

AN ABSTRACT OF THE THESIS OF

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Abstract Approved: _____
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In western Oregon the titratable acidity of grapes at harvest may in some seasons be higher than desirable for making quality wine, due to the retention of malic acid. The purposes of this study were 1) to investigate the effects of a vineyard cultural practice, cluster exposure at veraison by basal leaf removal, as a means of reducing the malate content and titratable acidity of grapes, and 2) to develop a rapid, simple, and inexpensive test procedure by which smaller wineries and vineyards could evaluate the effects of their own field experiments on the malate and tartrate content of their grapes.

1. At veraison, clusters of Chardonnay grapes were a) exposed to the sun by removal of all leaves opposite or below the clusters, b) treated as in a) but shaded with shade cloth, c) exposed to the sun by tying back leaves opposite or below the clusters, or d) left untreated as a control. Clusters of White Riesling were exposed to the sun by similar leaf removal a) 10 days before veraison, b) 10 days after veraison, or c) untreated. Clusters of Pinot

Noir were exposed a) at veraison, b) 2 weeks after veraison, or c) untreated. Exposed clusters received 3 to 3.5 times more light than shaded clusters and up to 32% more heat, with temperature differences between exposed and shaded treatments being most pronounced during cool, sunny weather. None of the treatments had any effect on juice or berry malate, tartrate, or potassium content; however, exposed clusters of Pinot Noir had a lower pH (.03) and higher titratable acidity (.06%) than the control at harvest. Cluster exposure of Chardonnay increased sunburning of grapes, and cluster exposure of Pinot Noir at veraison caused a 1% reduction in juice soluble solids concentration at harvest. The detrimental effects of cluster exposure by basal leaf removal at veraison, as well as the lack of any major effect on the acid content of the berries, suggest that the practice has no value for acid reduction during a warm, dry maturation season in western Oregon.

2. A rapid, simple procedure for the estimation of the malate and tartrate content of grape juice is described. The procedure, which requires only a pH meter for instrumentation, does not directly measure malate and tartrate but instead measures their buffering effect. Samples are titrated between pH 2.70-3.00 and pH 4.50-4.80 and the titrant volumes required are compared to two sets of empirically derived standard curves. The malate and

tartrate composition of the sample may be determined by a graphical or algebraic method. The use of the estimation method, its advantages, and its limitations are illustrated with different viticultural trials. The estimation error (estimated value - measured value) was influenced by many factors including maturity, season, vineyard location, and cultivar. Standard deviations of the estimation error for malate and tartrate in mature grapes were equal to 9% and 15%, respectively, of the mean malate and tartrate concentrations in pooled Pinot Noir and Chardonnay samples from different vineyards and years. The estimation error is probably due to interference from other buffers present in juice. Although not as accurate as existing analytical methods, the estimation method appears potentially useful for determining relative effects of treatments in vineyard trials where analytical equipment is unavailable or for monitoring malate decline during maturation of grapes.

Malate and Tartrate in Oregon Grapes

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The contribution of P.B. Lombard, the second author, to the first manuscript was as an advisor on the viticultural trials; and that of D.A. Heatherbell, the third author, was as major professor.

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Malate and Tartrate in Oregon Grapes

Introduction

Within the past twenty years, there have been numerous plantings of European wine grapes (Vitis vinifera L.) in western Oregon. The cool, maritime climate and long, dry summers have proved beneficial to the cultivation of the "noble" varieties of wine grapes and to the vinification of some very fine, flavorful wines in the northern European tradition.

Tartaric and malic acids and their potassium salts play an important role in determining eventual wine quality because they regulate the pH and titratable acidity of the grapes. Malic acid, in particular, is highly variable with respect to grape maturity and is the primary cause of poor grape and wine quality due to a high titratable acidity in overly cool growing seasons. It behooves winegrowers in cooler regions, therefore, to employ those cultural conditions in the vineyard which tend to minimize malic acid concentrations in the grapes at harvest. To this end, an analytical method for determining malate adaptable to smaller vineyards and wineries is desirable.

