONTENT DURING FRUIT MATURATION - 1960

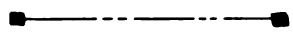
Spray Treatments



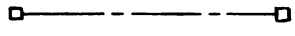
1. Fixed Copper, all season.



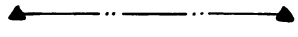
2. Fixed Copper, early (2 applications); Ferbam and Glyodin, late.



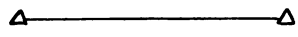
4. Ferbam and Glyodin, early (2 applications); Fixed Copper, late.



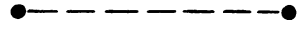
5. Ferbam and Glyodin, early (2 applications); Nu-iron and Glyodin, late.



6. Cyprex, all season.



7. Parathion, early (2 applications); Actidione and Ferbam, late.



8. Parathion, early (2 applications); Actidione and Nu-iron, late. (Sevin was also used for cherry fruit-fly control.)

Also, 1-7 were treated with lead arsenate all season.

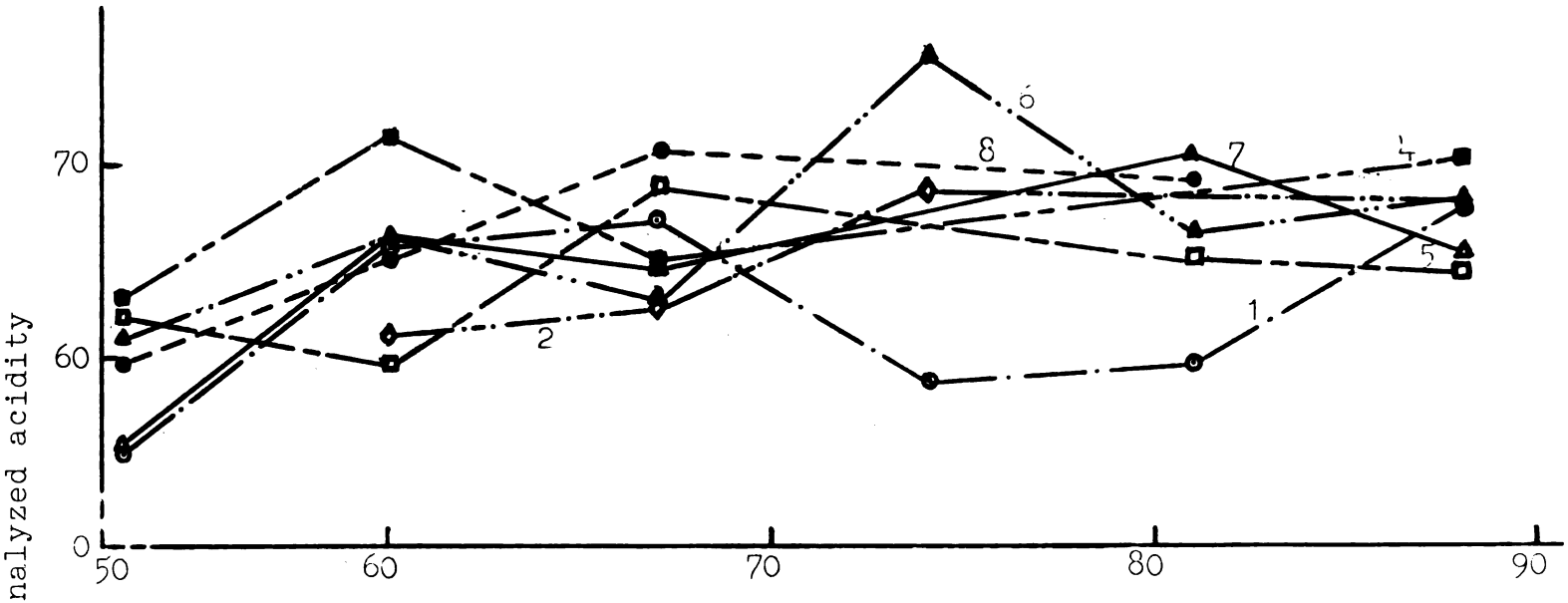
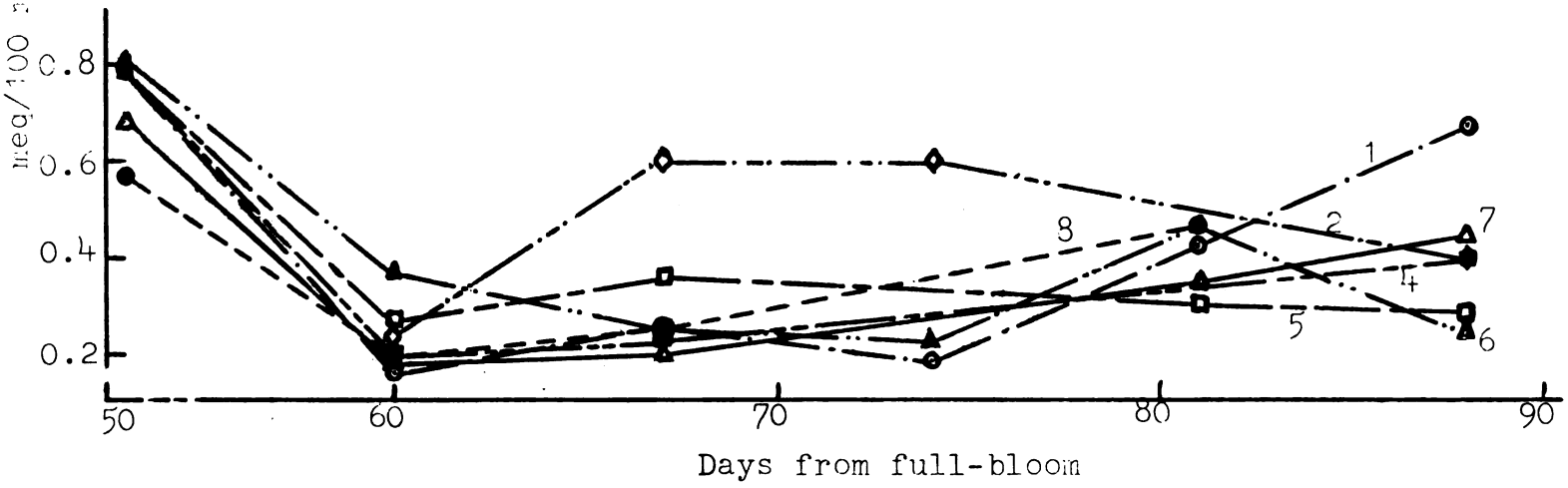


FIGURE 11. EFFECT OF SPRAY MATERIALS ON THE MALIC ACID CONTENT DURING FRUIT MATURATION - 1960



Days from full-bloom

FIGURE 12. EFFECT OF SPRAY MATERIALS ON THE CITRIC ACID CONTENT DURING FRUIT MATURATION - 1960

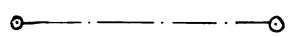
Polyphenolic Acids: The discussion on the changes in chlorogenic, isochlorogenic and neochlorogenic acids is based on the cumulative value of the three, not only because they belong to the same group of phenolic acids but also because they showed a similar trend of change during maturation. (Figure 13). It is possible that they are all responsible for the enzymatic or non-enzymatic browning in fruits and vegetables. A high concentration of these acids occurred in the green fruits, gradually dropped off until about a week before the commercial harvest, then they increased again, reaching a high concentration in the overripe fruits, approaching that in the green fruits.

Phosphoric Acid: Phosphoric acid decreased slightly during the first week, increased until the day of commercial harvest, and then leveled off (Figure 14). This change during fruit maturation paralleled that in aspartic and quinic acids, while was opposite to that of malic acid.

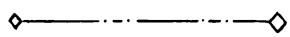
The data for the 1961 and 1962 copper-treated samples are given in Tables 14-15 and 18-19, respectively.

Aspartic Acid: In 1961, the aspartic acid content showed a small peak at the third picking, dropped and leveled off in the next two pickings, rose to a maximum at the time of commercial harvest, and then decreased markedly. In 1962, it did show a peak at the second picking, decreased to almost nothing and remained at this level. The maximum in aspartic

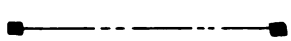
Spray Treatments



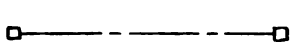
1. Fixed Copper, all season.



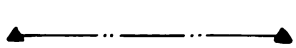
2. Fixed Copper, early (2 applications); Ferbam and Glyodin, late.



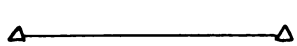
4. Ferbam and Glyodin, early (2 applications); Fixed Copper, late.



5. Ferbam and Glyodin, early (2 applications); Nu-iron and Glyodin, late.



6. Cyprex, all season.



7. Parathion, early (2 applications); Actidione and Ferbam, late.

8. Parathion, early (2 applications); Actidione and Nu-iron, late. (Sevin was also used for cherry fruit-fly control.)

Also, 1-7 were treated with lead arsenate all season.

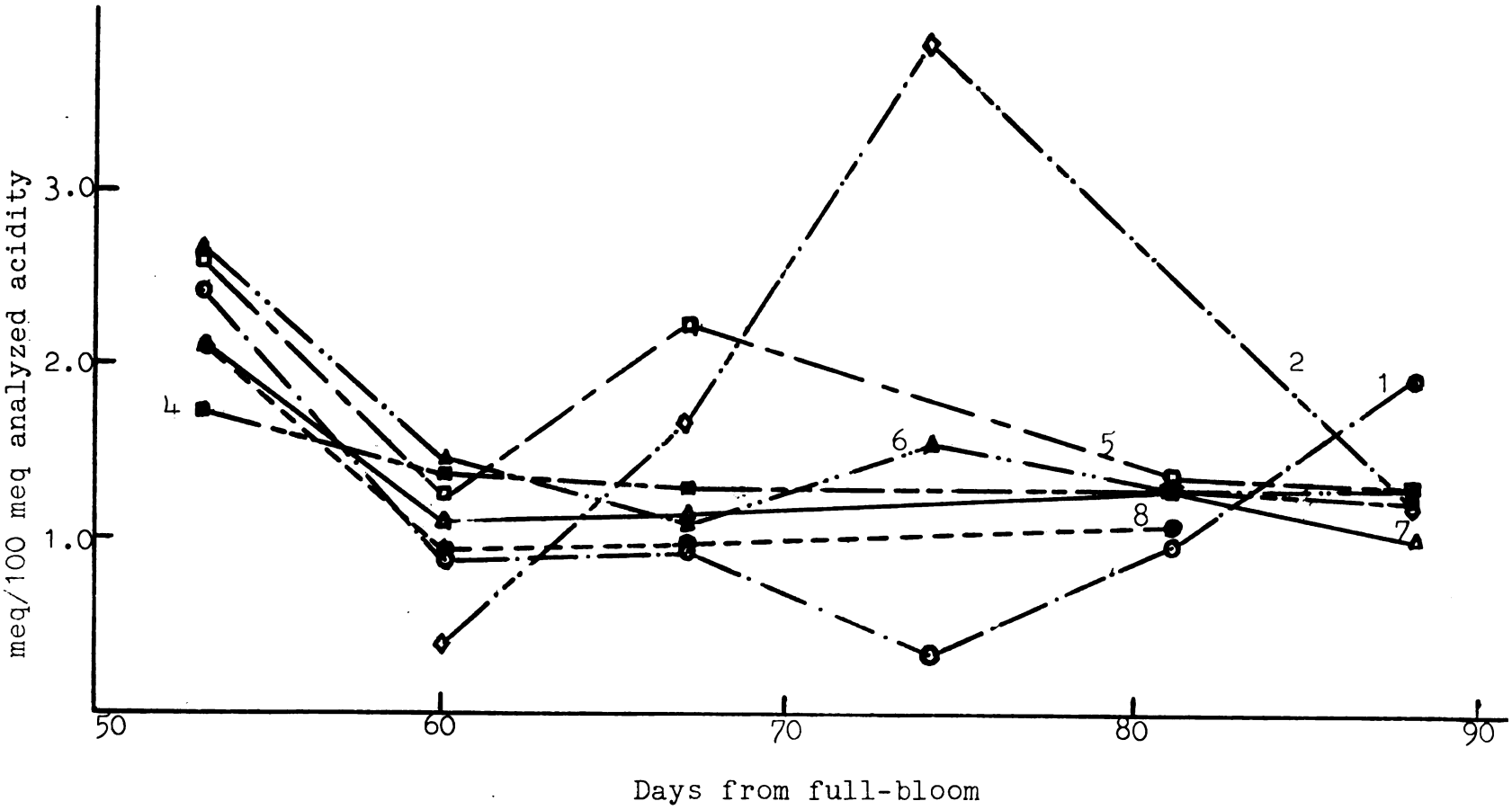
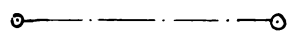
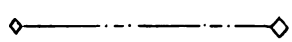