Part I of this study is concerned with the use of cluster exposure by basal leaf removal at veraison as a technique for the reduction of malate concentrations in the

grapes at harvest. Part II describes a rapid new technique for estimating must malate and tartrate concentrations which does not require the use of expensive analytical equipment.

Literature Review

Importance of acidity and pH in grapes and wine

Titrateable acidity and pH play a very important role in the perception of flavor in grapes and wine by providing a sense of freshness and balance. If too much acid is present, one will perceive an unpleasant, biting tartness; conversely, the lack of adequate acid will make the flavor seem flat and uninteresting. The human palate is capable of sensing differences in both pH and titrateable acidity (Amerine et al., 1965). The pH affects the perception of sourness - a wine with a given titrateable acidity will taste more acid at a lower pH (Nagel et al., 1982). This effect is complicated by the sweet taste of sugars and/or alcohols, which tends to mask the sour taste of the acids (Peynaud, 1984).

The pH of wine also affects many other quality parameters, including the color (Timberlake and Bridle, 1967; Berg and Akiohi, 1975; Timberlake, 1982; Sims and Morris, 1984, 1985), protein stability (Boulton, 1986), microbiological stability (Davis et al., 1985; Wibowo et al., 1985), effectiveness of sulfur dioxide (SO₂) addition (Amerine and Joslyn, 1970; Wibowo et al., 1985), and susceptibility to ferric casse (Amerine and Joslyn, 1970). All of these are adversely affected with increasing pH. Thus, a primary concern of the winemaker is to prevent

excessive increases in pH during the winemaking process.

The winemaker may, at his or her discretion, adjust the pH and titratable acidity of must or wine by any of several techniques. These include deacidification by malate-utilizing yeasts (Gallander, 1977) or malolactic bacteria (Rankine, 1977); chemical deacidification with carbonates using the "single-salt" or "double-salt" methods (Mattick et al., 1980; Steele and Kunkee, 1978, 1979); amelioration of the must with sugar and water (Kluba and Beelman, 1975); ion-exchange; cold stabilization to precipitate tartrates; and addition of tartaric acid (Beelman and Gallander, 1979). Most of these methods are used to deacidify the must or wine, although ion-exchange may be used for acidification or deacidification. Addition of tartaric acid will reduce pH and increase titratable acidity. Each technique has certain advantages and disadvantages which must be taken into account when the acidity modification is employed (Nagel et al., 1975; Munyon and Nagel, 1977; Boulton, 1985).

The major organic acids found in grapes are L-tartaric and L-malic acids (Fig. 1), which together comprise 90% or more of the total acidity (Winkler et al., 1974). Both are dicarboxylic organic acids, but their origins and apparent functions within the grape plant are dissimilar. Although the quantities present in the berries may vary with the cultivar, season, location, cultural practices, and degree

of maturity, tartaric acid is usually more abundant at harvest (Johnson and Nagel, 1976; Winkler et al., 1974). Besides malate and tartrate, smaller amounts of more than 20 other non-nitrogenous organic acids, including members of the Krebs cycle, glycolytic pathway, glyoxylic acid cycle, and shikimic acid pathway, are found in grapes (Winkler et al., 1974).

Tartaric acid

The grape is the only cultivated fruit of economic importance which contains tartaric acid in appreciable quantities, although tartaric acid does occur in the fruits of Morus indica and Tamarindus indica (Ulrich, 1970). Tartaric acid is present in grape leaves and berries in the form of the free acid and its calcium salt. In leaves, the highly insoluble calcium tartrates are stored in crystalline bundles, or raphides, within large specialized storage cells called idioblasts. The idioblasts are located in the intercostal sections of fully differentiated leaves, especially in the serrated leaf tips. The cellular fluid surrounding the raphides contains a highly concentrated solution of tartaric acid. Apparently the limiting factor affecting the calcium tartrate precipitation in the idioblasts is the calcium ion concentration. It is thought that the physiological role of tartaric acid in grape leaves may be the scavenging of

excess calcium ions from the leaf tissue (Ruffner, 1982a). The synthesis of tartaric acid in leaves seems limited to the period of initial growth and cell expansion in the young tissue. Maximum concentration occurs about when the laminae are fully expanded (at 40 days after unfolding) (Winkler et al., 1974).

In grape berries, free tartaric acid may also be stored in vacuoles as well as the insoluble salt (Ruffner, 1982a). The concentration of tartaric acid increases from berry set until just prior to the initiation of ripening (veraison), then declines gradually under hot conditions or remains constant under cool conditions (Johnson and Nagel, 1976). When the increases in berry volume during ripening are considered, the actual decrease in tartrate is very small (Ruffner, 1982a).

The exact biosynthetic pathway(s) of tartaric acid is not yet known. It appears to be a secondary product derived from a sugar. Two pathways, both light independent, have been proposed:

(1) biogenesis via gluconate-glucono- γ -lactone-ascorbic (or pretaric) acid, accompanied by a high hexose-monophosphate shunt activity.

(2) synthesis in a salvage-type reaction by transformation of galacturonic acid or other alduronic compounds. This mechanism may help maintain a physiological balance of calcium ions (Ruffner, 1982a).

Ripe California grapes may contain 0.38 to 1.08% tartaric acid, depending on the cultivar. It is very stable and appears susceptible to degradation only at temperatures above 30° C (Winkler et al., 1974) and in appreciable amounts only above 35° C (Kliewer and Lider, 1968). Free tartaric acid, but not the salt forms, may be catabolized to CO₂ at all stages of berry development (Winkler et al., 1974). The degradation mechanism is subject to debate, being attributed by some to fermentation within the berries (Ruffner, 1982a), and by others to respiration, although no enzymes capable of degrading tartaric acid have been identified (Winkler et al., 1974).

Malic acid

Malic acid is abundant in many cultivated fruits, including apples (80-90% of total organic acids), apricots (76%), bananas, cherries, peaches, pears, plums (Ulrich, 1970), blackberries (Thinman and Bonner, 1950), tamarillos (Heatherbell et al., 1982), and Chinese gooseberries (Kiwi fruit) (Heatherbell, 1975). The patterns of accumulation and decline vary with the fruit. In pears and bananas, there is a steady, slow decrease in malate until the fruit is ripe. In grapes and apples, the titratable acidity due to malate peaks as the fruit matures, then declines (Ulrich, 1970). These declines suggest that malate is used as an energy source during ripening. Chinese gooseberries,

which use carbohydrates as the energy source for respiration, show very little decline in malate at maturity (Heatherbell, 1975).

Malic acid, in contrast to tartaric acid, is very active in grape metabolism. It is the primary ^{14}C -labeled product in leaves in the dark after assimilation of $^{14}\text{CO}_2$. Analysis of carbon-isotope ratios in the leaves and berries indicate that ribulose-1,5-diphosphate carboxylase is the primary enzyme fixing CO_2 , and thus the grapevine is considered to be a C_3 plant. However, the green berries, after a 10 second exposure to $^{14}\text{CO}_2$ in the light, have been shown to incorporate about 40% of the radioactive label into malate and aspartate, which may indicate a trend toward C_4 metabolism. There is no diurnal fluctuation in acidity, and so the Crassulacean Acid Metabolism is not considered to exist (Ruffner, 1982b).

It is thought that the C_1 - C_3 moiety of malate derives from photosynthetic phosphoglyceric acid (PGA) (Ruffner et al., 1983). Another possible pathway within the leaf involves a carbon flow to PGA from a photosynthetic and transitory hexose or starch pool (Ruffner, 1982b). Both mechanisms may occur.

In the light, there is competition for atmospheric CO_2 between carboxylation of ribulose-1,5-diphosphate for carbohydrate synthesis and carboxylation of

phosphoenolpyruvate (PEP) for malic acid synthesis. In the leaves, the former process is thought to prevail, in the fruits the latter. At night, when there is no light to power photosynthesis, 90% of incorporated $^{14}\text{CO}_2$ radioactivity is found in malate in both leaves and fruits (Peynaud and Ribereau-Gayon, 1971).