FIGURE 13. EFFECT OF SPRAY MATERIALS ON THE POLYPHENOLIC ACIDS CONTENT DURING FRUIT MATURATION - 1960

Spray Treatments

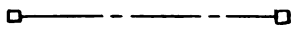


1. Fixed Copper, all season.

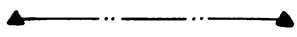


2. Fixed Copper, early (2 applications); Ferbam and Glyodin, late.

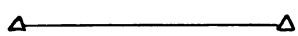
4. Ferbam and Glyodin, early (2 applications); Fixed Copper, late.



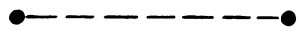
5. Ferbam and Glyodin, early (2 applications); Nu-iron and Glyodin, late.



6. Cyprex, all season.



7. Parathion, early (2 applications); Actidione and Ferbam, late.



8. Parathion, early (2 applications); Actidione and Nu-iron, late. (Sevin was also used for cherry fruit-fly control.)

Also, 1-7 were treated with lead arsenate all season.

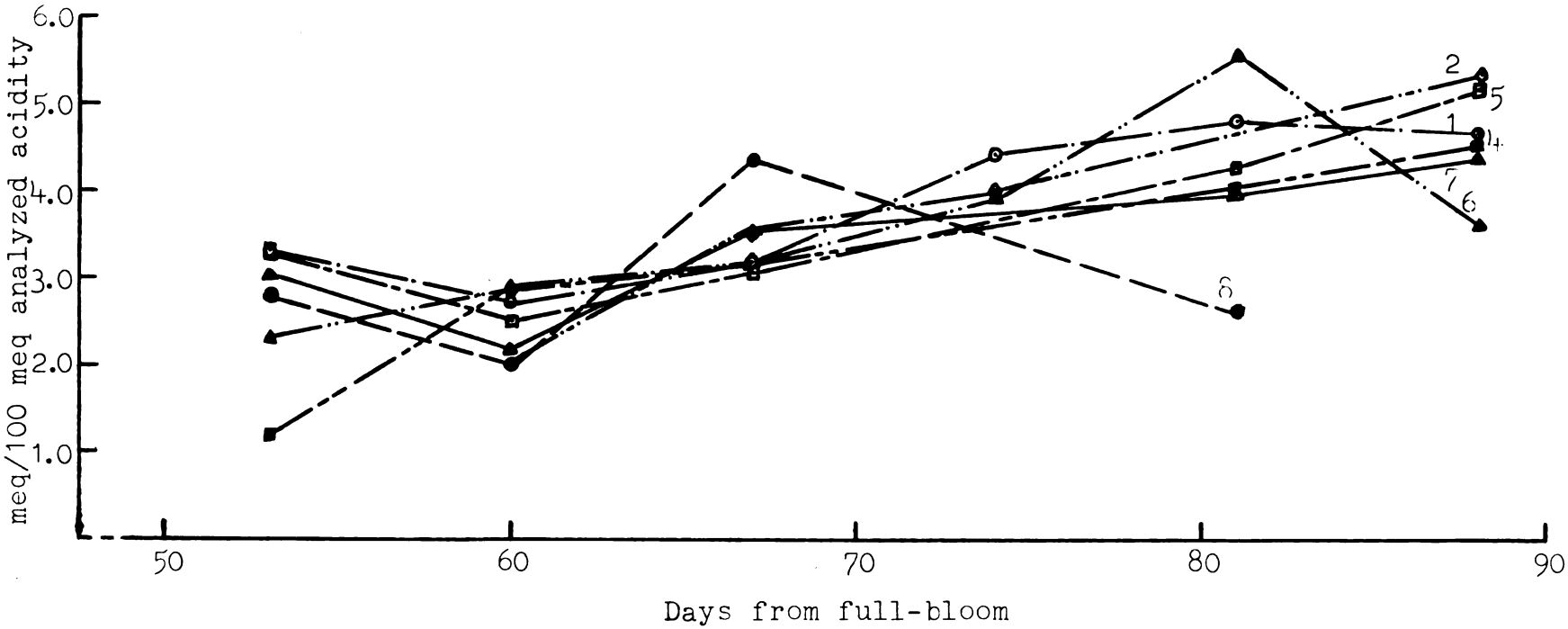


FIGURE 14. EFFECT OF SPRAY MATERIALS ON THE PHOSPHORIC ACID CONTENT DURING FRUIT MATURATION - 1960

acid content coincided with the time of commercial harvest in 1961, while no peak was obtained at this time in 1962. The maximum amount of this acid occurred at the second picking in 1962. (Figure 15)

Shikimic Acid: In both the 1961 and 1962 years, shikimic acid showed the same trend with the maximum in the green fruits. (Figure 17)

Quinic Acid: Maximum amount of quinic acid was found in the green fruits and decreased to nothing in the overripe fruits both in 1961 and 1962 seasons. Only in 1961 a small peak occurred at the time of commercial harvest (Figure 16). Changes in quinic acid paralleled those in aspartic and shikimic acids in both the years, with slight variations in the first one or two pickings.

Uronic Acids: Both galacturonic and glucuronic acids showed no maxima in the overripe fruits, either in 1961 or 1962. In 1961, these acids were very low throughout the harvest period, while in 1962 they showed a gradual decline to nothing in the ripe fruits. (Figures 18 and 19)

Succinic Acid: A high concentration of succinic acid occurred in the green fruits and showed a gradual decline in both the 1961 and 1962 seasons. (Figure 20)

Malic Acid: The changes in malic acid (in the two seasons 1961 and 1962) showed a parallel trend except in the second and the third week. The general trend was an increase

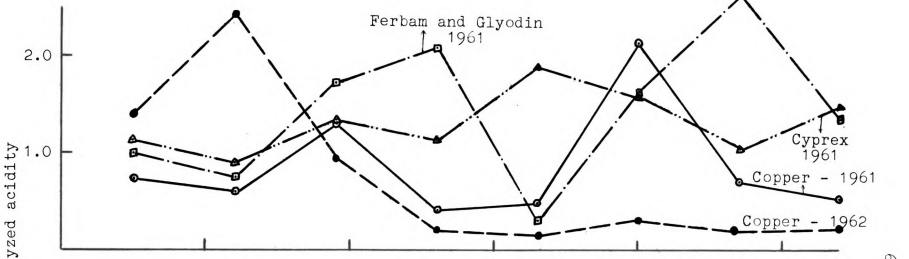


FIGURE 15. EFFECT OF SPRAY MATERIALS ON THE ASPARTIC ACID CONTENT DURING FRUIT MATURATION - 1961-1962

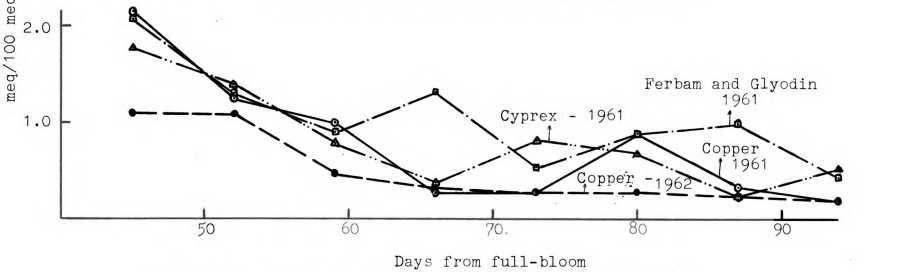


FIGURE 16. EFFECT OF SPRAY MATERIALS ON THE QUINIC ACID CONTENT DURING FRUIT MATURATION - 1961-1962

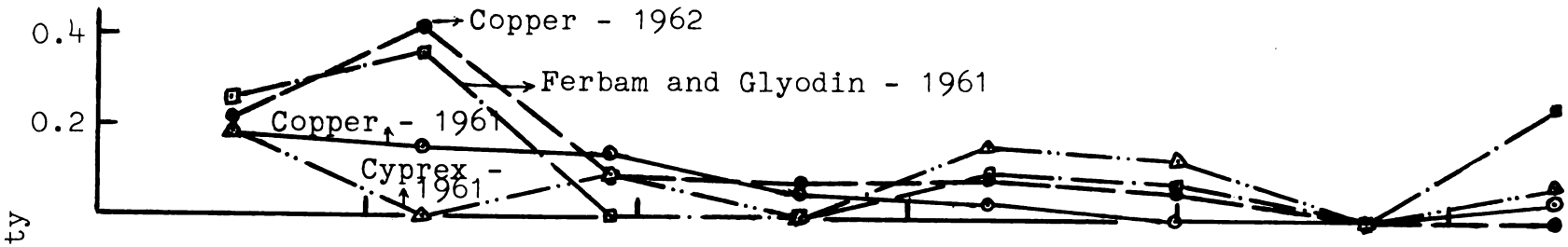


FIGURE 17. EFFECT OF SPRAY MATERIALS ON THE SHIKIMIC ACID CONTENT DURING FRUIT MATURATION - 1961-1962

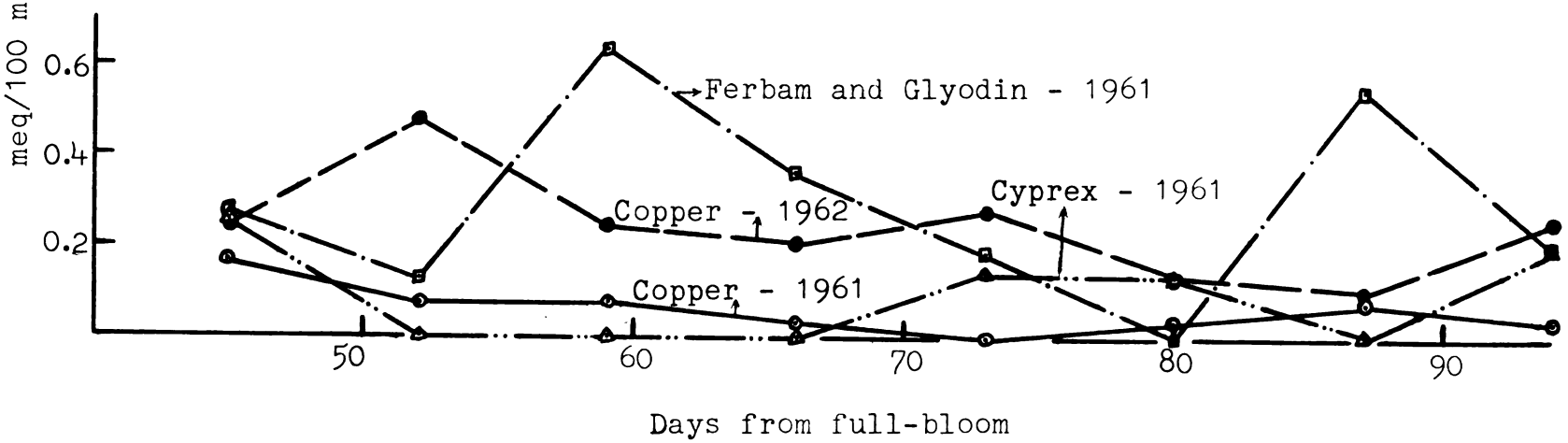


FIGURE 18. EFFECT OF SPRAY MATERIALS ON THE GLUCURONIC ACID CONTENT DURING FRUIT MATURATION - 1961-1962

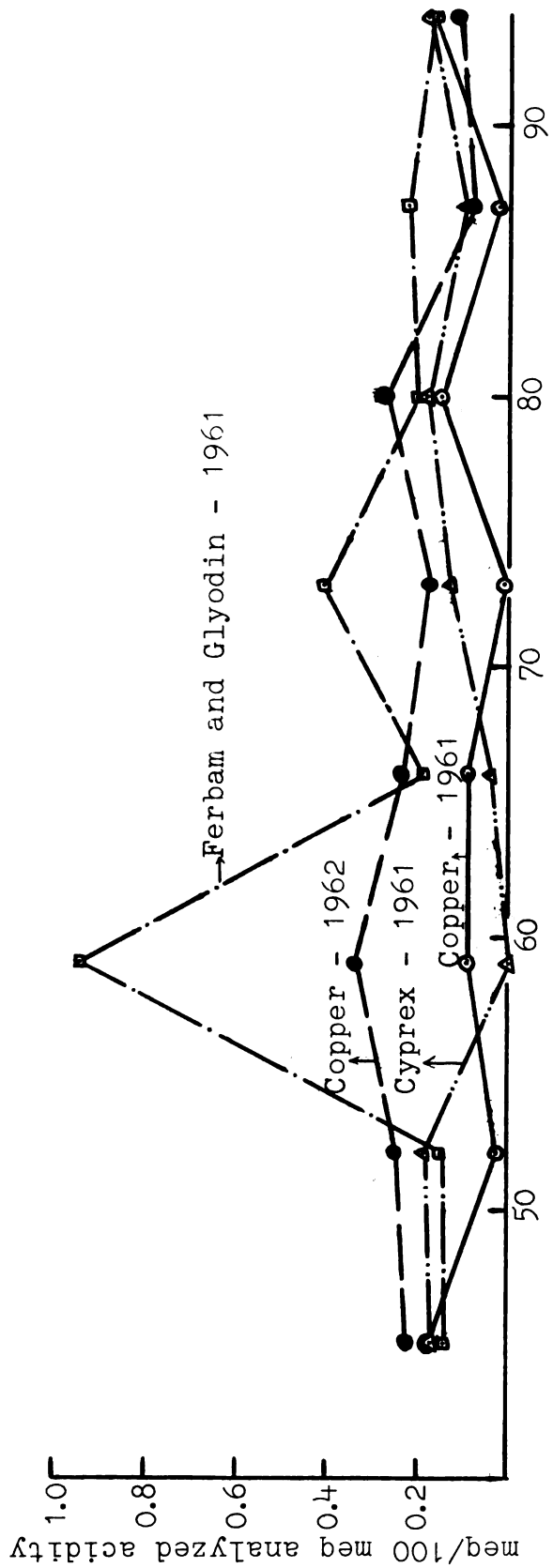


FIGURE 19. EFFECT OF SPRAY MATERIALS ON THE GALACTURONIC ACID CONTENT DURING FRUIT MATURATION - 1961-1962

in its quantity as the fruits matured. The maximum level of this acid occurred a week after the commercial harvest. (Figure 22)

Citric Acid: Citric acid showed a certain pattern during fruit development in 1961 and another in 1962. There was a high concentration of citric acid in the green fruits in 1961, decreased until the fourth picking and then leveled off. But, in 1962, it started with a low concentration in the green fruits, showed peaks at the third and the fifth pickings, and then leveled off (Figure 21). In 1961, changes in citric acid showed an inverse change relation to those in malic acid (only in the first six pickings), while in 1962, there was a positive inverse change relation between the two acids throughout the period of fruit development.

Polyphenolic Acids: The combined values of the three polyphenolic acids, both in 1961 and 1962, showed a high concentration in the green fruits, dropped gradually until the day of the commercial harvest, and then increased slightly after that. (Figure 23).

Phosphoric Acid: In both 1961 and 1962 seasons, phosphoric acid showed a general decline until the fourth picking, a peak at the fifth, and then decreased, reaching a maximum in the overripe fruits (Figure 24). In general, both in 1961 and 1962, phosphoric acid showed an inverse change relation to malic acid and a similar trend to that of citric acid.