Malate synthesis in the leaves begins when they are very young and reaches a plateau when the leaves are about one-fourth their final size. After this, the concentration remains constant for the photosynthetic lifetime of the leaves. The constant concentration of malate in the leaves (about 2 mg/g fresh weight (FW)) is probably due to a balance between synthesis, dissimilation, and possibly transport in the tissue (Ruffner, 1982b). During senescence, both malate and tartrate are translocated from the leaves to the woody tissue of the plant for storage (Winkler et al., 1974).

In the grape berries, malate is accumulated in the developing fruit until it reaches a maximum concentration at veraison, and then declines rapidly (Hrazdina et al., 1984; Winkler et al., 1974). Within a week after ripening begins, the malate concentration of the berries may decline from 15 mg/g FW to 2-3 mg/g FW. At the same time the berry undergoes a rapid accumulation of sugars, mostly glucose and fructose. It has been shown that some of the sugars formed during ripening are derived from stored malate by

gluconeogenesis, but the amount is small (Ruffner, 1982b).

The accumulation of malate in the grape berry is thought to occur as a result of the berry's role as a storage organ. Between berry set and veraison the photosynthesizing leaves produce sucrose, some of which is transported to the green berries. Grape berries lack the enzymes for starch synthesis, so the sucrose is metabolized via glycolysis and the hexose-monophosphate shunt, and the excess is stored as malate in vacuoles. There appear to be two malate storage pools, one being metabolically active and the other relatively inactive. During periods of stress or low photosynthetic activity, when the metabolically active malate pool becomes depleted and insufficient photosynthates are being received by the berries, malate may be withdrawn from the inactive pool and respired to provide energy to sustain cellular activity. When the import of excess photosynthates resumes, then the active malate pool is refilled first and the inactive pool last (Ruffner, 1982b).

At veraison, the green berries soften, accumulate sugars and potassium, and begin to develop flavor and color compounds. The rates of glycolysis and malate production become sharply reduced. Incoming sucrose, which prior to veraison would have been converted to malate or respired via glycolysis and the citric acid cycle, is instead converted to sucrose phosphate while passing through the

tonoplast, hydrolyzed into glucose and fructose, and stored in mesocarp vacuoles (Ruffner, 1982b).

Since glycolysis is inhibited during ripening, the berry must rely on other sources of energy to carry out phosphorylation of sucrose, biosynthesis of color and flavor compounds, and other energy requiring reactions. The metabolically active malate pool is respired to provide the necessary energy, and when it is depleted, malate is removed from the inactive pool and transported to the respiration site. Most malate respiration seems to occur in the outer layers of the berry, giving it a radial profile of decreasing malate concentration from the center outward (Ruffner, 1982b), which is associated with a similar decrease in titratable acidity (Amerine, 1956). The skins of the berries after veraison provide an exception to this pattern, being higher in malate than the pulp beneath but lower in titratable acidity. This is probably due to the formation of acid salts of potassium and calcium in the skins (Possner and Kliewer, 1985).

There are several enzymes directly involved in malate metabolism. Malate dehydrogenase (L-malate: NAD oxidoreductase, E.C. 1.1.1.37) (Hawker, 1969) catalyzes the NAD-linked oxidation of malate to oxaloacetate in the citric acid cycle (Fig. 2). There is some indication that it may be synthesized de novo after veraison in

mitochondria and may thus be more active at that time (Ruffner, 1982b).

Phosphoenolpyruvate carboxylase (orthophosphate: oxaloacetate carboxylase (phosphorylating), E.C. 4.1.1.31) (Hawker, 1969), in conjunction with malate dehydrogenase, is assumed to be responsible for malic acid synthesis by beta-carboxylation (Fig. 2). When grape leaves fix $^{14}\text{CO}_2$ in the dark, malic acid is the predominant radioactive compound formed, with most of the radioactivity located in the carboxyl (mainly C_4) atoms. There is, however, no detectable increase in malic acid in the tissues, indicating that PEP carboxylase is not the pace-setting enzyme in malic acid synthesis (Ruffner, 1982b).