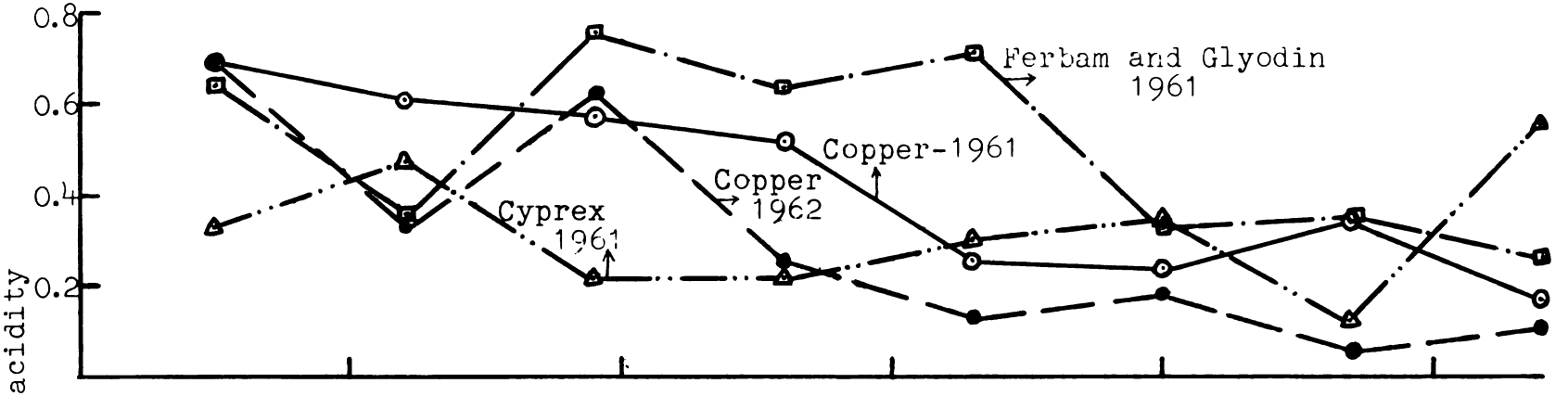


FIGURE 20. EFFECT OF SPRAY MATERIALS ON THE SUCCINIC ACID CONTENT DURING FRUIT MATURATION - 1961-1962

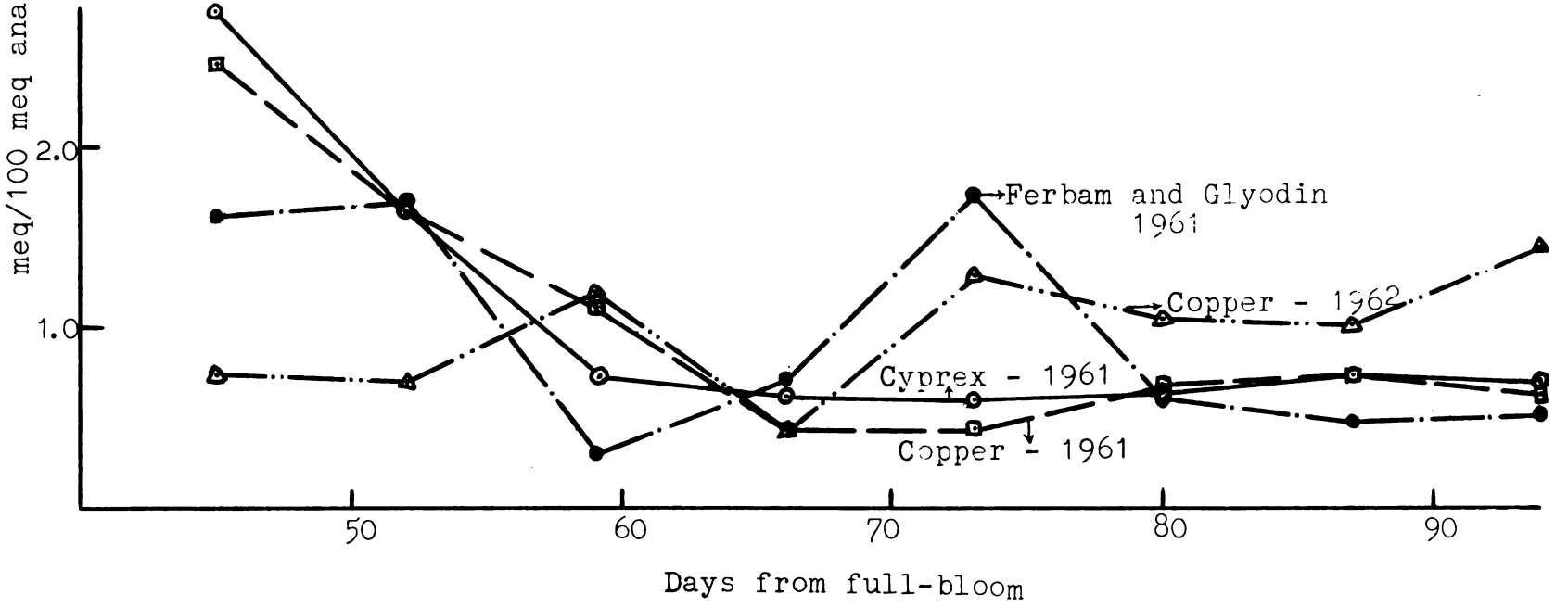


FIGURE 21. EFFECT OF SPRAY MATERIALS ON THE CITRIC ACID CONTENT DURING FRUIT MATURATION - 1961-1962

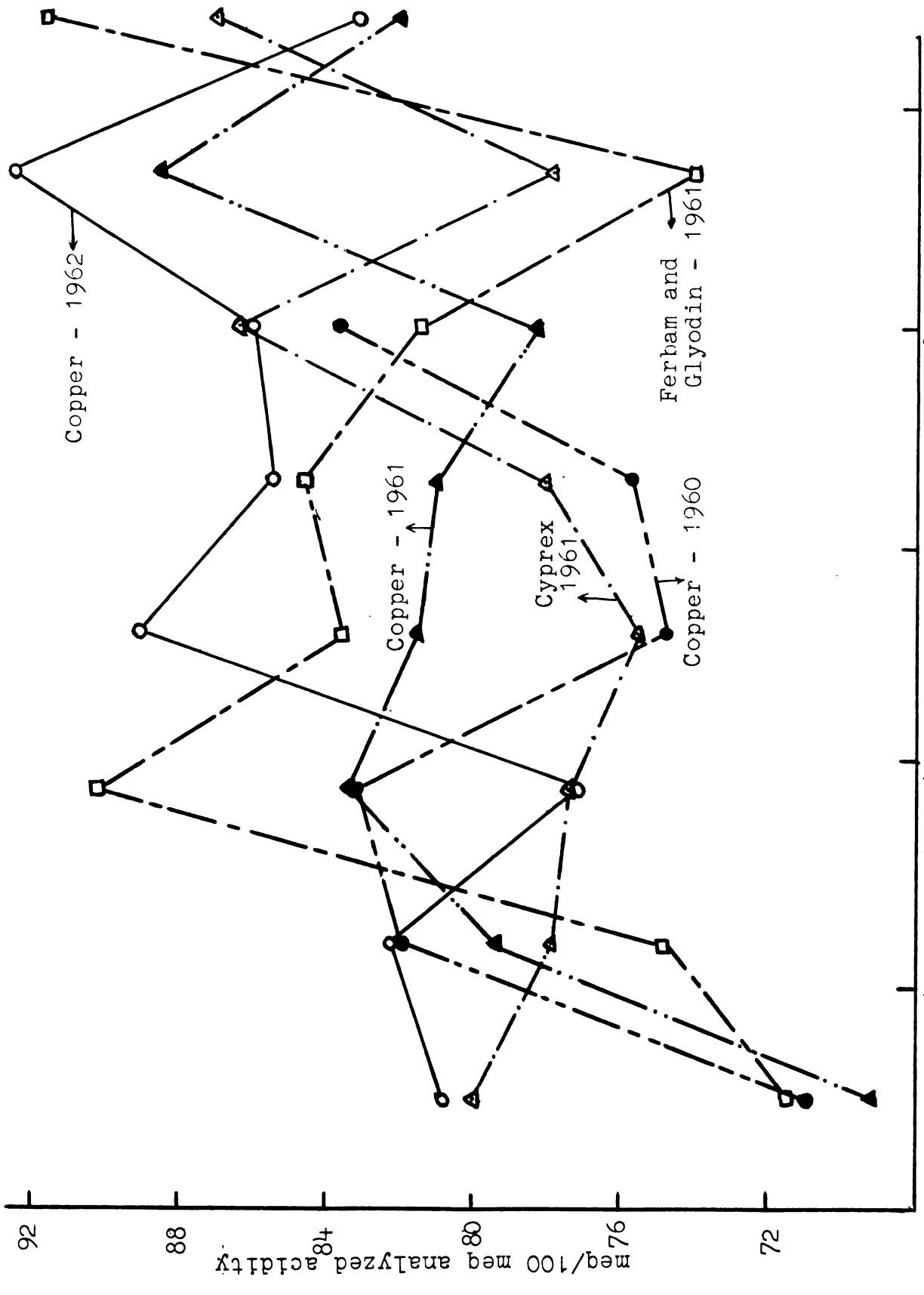


FIGURE 22. EFFECT OF SPRAY MATERIALS ON THE MALIC ACID CONTENT DURING FRUIT MATURATION - 1961-1962

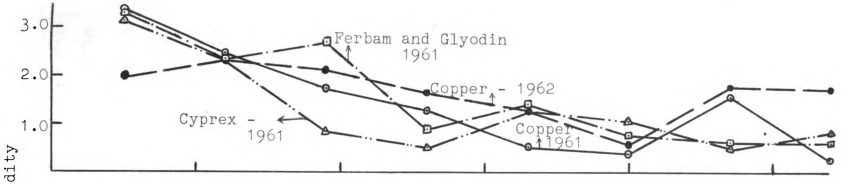


FIGURE 23. EFFECT OF SPRAY MATERIALS ON THE POLYPHENOLIC ACIDS CONTENT DURING FRUIT MATURATION - 1961-1962

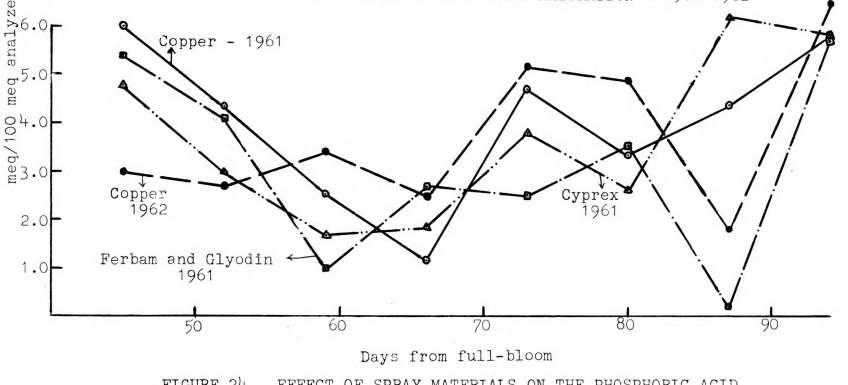


FIGURE 24. EFFECT OF SPRAY MATERIALS ON THE PHOSPHORIC ACID CONTENT DURING FRUIT MATURATION - 1961-1962

There was a very good general agreement among the copper-treated samples, as to the changes in the twelve acids during fruit development, in both 1961 and 1962 seasons. Wherever there was any disagreement it was only in one or two pickings, out of the eight for each acid.

E. EFFECT OF SPRAY TREATMENTS ON INDIVIDUAL ACIDS

The data for the eight fungicidal sprays studied in 1960 are given in Tables 5 through 12. For convenience, the sprays will be referred to by number in order given on Page 19 and as shown in the figures. Some of the samples were lost or discarded because they turned cloudy after a few weeks storage at -10°F. These cloudy samples showed a change in the total titratable acidity from that obtained right after extraction of the acids. The reason for this cloudiness was not known, since all samples were treated in a similar manner for the extraction of the acid fraction. Ferbam and Glyodin spray treatment was not included in the discussion since only the first three samples out of the six were analyzed and the data tabulated.

Aspartic (Figure 5): There seemed to be two different trends in its changes during fruit development. While sprays 1, 2, 5 and 6 showed no marked changes during the first two weeks after which it increased to a peak at commercial harvest, then leveled off; sprays 4, 7 and 8

showed almost an opposite trend, although this may not be true in the second half of the season since a few of the values are missing.

Shikimic (Figure 6): There was the same general trend in all treatments which paralleled the changes of the Copper spray, although some showed a high and some a low concentration of shikimic acid in the green fruits. Only in spray 7 was a peak obtained at the time of the commercial harvest (Table 11).

Quinic (Figure 7): Changes in all the treatments followed the same general trend as the Copper spray, except at the time of the commercial harvest, when sprays 1, 5 and 6 showed a peak. Also, spray 6 showed a very high peak at the third picking (Table 10), while all others showed only a very slight rise, if any, at this stage.

Galacturonic (Figure 8): There was no change in the concentration of this acid throughout the period of fruit development in any of the spray treatments, except the Copper spray. In the copper treatment, as already discussed, it showed a very high concentration in both the very green and the overripe fruits. It is hard to say if this is typical of Copper sprays; since 1961 and 1962, Copper spray treatments did not show this trend.

Glucuronic (Figure 9): It did not show any regular pattern in its changes during the whole period of fruit develop-

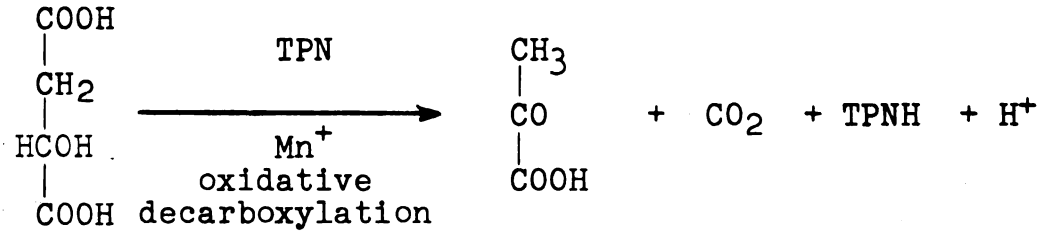
ment in the various treatments. But, either toward the end of this period or in the overripe fruits, there is a definite indication of a higher concentration of this acid than in the green fruits, in most treatments or just about the same concentration in some (spray 4 and 5). Only spray 2 showed a peculiarly big peak at the fourth picking which declined as the fruits became overripe. (Table 6)

Succinic (Figure 10): As far as the changes in this acid are concerned, all the treatments seemed to agree with the trend in the control. They all showed a higher concentration at the time of commercial harvest, than in the green fruits, except spray 7 where succinic acid seemed to drop off in the ripe fruits.

Malic (Figure 11): There was no regular pattern in its changes in the various treatments during fruit development but the general trend was towards an increasing concentration of malic acid as the fruits matured, with a dip either at the time of harvest or a week before this time in most of the seven treatments. This dip as mentioned before, was noticed not only in most of the 1960 treatments, but also in all of the 1961 and 1962 treatments. It generally occurred between sixty and seventy-four days in 1960 and between sixty-six and eighty days from full-bloom in 1961 and 1962.

Whether or not this decrease in malic acid is related to a climacteric in red cherries cannot be established.

Graham (37) found an indication of a climacteric in Montmorency cherries about five to seven days before the period of commercial harvesting which corresponds to the drop in malic acid content. However, Pollack, et al. (100) found no evidence of a climacteric rise either while the fruit was on the trees or after harvest. Hulme and Neal (58) have theorized that at the time of the climacteric in apples a malate decarboxylation reaction occurs which results in the decrease of malic acid. So, possibly, there is a climacteric in red tart cherries which coincides with a significant drop in malic acid, due probably to the malate-decarboxylation reaction (33):



Malic Acid Pyruvic Acid

Citric (Figure 12): There is a general similar trend in all treatments, except spray 2, with regard to the changes in citric acid, which was identical to the one in the control, high concentration in the green, ripe and overripe fruits with a low plateau in the middle. Spray 2 showed a plateau at the stage where the acid was the highest.

Polyphenolic Acids Fraction (Figure 13): It showed a high concentration in the green fruits in all treatments, which dropped during the first week and leveled off to the end in most of the treatments. In the control treatment, it dropped until the fourth picking (Table 5), after which it increased, while with spray 2, it showed a gradual increase up to the fourth picking (Table 6) where it showed a very high peak and then declined. Spray 5 (Table 9) showed a small peak at the time of the third picking.

Phosphoric: Except during the first week, where some treatments showed an increase, and others showed a decrease in phosphoric acid content, the changes in this acid were similar during the rest of the period of fruit development. All treatments showed a gradual increase in this acid until the time of the commercial harvest, after which spray 6 and 8 showed a drop while all others continued to show an increase. Therefore, with the exception of sprays 6 and 8, the spray treatments paralleled the trend shown by the copper treatment.

In summary, the results of 1960 indicate no great variations between the various treatments in their effect on the acids during fruit maturation. Treatments 2 through 8 differed from the control only in two acids, polyphenolic and galacturonic, and treatments 4, 7 and 8 only in aspartic acid. For all other acids there was a fairly good agreement among the treatments and the control. There were minor variations among treatments, as to the trends, but they were

not consistent as to the time when they occurred.

In 1961, three fungicidal sprays, Ferbam and Glyodin, Copper and Cyprex were used and the data under each of them tabulated in Tables 12-13, 14-15 and 16-17, respectively. The 1962 Copper treatment was not included in this discussion since it has already been discussed with the 1961 Copper treatment.

Aspartic (Figure 15): The changes in this acid during the fruit maturation, follow the same trend of a gradual increase in all three spray treatments, although the maximum reached was one week earlier in Cyprex and one week later in Ferbam and Glyodin treatment, as compared to the Copper spray.