Phosphoenolpyruvate carboxykinase (E.C. 4.1.1.32) is probably the key enzyme in gluconeogenesis (Ruffner, 1982b), catalyzing the reversible decarboxylation of oxaloacetate to form phosphoenolpyruvate (Ruffner and Kliever, 1975). Since the reaction is reversible, it may also play a role in malic acid synthesis (Lakso and Kliever, 1975). In Pinot Noir berries, PEP carboxykinase had an activity one-fourth to one-half that of PEP carboxylase throughout fruit development, and was not affected by temperature (Ruffner and Kliever, 1975). This indicates that at high temperatures the reduction in malate concentrations in grape berries must be caused by

respiration rather than by gluconeogenesis (Ruffner, 1982b).

Little correlation has been found between the activities of PEP carboxylase and PEP carboxykinase during development of Pinot Noir berries. The activity of PEP carboxylase remained high throughout the entire fruit development and ripening period, with a temporary sharp decline at veraison. PEP carboxykinase displayed peak activity 4 weeks after anthesis (bloom), when berry growth rate was maximal. Both enzymes had very high optimal temperatures (about 40° C) (Ruffner and Kliewer, 1975).

Since the optimal temperature for malic acid synthesis (Kliewer, 1964) and gluconeogenesis in grape berries (Ruffner et al., 1975) is about 20° C and because the size of the malic acid pool has been found to decrease with sharply increasing temperature (Ruffner and Kliewer, 1975), it is possible that the activities of the malic acid synthesizing and degrading enzymes may be controlled by substrate availability. Prior to veraison, the vacuoles containing stored malic acid may isolate it from the degradative enzymes. At veraison, the vacuole membranes may become more permeable, allowing greater gluconeogenetic and respiratory activity (Ruffner and Kliewer, 1975; Lakso and Kliewer, 1978). This increase in permeability may be due to the large increase in cell osmotic pressure which occurs during maturation as a result of the influx of

potassium (Possner and Kliewer, 1985). Malic acid metabolism may also be regulated by inhibitors and activators. Malic acid, for instance, is known to inhibit PEP carboxylase (Lakso and Kliewer, 1975). The control mechanisms of malic acid metabolism in grapes are still poorly understood and remain open to speculation (Lakso and Kliewer, 1978).

Malic enzyme (L-malate; NADP oxidoreductase (decarboxylating), E.C. 1.1.1.40) catalyzes the NADP-linked decarboxylation of malate to pyruvate (Hawker, 1969). It is probably not involved in malate formation, due to unfavorable pH optima and energetic considerations, but it is presumed to be important in malate catabolism. Like PEP carboxykinase, malic enzyme does not appear to be temperature dependent. It is, however, very sensitive to changes in NADP⁺/NADPH ratios, with higher NADP⁺ concentrations causing increased enzymatic activity (Ruffner, 1982b). During berry seed development, the maximal activity of grape malic enzyme occurs near the center of the berry. After ripening begins, it moves to the outer layers of the berry where it is presumed that there is a need for NADPH in the biosynthetic reactions occurring there (Possner *et al.*, 1983). Accordingly, it has been suggested that malic enzyme plays a regulatory role in malate decarboxylation, governed by the berry's requirements for reducing power (Ruffner, 1982b), in a

manner analogous to that of glucose-6-phosphate dehydrogenase in the hexose monophosphate shunt (Ruffner et al., 1984).

Factors affecting the pH, titratable acidity, and the concentrations of potassium and malic and tartaric acids in grapes

Temperature. Temperature is the most important factor governing the acid content of grape berries (Kliewer, 1968; Winkler et al., 1974). Tartaric acid is respired only at temperatures above 30° C (Winkler et al., 1974), while malic acid is quickly respired at temperatures above 25° C (Lakso and Kliewer, 1978).

In 1897 Gerber, using a seedless grape to prevent seed respiration from interfering with fruit respiration, found that the respiratory quotient (RQ) increased in grape berries with increasing temperature. This he interpreted to mean that at very low temperatures sugars were incompletely respired and malate was formed, while at higher temperatures (30° C and above) malate and eventually tartrate were respired. Fruits high in tartaric acid, such as grapes, would therefore require higher ripening temperatures than those rich in malic acid, such as apples. Fruits containing citric acid (currants, citrus), which has an even higher RQ, would stay acid at maturity (Gerber, 1897).