Shikimic (Figure 17): All treatments showed the same trend in changes in this acid -- a gradual decline as the fruit ripened. Ferbam and Glyodin treatment showed a peak at the second picking (Table 13) like the 1962 Copper treatment but was not found in the other treatments.

Quinic (Figure 16): In all three treatments it decreased to almost nothing, the only difference among them being the time when a small peak showed up during the latter part of fruit development. While the peak coincided with the time of commercial harvest in the control, it was a week early in Cyprex and a week later in the Ferbam and Glyodin treatment. The peaks occurred at the same time as the aspartic acid peaks.

Uronic Acids (Figures 18 and 19): The galacturonic and glucuronic acid values of both Copper and Cyprex were low and consistent during the harvest period. However, in the Ferbam and Glyodin treatment a high peak (maximum) was obtained in both the acids at the time of the third picking (Table 13), then declined gradually with a small peak at the seventh picking in the case of galacturonic acid and at the fifth picking in the case of glucuronic acid.

Succinic (Figure 20): The changes in this acid in the Ferbam and Glyodin treatment were parallel to those of the copper, except at the fifth picking (Table 13) where it showed a peak, while those in the Cyprex treatment were exactly opposite to the ones in the copper. But the general trend in all was toward a gradual decline with fruit maturation although Cyprex treatment showed a maximum of succinic acid in the overripe fruits.

Malic (Figure 22): Here changes in Ferbam and Glyodin treatment were just the opposite to the ones in Cyprex, except in the last two weeks, when the same trend occurred. On the other hand, the trend in the changes of this acid in Ferbam and Glyodin treatment followed the one in the copper for this year except in the last two weeks, when it went the opposite direction. The dip that was discussed earlier was seen in all three treatments, with a difference as to the time when it occurred. While it appeared at the time of commercial harvest in the Copper treatment, it showed up two weeks before this

date in both the other treatments. This, again, may or may not be a treatment difference since the dip in the 1962 Copper treatment occurred two weeks earlier to the date of commercial harvest.

Citric (Figure 21): Again, there was agreement between Cyprex and the Copper regarding the changes in this acid during fruit development. Also, Ferbam and Glyodin treatment showed the same trend as the copper, with the exception that it showed a peak at the fifth picking (Table 13), while there is none in the copper. This peak coincides with the one in the 1962 Copper treatment. This acid showed a high concentration in the green fruits, declined until the third picking (59 days from full-bloom) and then leveled off.

Polyphenolic Acid Fraction (Figure 22): There was a good agreement among the three treatments as to the trend in changes in this fraction, during fruit development. Starting with a high concentration in green fruits, they all showed a gradual decline with fruit maturation.

Phosphoric (Figure 23): The changes in this acid also were very much the same in all three treatments. A high concentration was found in the green fruits, then it declined in the next picking and then increased again to a concentration as high as in the green fruits.

There was a very good agreement among the three spray treatments as to the changes in aspartic, shikimic, quinic, polyphenolic acid fraction and phosphoric, during fruit development. Cyprex and Copper treatments were in full agreement on the changes in uronic, citric and malic acids, while they differed very much from those in the Ferbam and Glyodin treatment. Succinic acid was the only acid whose changes in Ferbam and Glyodin treatment paralleled those of the Copper treatment and showed an opposite trend to that of Cyprex treatment. This could have been a chance variation. Generally, the changes in all acids, except succinic acid, in the Cyprex and Copper treatments were identical while Ferbam and Glyodin treatment showed dissimilarities or opposite trends in citric, malic and uronic acids.

SUMMARY AND CONCLUSIONS

1. The non-volatile acids of Montmorency cherries were determined during the maturation of the fruit and after the application of several fungicidal sprays.
2. Acidified water extraction, lead precipitation, gradient elution column chromatography, paper chromatography and titration were used to identify and quantitatively determine the acids.
3. Twenty non-volatile organic acids and one non-volatile inorganic acid were identified: glutamic, aspartic, lactic, shikimic, quinic, galacturonic, glyceric, glycolic, glutaric, glucuronic, succinic, citramalic, malic, tartaric, malonic, citric, neochlorogenic, isochlorogenic, fumaric, chlorogenic, and phosphoric.
4. In 1961-1962, the total titratable acidity showed a marked decline as the fruits matured, with a plateau at the time of the commercial harvest, while in 1960, it showed only a slight decline during maturation.
5. During fruit development changes in all acids were studied, but the results are tabulated for only twelve of them: aspartic, shikimic, quinic, galacturonic, glucuronic, succinic, malic, citric, neochlorogenic, isochlorogenic, chlorogenic, and phosphoric, since these were the major acids found in cherries.

6. The average amounts of the major acids for all three seasons found at harvest maturity (for copper treatment)

	meq./100 meq. Analyzed Acidity	mg./100 gm. Fresh Fruit
1. Aspartic	2.16	33.20
2. Shikimic	0.06	2.57
3. Quinic	0.71	31.00
4. Galacturonic	0.19	7.90
5. Glucuronic	0.06	3.40
6. Succinic	0.33	5.77
7. Malic	83.71	1259.00
8. Citric	0.66	6.30
9. Polyphenolic acids	0.45	61.40
10. Phosphoric	4.56	28.10

7. The data obtained for Fixed Copper (proprietary copper) with lime treatment for the three seasons 1960, 1961, and 1962, showed similar changes in all individual acids during fruit maturation, with the exception of succinic and the uronic acids.

8. Aspartic, quinic and phosphoric acids showed similar trends in their changes during fruit maturation for all spray treatments.

9. Phosphoric acid showed an opposite trend in changes to that of malic acid in all three years in the copper spray treatment and in most of the other spray treatments.

10. There was an inverse change relation between malic and citric in the Copper treatment in 1961 and 1962 and in Ferbam and Glyodin treatment in 1961. None was found in the other spray treatments.

11. Either at the time of commercial harvest or a week before it, there was a dip in the malic acid content in almost all treatments in 1960 and in all the treatments in 1961-1962. There is a possibility that this dip may be due to a malate-decarboxylation reaction.
12. The fungicidal sprays used in 1960 had no effect on the changes in individual acids during fruit maturation.
13. In 1961, the changes in all acids, except succinic acid, in the Cyprex and Copper spray treatments were similar. An opposite trend was found for citric, malic and uronic acids in the Ferbam and Glyodin spray treatment to that obtained for the Copper and Cyprex treatments.

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APPENDIX

TABLE 1. Rf x 100 VALUES IN TWO DIFFERENT SOLVENTS, THE % RECOVERIES ON DOWEX-1 AND 50, AND pK VALUES OF ACIDS OF RED CHERRIES. (Acids are listed in order of their emergence from the anion exchange column.)

ACIDS	Rf x 100 Values				% Recovery		pK Values
	Solvent I		Solvent II				
	K	Unk	K	Unk	Dowex-1	Dowex-50	
1. Glutamic	10	11	36	35	93.0	13.0	2.19, 4.25
2. Aspartic	9	9	29	28	97.0	3.0	1.88, 3.65
3. Lactic	73	74	79	80	Trace	-	3.86
4. Shikimic	39	38	61	60	101.5	100.0	-
5. Quinic	26	25	64	63	100.0	100.0	-
6. Galacturonic	9	10	46	45	93.0	100.0	-
7. Glyceric	45	46	68	67	100.0	100.0	1.47, 6.19
8. Glycolic	62	60	65	64	100.0	100.0	3.82
9. Glutaric	79	78	61	60	90.6	100.0	4.34
10. Glucuronic	10	10	48	48	71.0	100.0	-
11. Succinic	73	71	55	55	100.0	100.0	4.19, 5.57
12. Citramalic	62	61	-	-	100.0	100.0	2.48
13. Malic	51	50	45	46	100.0	100.0	3.40, 5.05
14. Tartaric	34	33	17	17	-	-	3.02, 4.54
15. Malonic	68	66	48	48	100.0	100.0	2.85, 6.10
16. Citric	44	44	22	22	100.0	100.0	3.06, 4.74, 5.40

TABLE 1. continued

ACIDS	Rf x 100 Values				% Recovery		pK Values
	Solvent I		Solvent II				
	K	Unk	K	Unk	Dowex-1	Dowex-50	
17. Neochlorogenic	36	35	-	-	-	-	-
18. Isochlorogenic	69	68	81	82	-	-	-
19. Fumaric	84	83	48	47	100.0	100.0	3.03, 4.47
20. Chlorogenic	61	60	68	69	61.0	100.0	-
21. Phosphoric	39	40	10	10	100.0	100.0	2.12, 7.21, 12.32

K = Known or pure acid.

Unk = Unknown from cherries.

Solvent I - n-Butanol + 3N Formic Acid, 1:1

Solvent II - Ethanol + ammonium hydroxide + water, 20:1:4

TABLE 2. Rf x 100 VALUES OF OTHER PURE ACIDS STUDIED

	ACID	Rf x 100 Values Solvent I	Solvent II
1.	Aconitic	78	26
2.	Adipic	86	59
3.	p - Anisic	14	-
4.	Anthranilic (O-NH_2 Benzoic)	-	-
5.	Ascorbic	31	-
6.	Benzoic	89	-
7.	p - hydroxybenzoic	-	-
8.	Caffeic	76	75
9.	Chelidonic	-	51
10.	Cinnamic	-	52
11.	Isocitric	49	18
12.	Citraconic	73	61
13.	o - coumaric	88	79
14.	p - Coumaric	86	-
15.	Ferulic	-	-
16.	Gallic	60	55, 61
17.	Gentisic	61, 87	83
18.	Glyoxylic	73	50
19.	Homogentisic	-	-
20.	Hydrocaffeic	88	-
21.	α-Ketoglutaric	64	48
22.	Maleic (dihydroxy)	24	26, 48

TABLE 2. continued

ACID	Rf x 100 Values Solvent I	Solvent II
23. Meconic	15	57
24. Mucic	-	-
25. Oxalic	55	14
26. Oxalacetic	57	43
27. 1, 2, 3-Propanetricarboxylic	70, 86	83
28. Protocatechuic	78	68
29. o - pyrocatechuic	69, 86	81
30. ∝- Pyrrolidone carboxylic	56	65
31. Pyruvic	79	48, 55
32. Saccharic	-	-
33. Salicylic	90	89
34. Sulfuric	33	15
35. Tartaric (meso)	34	17
36. Trimesic	88	25
37. DL - Tropic	88	-
38. Veratric	-	-

TABLE 3. TOTAL TITRATABLE ACIDITY FOR ALL TREATMENTS IN 1960

PICKING NO.		1	2	3	4	5	6
DAYS FROM FULL BLOOM		53	60	67	74	81	88
	TREATMENT	meq/100 GMS FRESH FRUIT					
1.	Fixed Copper	32.48	26.77	25.98	25.54	23.17	25.76
2.	Copper and Ferbam and Glyodin	33.54	25.98	23.28	26.05	-	23.18
3.	Ferbam and Glyodin	27.04	25.30	21.36	-	-	18.24
4.	Ferbam and Glyodin and Copper	26.30	24.90	23.67	-	-	22.36
5.	Ferbam and Glyodin and Nu-Iron and Glyodin	25.20	22.85	26.59	-	25.74	23.36
6.	Cyprex	26.40	24.62	23.54	20.66	23.66	22.98
7.	Parathion and Actidione and Ferbam	27.88	28.26	25.68	24.08	24.12	27.06
8.	Parathion and Actidione and Nu-Iron	27.84	24.82	25.27	28.80	23.86	-

TABLE 4. TOTAL TITRATABLE ACIDITY FOR ALL TREATMENTS IN 1961 and 1962.