Warmer seasons consistently produce less acid fruit. The titratable acidity (TA) of Tokay grapes, for example, was found to be inversely correlated with the heat summation from bloom to harvest where the heat summation was defined as "the sum of the mean daily temperature above 50° F (10° C) for the period concerned", expressed as day-degrees (Winkler and Williams, 1939).

Low temperatures during ripening are conducive to high levels of malic acid in grapes. The acidity of grapes ripened under hot field conditions (average 37° C during the day, 14° C at night) was compared with that of those ripened in a cool, sunlit phytotron (20° C days, 15° C nights). Fruits ripened at low temperatures had greater TA, malic acid and tartaric acid, and a lower pH than fruits ripened at high temperatures. The percentage of total titratable acidity due to the malate fraction was greater in the cool-ripened phytotron fruits than in the hot field-ripened fruits at the same degree of maturity (Kliewer, 1968).

At Davis, California, the acidity of grapes from Thompson Seedless clusters which were naturally exposed to the sun was measured periodically from before veraison until fruit maturity and compared with the acidity of grapes from similar clusters hidden inside the shady leaf canopy. "Sun" clusters at harvest were found to have a lower TA and higher pH than "shade" clusters. Although

tartrate levels were little affected by sun exposure, the malate concentrations in shade berries were 2-3 times greater at maturity than in sun berries. The differences between the two treatments were attributed to the higher temperatures of the sun berries, which received 43-62% more heat daily than shade berries (Kliewer and Lider, 1968). In another experiment, clusters of Sultana grapes were artificially shaded, and their acidity was determined to be higher than an unshaded control. Again, the conclusion was that the more acidic, shaded clusters received less heat (Kliewer and Antliff, 1970).

In field experiments, it is often difficult to differentiate between effects which are the result of heat intensity and those which are the result of light intensity because the major source of both heat and light is the sun. A stationary and rotating phytotron was used at Davis, California to grow three-year-old Cardinal and Pinot Noir grapevines from veraison to fruit maturity under a combination of high (30° C) and low (20° C) day temperatures and high (>2500 footcandle (ft-c)) and low (<1200 ft-c) light intensities. Night temperatures were held constant at 15° C. Grapes ripened at high light levels had a lower TA and higher pH than grapes ripened at low light levels. Since leaf and berry temperatures at low light levels were often much lower than those at high light levels, the difference in this case was considered to be

due to temperature rather than light intensity, since the rate of respiration would be reduced at lower temperatures. Reduced light intensity also delayed maturation, which probably contributed to a lower pH and higher TA in the fruit. In all treatments, malic acid was the source of the change in the TA, as tartaric acid levels were little affected (Kliewer and Lider, 1970).

The effect of temperature on the acidity of grapes does not seem to be limited to the ripening period. In another controlled temperature experiment, Cabernet Sauvignon vines, otherwise grown at 20° C day temperatures, were subjected to a 10-day "hot spell" of 30° C day temperatures. This "hot spell" lowered the concentration of malate at harvest by 25%, as compared to a 20° C control. The result was the same whether the 30° C temperatures occurred prior to veraison or late in the ripening period (Buttrose et al., 1971).

Nyctotemperature (night temperature), as well as phototemperature (day temperature), is important in determining the malate, TA, and pH of grapes. The lower the nyctotemperature for a given phototemperature, the greater the increase in TA and malate levels, and the greater the decrease in pH as compared to a control (Kliewer, 1973).

The preceding experimental evidence indicates that the amount of heat which the grapevine receives affects the

malic acid composition of the fruit at maturity. This was substantiated by a recent experiment in which individual bunches of grapes on the north side of grapevines (in the shade) were enclosed in electrically warmed plastic bags. The temperature of the clusters in the bags were artificially raised by 2-6°C during different phases of growth. Raising the temperature after veraison reduced the TA by 2.1 g/l at harvest. Raising the temperature from flowering to veraison increased the TA by 2.3 g/l (Klenert et al., 1978).