PICKING NO.	DAYS FROM FULL BLOOM	TREATMENT 1961 COPPER	1961 CYPREX	1961 FERBAM AND GLYODIN	1962 COPPER
		meq/100 GMS FRESH FRUIT			
1	46	24.06	25.25	24.18	23.44
2	53	28.86	29.21	26.66	23.60
3	60	27.00	27.90	16.00	24.38
4	67	19.24	18.00	22.23	23.33
5	74	20.91	18.50	18.65	21.77
6	81	19.72	26.58	26.31	21.46
7	88	7.82	13.13	7.09	18.28
8	96	14.09	12.94	14.44	20.23

TABLE 5. NON-VOLATILE ACIDS OF MONTMORENCY CHERRIES DURING FRUIT DEVELOPMENT. - 1960

Fixed Copper Spray, all season.

PICKING NO.	1		2		3		4		5		6	
DAYS FROM FULL BLOOM	53		60		67		74		81		88	
ACID	A	B	A	B	A	B	A	B	A	B	A	B
1. Aspartic	3.22	69.6	2.65	47.2	3.06	52.8	4.04	68.7	5.06	78.0	4.88	83.7
2. Shikimic	1.03	58.3	0.20	9.3	0.08	3.7	0.11	5.1	-	-	-	-
3. Quinic	1.57	98.0	0.81	41.9	1.26	63.0	0.92	45.2	2.56	114.0	1.69	83.8
4. Galacturonic	0.55	34.8	0.10	5.2	0.12	6.0	0.15	7.6	0.16	7.2	0.87	43.3
5. Glucuronic	-	-	0.28	14.4	0.24	12.0	-	-	0.27	12.0	0.63	31.3
6. Succinic	0.42	8.1	0.26	4.1	0.22	3.4	0.57	8.6	0.79	10.7	0.56	8.6
7. Malic	74.98	1632.8	85.97	1542.8	87.26	1519.8	78.80	1349.3	79.69	1238.0	87.67	1514.2
8. Citric	0.98	20.4	0.36	6.1	0.45	7.4	0.38	6.2	0.62	9.2	0.86	14.2
9. Neochlorogenic*	0.45	51.4	0.13	12.4	0.26	23.7	0.10	9.2	0.30	24.4	0.70	64.1
10. Isochlorogenic*	0.41	47.5	0.16	15.2	0.20	18.4	0.11	10.3	0.18	14.5	0.48	43.6
11. Chlorogenic	1.57	180.7	0.59	55.6	0.48	43.9	0.13	12.0	0.50	41.5	0.75	68.4
12. Phosphoric	4.31	45.7	3.74	32.7	4.18	35.5	5.43	45.3	5.79	43.8	5.69	47.9
Fluorescent	2.43	279.6	0.88	83.2	0.94	86.0	0.34	31.5	0.98	80.4	1.93	176.1

A = meq/100 meq of analyzed acidity
B = Mg/100 gm fresh fruit
* = The molecular weight of these acids was assumed to be the same as that of Chlorogenic acid, since they are also caffeoyl-quinic acids.

TABLE 6. NON-VOLATILE ACIDS OF MONTMORENCY CHERRIES DURING FRUIT DEVELOPMENT. - 1960

Copper Spray, early (two applications); Ferbam and Glyodin Sprays, late[a]

	PICKING NO.	2		3		4		6	
	DAYS FROM FULL BLOOM	60		67		74		88	
	ACID	A	B	A	B	A	B	A	B
1.	Aspartic	3.73	64.6	3.53	54.6	3.68	63.8	3.93	60.6
2.	Shikimic	0.22	10.0	0.16	6.7	0.23	10.3	0.11	4.4
3.	Quinic	1.32	65.9	1.62	72.3	1.00	50.0	1.48	66.1
4.	Galacturonic	0.12	6.0	0.23	10.5	0.09	4.5	0.16	7.0
5.	Glucuronic	0.27	13.6	0.37	16.9	0.99	50.1	0.33	14.9
6.	Succinic	0.28	43.1	0.23	31.9	0.20	30.7	0.49	67.3
7.	Malic	81.07	1412.1	82.58	1288.9	88.42	1544.2	88.31	1372.5
8.	Citric	0.44	73.0	0.80	190.8	0.80	13.3	0.59	87.7
9.	Neochlorogenic*	0.10	9.2	0.27	22.7	1.25	115.1	0.20	16.3
10.	Isochlorogenic*	0.20	18.4	0.42	34.4	1.00	92.1	0.40	32.9
11.	Chlorogenic	0.10	9.2	0.98	80.8	1.61	148.8	0.61	50.0
12.	Phosphoric	3.05	25.9	4.54	34.5	5.01	42.6	6.36	48.2
	Fluorescent	0.40	36.8	1.67	137.9	3.86	356.0	1.21	99.2

A = meq/100 meq of analyzed acidity
B = Mg/100 gm fresh fruit
* = The molecular weight of these acids was assumed to be the same as that of Chlorogenic acid, since they are also caffeoylquinic acids.
a = No data obtained for the 1st and 5th pickings.

TABLE 7. NON-VOLATILE ACIDS OF MONTMORENCY CHERRIES DURING FRUIT DEVELOPMENT. - 1960

Ferbam and Glyodin Sprays, all season[a]

PICKING NO.		1		2		3	
	DAYS FROM FULL BLOOM	53		60		67	
	ACID	A	B	A	B	A	B
1.	Aspartic	2.40	43.1	3.30	55.6	3.20	45.5
2.	Shikimic	0.37	17.5	0.47	20.7	-	-
3.	Quinic	1.17	60.9	1.41	68.4	1.17	48.0
4.	Galacturonic	0.44	22.9	0.41	20.0	0.13	5.4
5.	Glucuronic	0.18	9.3	0.68	39.4	0.18	7.4
6.	Succinic	0.40	6.3	0.70	10.5	0.11	1.4
7.	Malic	88.29	1600.6	82.21	1394.5	83.70	1198.6
8.	Citric	0.87	15.1	0.49	7.9	0.37	5.0
9.	Neochlorogenic*	0.45	43.6	0.20	17.7	0.08	6.0
10.	Isochlorogenic*	0.27	25.9	-	-	0.13	9.9
11.	Chlorogenic	2.13	203.7	0.38	33.7	0.54	40.4
12.	Phosphoric	2.51	22.2	4.27	35.3	4.40	30.7
	Fluorescent	2.85	273.2	0.58	51.4	0.75	56.3

A = meq/100 meq of analyzed acidity
B = Mg/100 gm fresh fruit
* = The molecular weight of these acids was assumed to be the same as that of Chlorogenic acid, since they are also caffeoylquinic acids.
a = No data obtained for the 4th, 5th, and 6th pickings.

TABLE 8. NON-VOLATILE ACIDS OF MONTMORENCY CHERRIES DURING FRUIT DEVELOPMENT. – 1960

TABLE 8. NON-VOLATILE ACIDS OF MONTMORENCY CHERRIES DURING FRUIT DEVELOPMENT. - 1960

Ferbam and Glyodin Sprays, early (two applications); Fixed Copper Spray, late[a]

	PICKING NO.	1		2		3		6	
	DAYS FROM FULL BLOOM	53		60		67		88	
	ACID	A	B	A	B	A	B	A	B
1.	Aspartic	3.70	64.8	3.67	60.8	2.67	42.1	1.20	17.9
2.	Shikimic	-	-	0.08	3.5	0.20	8.2	0.09	3.5
3.	Quinic	1.81	91.3	1.43	68.2	1.02	46.5	0.95	40.9
4.	Galacturonic	0.15	7.6	0.12	5.8	0.10	4.7	0.23	10.1
5.	Glucuronic	0.55	28.0	0.21	10.1	0.38	17.7	0.27	11.6
6.	Succinic	0.19	3.0	0.26	3.8	0.22	3.0	0.37	4.9
7.	Malic	82.96	1462.8	91.30	1524.1	84.99	1348.7	90.52	1357.1
8.	Citric	0.97	16.4	0.39	6.2	0.41	6.3	0.59	8.5
9.	Neochlorogenic*	0.37	34.4	0.23	20.2	0.22	18.1	0.31	24.4
10.	Isochlorogenic*	0.34	31.5	0.44	38.6	0.25	20.5	0.33	26.2
11.	Chlorogenic	1.02	95.3	0.71	62.4	0.84	70.9	0.61	48.5
12.	Phosphoric	2.19	18.8	3.86	31.4	4.19	32.4	5.55	40.5
	Fluorescent	1.73	160.2	1.38	121.2	1.31	109.5	1.55	99.1

A = meq/100 meq of analyzed acidity
B = Mg/100 gm fresh fruit
* = The molecular weight of these acids was assumed to be the same as that of Chlorogenic acid, since they are also caffeoylquinic acids.
a = No data obtained for the 4th and 5th pickings.

TABLE 9. NON-VOLATILE ACIDS OF MONTMORENCY CHERRIES DURING FRUIT DEVELOPMENT - 1966

TABLE 9. NON-VOLATILE ACIDS OF MONTMORENCY CHERRIES DURING FRUIT DEVELOPMENT. - 1960

Ferbam and Glyodin Sprays, early (two applications); Nu-Iron and Glyodin Sprays, late[a]

PICKING NO.	1		2		3		5		6	
DAYS FROM FULL BLOOM	53		60		67		81		88	
ACID	A	B	A	B	A	B	A	B	A	B
1. Aspartic	5.08	85.3	3.00	45.7	3.04	53.8	5.10	87.4	4.24	65.9
2. Shikimic	-	-	0.15	6.0	0.16	7.4	-	-	0.14	5.6
3. Quinic	2.67	129.3	1.21	53.0	1.31	67.1	2.44	120.9	1.70	76.1
4. Galacturonic	0.07	3.5	0.11	4.9	0.19	9.9	0.17	8.3	0.17	7.6
5. Glucuronic	0.40	19.4	0.31	13.8	0.33	17.1	0.46	22.9	0.42	19.0
6. Succinic	0.46	6.8	0.25	3.4	0.39	6.2	0.95	14.4	0.28	3.9
7. Malic	82.10	1387.1	79.65	1220.2	87.66	1562.7	85.21	1470.5	84.36	1321.3
8. Citric	0.98	15.8	0.47	6.9	0.56	9.5	0.50	8.2	0.48	7.2
9. Neochlorogenic*	0.91	81.1	0.30	24.1	0.69	64.8	0.26	23.4	0.32	26.6
10. Isochlorogenic*	0.06	5.0	0.46	37.2	0.54	51.0	0.40	36.8	0.24	20.2
11. Chlorogenic	1.63	145.6	0.48	38.6	1.01	95.3	0.71	64.5	0.69	57.4
12. Phosphoric	4.31	35.5	3.53	26.3	4.13	35.8	5.32	44.7	6.19	47.3
Fluorescent	2.60	231.7	1.24	99.9	2.24	211.1	1.37	124.7	1.25	104.2

A = meq/100 meq of analyzed acidity
B = Mg/100 gm fresh fruit
* = The molecular weight of these acids was assumed to be the same as that of Chlorogenic acid, since they are also caffeoylquinic acids.
a = No data obtained for the 4th picking.

TABLE 10. NON-VOLATILE ACIDS OF MONTMORENCY CHERRIES DURING FRUIT DEVELOPMENT. - 1960

TABLE 10. NON-VOLATILE ACIDS OF MONTMORENCY CHERRIES DURING FRUIT DEVELOPMENT. - 1960

Cyprex Spray, all season.

PICKING NO.	1		2		3		4		5		6	
DAYS FROM FULL BLOOM	53		60		67		74		81		88	
ACID	A	B	A	B	A	B	A	B	A	B	A	B
1. Aspartic	2.82	49.6	3.07	50.4	3.97	46.6	4.38	60.2	5.69	89.6	2.43	37.1
2. Shikimic	0.35	16.1	0.13	5.6	0.05	2.1	0.12	4.2	-	-	0.14	5.6
3. Quinic	1.61	81.9	1.39	65.7	3.95	178.9	1.54	61.3	2.04	92.8	1.21	53.2
4. Galacturonic	0.09	4.7	0.11	5.2	0.11	5.0	0.15	6.2	0.16	7.2	0.23	10.3
5. Glucuronic	0.17	8.5	0.38	18.2	0.25	11.5	0.30	12.2	0.33	15.1	0.38	16.9
6. Succinic	0.45	7.1	0.34	5.0	0.19	2.7	0.45	5.5	0.48	6.7	0.53	7.1
7. Malic	80.87	1431.3	86.21	1423.1	82.83	1307.3	95.71	1325.7	86.47	1371.7	87.45	1347.4
8. Citric	0.98	16.6	0.56	8.8	0.45	6.8	0.42	5.6	0.66	10.0	0.44	6.5
9. Neochlorogenic*	0.60	56.3	0.30	26.2	0.31	25.9	0.53	38.6	0.20	17.0	0.43	34.7
10. Isochlorogenic*	0.45	42.5	0.38	23.3	0.23	19.1	0.27	20.2	0.32	26.9	0.25	20.2
11. Chlorogenic	1.59	149.2	0.79	69.1	0.56	46.8	0.75	54.9	0.78	65.2	0.62	50.3
12. Phosphoric	3.33	28.7	3.91	31.4	4.24	32.6	4.96	33.4	6.61	51.0	4.60	34.5
Fluorescent	2.64	248.0	1.47	118.6	1.10	91.8	1.55	113.7	1.30	109.1	1.30	105.2

A = meq/100 meq of analyzed acidity
B = Mg/100 gm fresh fruit
* = The molecular weight of these acids was assumed to be the same as that of Chlorogenic acid, since they are also caffeoylquinic acids.

TABLE 10. NON-VOLATILE ACIDS OF MONTMORENCY CHERRIES DURING FRUIT DEVELOPMENT. - 1960

Cyprex Spray, all season.

PICKING NO.	1		2		3		4		5		6	
DAYS FROM FULL BLOOM	53		60		67		74		81		88	
ACID	A	B	A	B	A	B	A	B	A	B	A	B
1. Aspartic	2.82	49.6	3.07	50.4	3.97	46.6	4.38	60.2	5.69	89.6	2.43	37.1
2. Shikimic	0.35	16.1	0.13	5.6	0.05	2.1	0.12	4.2	-	-	0.14	5.6
3. Quinic	1.61	81.9	1.39	65.7	3.95	178.9	1.54	61.3	2.04	92.8	1.21	53.2
4. Galacturonic	0.09	4.7	0.11	5.2	0.11	5.0	0.15	6.2	0.16	7.2	0.23	10.3
5. Glucuronic	0.17	8.5	0.38	18.2	0.25	11.5	0.30	12.2	0.33	15.1	0.38	16.9
6. Succinic	0.45	7.1	0.34	5.0	0.19	2.7	0.45	5.5	0.48	6.7	0.53	7.1
7. Malic	80.87	1431.3	86.21	1423.1	82.83	1307.3	95.71	1325.7	86.47	1371.7	87.45	1347.4
8. Citric	0.98	16.6	0.56	8.8	0.45	6.8	0.42	5.6	0.66	10.0	0.44	6.5
9. Neochlorogenic*	0.60	56.3	0.30	26.2	0.31	25.9	0.53	38.6	0.20	17.0	0.43	34.7
10. Isochlorogenic*	0.45	42.5	0.38	23.3	0.23	19.1	0.27	20.2	0.32	26.9	0.25	20.2
11. Chlorogenic	1.59	149.2	0.79	69.1	0.56	46.8	0.75	54.9	0.78	65.2	0.62	50.3
12. Phosphoric	3.33	28.7	3.91	31.4	4.24	32.6	4.96	33.4	6.61	51.0	4.60	34.5
Fluorescent	2.64	248.0	1.47	118.6	1.10	91.8	1.55	113.7	1.30	109.1	1.30	105.2

A = meq/100 meq of analyzed acidity
B = Mg/100 gm fresh fruit
* = The molecular weight of these acids was assumed to be the same as that of Chlorogenic acid, since they are also caffeoylquinic acids.

TABLE 11. NON-VOLATILE ACIDS OF MONTMORENCY CHERRIES DURING FRUIT DEVELOPMENT. - 1960

Parathion Spray, early (two applications); Actidione and Ferbam Sprays, late[a].

PICKING NO.	1		2		3		5		6	
DAYS FROM FULL BLOOM	53		60		67		81		88	
ACID	A	B	A	B	A	B	A	B	A	B
1. Aspartic	4.26	79.1	3.48	63.1	2.99	51.2	1.67	26.9	1.36	24.5
2. Shikimic	-	-	0.12	5.8	0.06	2.6	0.42	17.9	0.13	6.1
3. Quinic	1.45	77.6	1.23	64.2	1.02	50.3	0.87	40.2	0.76	39.4
4. Galacturonic	0.19	10.1	0.10	5.2	0.12	6.0	0.19	8.7	0.14	7.2
5. Glucuronic	-	-	0.29	15.3	0.22	10.9	0.63	29.5	0.38	20.2
6. Succinic	0.36	5.8	0.27	4.3	0.35	5.3	0.35	5.0	0.56	9.0
7. Malic	75.22	1406.1	86.34	1577.9	84.60	1456.5	90.55	1464.3	85.62	1553.3
8. Citric	0.87	15.5	0.39	6.8	0.40	6.5	0.55	8.5	0.64	11.1
9. Neochlorogenic*	0.30	29.1	0.03	2.8	0.15	13.5	0.14	12.8	0.23	21.6
10. Isochlorogenic*	0.23	22.3	0.28	26.9	0.23	20.9	0.29	25.5	0.18	17.0
11. Chlorogenic	1.59	156.6	0.79	76.2	0.78	71.2	0.86	73.3	0.60	57.8
12. Phosphoric	4.08	37.1	3.23	28.7	4.54	38.1	4.98	39.2	5.44	48.1
Fluorescent	2.12	208.0	1.10	105.9	1.16	105.6	1.29	111.6	1.01	96.4

A = meq/100 meq of analyzed acidity
B = Mg/100 gm fresh fruit
* = The molecular weight of these acids was assumed to be the same as that of Chlorogenic acid, since they are also caffeoylquinic acids.
a = No data obtained for the 4th picking.

TABLE 13. NON-VOLATILE ACIDS OF [illegible] Samples dated[a].

TABLE 12. NON-VOLATILE ACIDS OF MONTMORENCY CHERRIES DURING FRUIT DEVELOPMENT. - 1960

Parathion Spray, early (two applications); Actidione and Nu-Iron Sprays, late[a].
(Sevin Spray: For cherry fruit fly control)

	PICKING NO.	1		2		3		5	
	DAYS FROM FULL BLOOM	53		60		67		81	
	ACID	A	B	A	B	A	B	A	B
1.	Aspartic	2.59	48.0	2.86	47.2	3.90	65.6	2.76	43.8
2.	Shikimic	0.46	22.4	0.10	4.2	-	-	-	-
3.	Quinic	1.75	93.8	1.01	48.2	1.31	63.6	1.02	57.5
4.	Galacturonic	0.26	13.8	0.10	4.7	0.14	6.8	0.23	10.7
5.	Glucuronic	-	-	0.17	8.0	0.29	14.2	0.73	34.0
6.	Succinic	0.50	8.1	0.23	3.4	0.19	2.8	0.19	2.7
7.	Malic	79.55	1484.8	85.17	1417.2	90.70	1536.7	89.19	1426.7
8.	Citric	0.77	13.7	0.40	6.4	0.45	7.3	0.66	10.1
9.	Neochlorogenic*	0.51	50.0	0.05	4.3	0.10	8.9	0.31	25.9
10.	Isochlorogenic*	0.33	32.6	0.10	9.2	0.15	13.5	0.10	8.9
11.	Chlorogenic	1.28	125.8	0.79	69.4	0.72	64.5	0.70	58.8
12.	Phosphoric	3.79	34.5	3.00	24.3	5.42	44.7	3.63	28.3
	Fluorescent	2.12	208.4	0.94	82.9	0.97	86.9	1.11	93.6

A = meq/100 meq of analyzed acidity
B = Mg/100 gm fresh fruit
* = The molecular weight of these acids was assumed to be the same as that of Chlorogenic acid, since they are also caffeoylquinic acids.
a = No data obtained for the 4th and 6th pickings.

TABLE 13. NON-VOLATILE ACIDS OF MONTMORENCY CHERRIES DURING FRUIT DEVELOPMENT. - 1961

Ferbam and Glyodin Sprays.

Acidity expressed as meq/100 meq of analyzed acidity.

	PICKING NO.	1	2	3	4	5	6	7	8
	DAYS FROM FULL BLOOM / ACID	45	52	59	66	73	80	87	94
1.	Aspartic	0.99	0.74	1.72	2.09	0.31	1.64	2.64	1.34
2.	Shikimic	0.26	0.36	-	-	0.11	0.08	-	0.26
3.	Quinic	2.04	1.31	0.88	1.36	0.54	0.90	0.99	0.44
4.	Galacturonic	0.14	0.15	0.94	0.19	0.41	0.21	0.23	0.17
5.	Glucuronic	0.28	0.13	0.64	0.37	0.19	-	0.55	0.20
6.	Succinic	0.64	0.36	0.76	0.64	0.72	0.33	0.36	0.26
7.	Malic	75.55	78.82	94.22	87.58	88.65	85.43	78.11	95.73
8.	Citric	1.62	1.71	0.29	0.72	1.73	0.62	0.49	0.54
9.	Neochlorogenic*	0.61	1.29	1.45	0.61	0.30	0.51	0.38	0.45
10.	Isochlorogenic*	1.39	-	0.43	0.12	0.40	-	0.13	-
11.	Chlorogenic	1.27	1.06	0.73	0.15	0.72	0.26	0.13	0.17
12.	Phosphoric	5.40	4.11	0.98	2.73	2.50	3.55	0.26	5.71
	Fluorescent	3.27	2.35	2.67	0.88	1.42	0.77	0.64	0.62

* = The molecular weight of these acids was assumed to be the same as that of Chlorogenic acid, since they are also caffeoylquinic acids.

TABLE 14. NON-VOLATILE ACIDS OF MONTMORENCY CHERRIES DURING FRUIT DEVELOPMENT. - 1961

Ferbam and Glyodin Sprays

Acidity expressed as mg/100 gm of fresh fruit.

	PICKING NO.	1	2	3	4	5	6	7	8
	DAYS FROM FULL BLOOM / ACID	45	52	59	66	73	80	87	94
1.	Aspartic	16.0	13.2	18.3	30.9	3.8	28.7	12.4	12.8
2.	Shikimic	10.9	16.6	-	-	3.5	0.2	-	6.7
3.	Quinic	94.7	66.9	26.9	58.0	19.4	45.7	13.5	12.3
4.	Galacturonic	6.8	7.8	29.3	8.2	14.9	10.7	3.1	4.9
5.	Glucuronic	13.0	6.6	19.8	16.1	7.0	-	7.6	7.2
6.	Succinic	9.3	5.6	7.1	8.4	8.0	5.1	1.5	2.2
7.	Malic	1224.4	1408.8	1010.7	1305.3	1108.5	1506.9	371.3	926.8
8.	Citric	25.1	29.2	2.9	10.2	20.6	10.4	2.2	5.0
9.	Neochlorogenic*	52.1	121.5	82.2	47.8	19.8	47.8	9.6	23.0
10.	Isochlorogenic*	119.0	-	24.1	9.2	26.2	-	3.2	-
11.	Chlorogenic	108.4	99.9	41.4	12.0	47.8	24.4	3.2	8.9
12.	Phosphoric	42.7	35.8	5.1	19.8	15.3	30.5	0.6	26.9

* = The molecular weight of these acids was assumed to be the same as that of Chlorogenic acid, since they are also caffeoylquinic acids.

TABLE 15. NON-VOLATILE ACIDS OF MONTMORENCY CHERRIES DURING FRUIT DEVELOPMENT. - 1961 Copper Spray.

Acidity expressed as meq/100 meq of analyzed acidity.

	PICKING NO.	1	2	3	4	5	6	7	8
	DAYS FROM FULL BLOOM / ACID	45	52	59	66	73	80	87	94
1.	Aspartic	0.76	0.60	1.31	0.42	0.49	2.14	0.69	0.53
2.	Shikimic	0.18	0.16	0.14	0.05	0.04	-	-	0.05
3.	Quinic	2.15	1.23	0.98	0.31	0.30	0.91	0.35	0.18
4.	Galacturonic	0.18	0.03	1.10	0.10	0.02	0.16	0.03	0.17
5.	Glucuronic	0.17	0.07	0.08	0.03	-	0.04	0.08	0.04
6.	Succinic	0.69	0.33	0.63	0.25	0.13	0.18	0.06	0.11
7.	Malic	73.21	83.52	87.41	85.58	84.94	82.29	92.49	86.10
8.	Citric	2.45	1.66	1.10	0.45	0.45	0.54	0.74	0.66
9.	Neochlorogenic*	0.82	1.03	0.44	0.22	0.10	0.35	0.15	0.13
10.	Isochlorogenic*	0.80	-	-	0.21	0.01	0.02	0.05	0.03
11.	Chlorogenic	1.80	1.48	1.32	0.85	0.44	0.02	1.37	0.10
12.	Phosphoric	6.02	4.32	2.58	1.19	4.73	3.35	4.40	5.86
	Fluorescent	3.42	2.51	1.76	1.28	0.55	0.39	1.57	0.26

* = The molecular weight of these acids was assumed to be the same as that of Chlorogenic acid, since they are also caffeoylquinic acids.

TABLE 16. NON-VOLATILE ACIDS OF MONTMORENCY CHERRIES DURING FRUIT DEVELOPMENT. - 1961

Copper Spray

Acidity expressed as mg/100 gm of fresh fruit.

	PICKING NO.	1	2	3	4	5	6	7	8
	ACID \ DAYS FROM FULL BLOOM	45	52	59	66	73	80	87	94
1.	Aspartic	12.2	11.4	23.6	5.4	6.8	28.1	3.6	5.0
2.	Shikimic	7.5	8.1	6.7	1.6	1.6	-	-	1.2
3.	Quinic	99.4	68.4	50.9	11.5	11.9	34.6	5.2	5.0
4.	Galacturonic	8.5	0.2	5.2	3.5	1.0	6.0	0.4	4.7
5.	Glucuronic	8.2	0.4	4.1	1.2	-	1.4	1.2	1.2
6.	Succinic	9.8	5.6	10.1	2.9	1.6	2.1	0.3	0.9
7.	Malic	1180.9	1616.1	1582.3	1103.9	1190.8	1087.9	484.9	813.4
8.	Citric	37.7	30.7	90.0	5.6	6.1	6.8	3.7	6.0
9.	Neochlorogenic*	69.8	104.9	41.8	15.2	7.1	24.8	4.3	6.7
10.	Isochlorogenic*	68.0	-	-	14.2	1.1	1.4	1.4	1.4
11.	Chlorogenic	153.4	151.6	126.11	58.1	32.6	1.4	37.9	5.0
12.	Phosphoric	47.3	40.7	22.7	7.5	32.3	21.6	11.2	26.9

* = Molecular weight of these acids was assumed to be the same as that of Chlorogenic acid, since they are also caffeoylquinic acids.

TABLE 17. NON-VOLATILE ACIDS OF MONTMORENCY CHERRIES DURING FRUIT DEVELOPMENT. - 1961

Cyprex Spray.

Acidity expressed as meq/100 meq of analyzed acidity.

	PICKING NO.	1	2	3	4	5	6	7	8
	DAYS FROM FULL BLOOM / ACID	45	52	59	66	73	80	87	94
1.	Aspartic	1.14	0.89	1.34	1.16	1.90	1.58	1.06	1.49
2.	Shikimic	0.18	-	0.10	-	0.17	0.14	-	0.08
3.	Quinic	1.81	1.39	0.82	0.41	0.85	0.68	0.26	0.55
4.	Galacturonic	0.18	0.19	-	0.05	0.14	0.19	0.11	0.19
5.	Glucuronic	0.27	-	-	-	0.15	0.14	0.01	0.20
6.	Succinic	0.34	0.48	0.22	0.22	0.31	0.35	0.12	0.56
7.	Malic	84.07	81.90	81.48	79.54	82.08	90.32	81.90	91.04
8.	Citric	2.74	1.65	0.76	0.64	0.60	0.63	0.74	0.70
9.	Neochlorogenic*	1.32	1.44	0.39	0.23	0.56	0.51	0.20	0.39
10.	Isochlorogenic*	-	0.47	-	-	0.13	0.15	0.10	0.15
11.	Chlorogenic	1.80	0.46	0.48	0.31	0.61	0.47	0.19	0.32
12.	Phosphoric	4.82	3.03	1.72	1.87	3.77	2.64	6.18	5.84
	Fluorescent	3.12	2.37	0.87	0.54	1.30	1.13	0.49	0.86

* = The molecular weight of these acids was assumed to be the same as that of Chlorogenic acid, since they are also caffeoylquinic acids.

TABLE 18. NON-VOLATILE ACIDS OF MONTMORENCY CHERRIES DURING FRUIT DEVELOPMENT. - 1961

Cyprex Spray

Acidity expressed as mg/100 gm of fresh fruit.

	PICKING NO.	1	2	3	4	5	6	7	8
	DAYS FROM FULL BLOOM / ACID	45	52	59	66	73	80	87	94
1.	Aspartic	19.2	17.3	24.8	13.9	23.4	28.0	9.3	12.8
2.	Shikimic	8.1	-	4.9	-	5.6	6.5	-	1.8
3.	Quinic	88.0	77.8	44.2	14.0	30.4	34.6	6.5	13.6
4.	Galacturonic	8.9	10.7	-	1.7	5.0	9.7	2.9	4.9
5.	Glucuronic	13.0	-	-	-	5.4	7.2	0.2	5.0
6.	Succinic	5.0	8.2	3.7	2.3	3.4	5.6	0.9	4.3
7.	Malic	1423.2	1604.3	1524.1	960.0	1018.1	1609.6	720.9	790.0
8.	Citric	44.3	30.9	13.6	7.4	7.1	10.7	6.2	5.8
9.	Neochlorogenic*	118.3	149.9	38.6	14.5	36.5	48.2	9.2	18.1
10.	Isochlorogenic*	-	49.2	-	-	8.5	13.8	4.6	7.1
11.	Chlorogenic	161.2	47.1	47.8	19.5	40.0	44.6	8.9	14.5
12.	Phosphoric	39.7	28.9	15.7	11.0	22.8	22.9	26.5	24.7

* = The molecular weight of these acids was assumed to be the same as that of Chlorogenic acid, since they are also caffeoylquinic acids.

TABLE 19. NON-VOLATILE ACIDS ON MONTMORENCY CHERRIES DURING FRUIT DEVELOPMENT. - 1962

Copper Spray

Acidity expressed as meq/100 meq of analyzed acidity

	PICKING NO.	1	2	3	4	5	6	7	8
	DAYS FROM FULL BLOOM / ACID	45	52	59	66	73	80	87	94
1.	Aspartic	1.42	2.44	0.96	0.21	0.16	0.31	0.21	0.23
2.	Shikimic	0.22	0.42	0.15	0.07	0.10	0.06	-	-
3.	Quinic	1.13	1.10	0.48	0.32	0.30	0.31	0.24	0.20
4.	Galacturonic	0.23	0.25	0.34	0.24	0.18	0.27	0.09	0.12
5.	Glucuronic	0.26	0.48	0.25	0.21	0.28	0.14	0.10	0.26
6.	Succinic	0.70	0.62	0.58	0.52	0.26	0.24	0.35	0.17
7.	Malic	84.83	86.25	81.24	93.13	89.50	90.05	96.50	87.17
8.	Citric	0.76	0.69	1.22	0.44	1.30	1.07	1.03	1.44
9.	Neochlorogenic*	0.53	1.05	0.63	0.40	0.49	0.21	0.27	0.52
10.	Isochlorogenic*	0.55	0.88	0.69	0.57	0.46	0.21	0.50	0.60
11.	Chlorogenic	0.90	0.50	0.82	0.67	0.33	0.20	1.00	0.62
12.	Phosphoric	2.97	2.73	3.43	2.47	5.19	4.89	1.78	6.48
	Fluorescent	1.98	2.43	2.14	1.64	1.28	0.62	1.77	1.74

* = The molecular weight of these acids was assumed to be the same as that of Chlorogenic acid, since they are also caffeoylquinic acids.

TABLE 20. NON-VOLATILE ACIDS OF MONTMORENCY CHERRIES DURING FRUIT DEVELOPMENT. - 1962

Copper Spray.

Acidity expressed as mg/100 gm of fresh fruit.

	PICKING NO.	1	2	3	4	5	6	7	8
	DAYS FROM FULL BLOOM / ACID	45	52	59	66	73	80	87	94
1.	Aspartic	22.2	38.3	15.5	4.8	2.3	2.9	2.6	3.1
2.	Shikimic	8.9	17.3	6.3	2.5	3.7	2.6	-	-
3.	Quinic	50.7	49.8	22.5	13.8	12.5	13.3	8.5	7.9
4.	Galacturonic	10.7	11.6	16.1	12.2	7.8	10.1	3.1	4.7
5.	Glucuronic	12.0	21.9	11.6	6.2	11.6	8.9	3.4	10.3
6.	Succinic	9.6	8.6	8.3	3.4	3.3	6.6	3.8	2.0
7.	Malic	1333.2	1364.7	1327.9	1408.5	1306.4	1339.8	1182.7	1182.5
8.	Citric	11.5	10.4	19.0	15.9	18.1	6.0	12.0	18.6
9.	Neochlorogenic*	43.9	87.5	54.6	17.0	37.6	30.5	17.7	37.6
10.	Isochlorogenic*	45.7	73.7	59.5	17.0	35.4	43.6	32.2	42.9
11.	Chlorogenic	74.8	42.5	71.2	16.3	35.2	51.0	64.8	44.3
12.	Phosphoric	22.7	21.0	27.3	37.3	36.9	17.3	10.6	42.8

* = The molecular weight of these acids was assumed to be the same as that of Chlorogenic acid, since they are also caffeoylquinic acids.

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