The temperature of the grapes themselves is not the only heat factor that influences the titratable acidity of grapes. In a University of California (UC) Davis experiment, grapevines were grown in a greenhouse at a constant air temperature but at different root temperatures. Root temperatures of 12° C caused a lower pH and higher malate and TA levels in the fruit as compared to root temperatures of 25° C. The higher TA at 12° C root temperature was closely related to a lower level of total soluble solids in the fruits, reflecting a general delay in maturity. The higher pH at 25° C may have been due to the greater uptake of cation, mostly potassium (K), at that temperature (Zelleke and Kliever, 1979).

Temperature indubitably affects the malate content of grape berries; however, the mechanisms by which it affects malate metabolism are complicated and not entirely known.

As related previously, the malate synthesizing and degrading enzymes seem to be directly regulated by cosubstrate availability rather than temperature itself (Ruffner, 1982b; Ruffner et al., 1984).

Moisture availability. The amount of soil moisture available to the vine during the growing season affects the levels of titratable acidity in the fruit. Researchers in Bordeaux, France, working with field planted Merlot Noir vines, found that the levels of tartaric and malic acids in the must were correlated with water uptake. Low water uptake was associated with reduced acidity (Duteau et al., 1981). Another French experiment involved 5 potted grapevine cultivars over a 5-year period during which the transpiration rate of the vines was measured. The vines were watered either at maximum transpiration (MT) or at a fraction of MT. The berry acidity was negatively correlated with the temperature sum during berry ripening, and water stress always reduced acidity (Meriaux et al., 1981). Similarly, in a long-term South African trial, moisture stress which occurred between fruit set and harvest resulted in a decrease in tartaric and malic acid (van Zyl, 1982). A German experiment using Bacchus and Muller-Thurgau vines concluded that sufficient water provided early after berry set led to a greater acid content in the berries (Eibach and Alleweldt, 1985).

An irrigation study at UC Davis concluded that frequent

irrigation of Carignane vines delayed maturity but did not affect titratable acidity at a given level of soluble solids. Irrigation increased wine pH and K concentration at a given level of soluble solids, while water stress delayed K accumulation in the berries during the final ripening period (Freeman and Kliewer, 1983). A similar experiment in Arkansas with Concord vines (Vitis labrusca L.) found little variation in TA or pH due to irrigation (Morris et al., 1983).

Nitrogen fertilization. Nitrogen fertilization may have an effect on the titratable acidity of grapes. Fruit from Thompson Seedless vines in a California vineyard which had been fertilized with various levels of ammonium nitrate showed a significant increase in pH and TA with increasing nitrogen fertilization. These effects may have been the result of increased vine vigor and leaf area in the fertilized vines, with a concomitant increase in acid production, or the denser shade resulting from the leafier canopy may have caused lower fruit temperatures (Bell et al., 1979). Conversely, an Arkansas experiment with Concord grapevines concluded that nitrogen fertilization tended to increase pH and reduce TA. The differences, though significant, were inconsistent and varied with growing season, irrigation level, and pruning level (Morris et al., 1983). The apparent discrepancy in results between the California and Arkansas trials may have been due to

varying cultivar responses, to differences in the actual need for nitrogen at the two locations, or to application rates, since the application rates in the Arkansas trial were only half as much as in the California trial.

Potassium (K) fertilization. Reports concerning the effects of potassium fertilization upon the titratable acidity and pH of grapes are varied. When excessive (225-900 kg/ha) levels of KCl fertilizer were applied to Concord vines in an Arkansas vineyard which already showed adequate initial levels of petiole K, the levels of juice K increased with high levels of K fertilization, resulting in a higher pH and lower TA in the fresh and stored juices. The positive correlation between total acidity loss and K levels were partly due to the hot Arkansas growing conditions, which has resulted in Concord grapes with malate levels as low as 28% of total acids. The correlation between K levels in the fresh juice and pH was much higher than that between K and TA. The authors concluded that in warm climates, where grapes often have a low acid content, overfeeding of vines with K fertilizer might result in poor quality juice products due to excessive potassium bitartrate precipitation (Morris et al., 1980).

Researchers working with Concord grapes in cooler, upstate New York were faced with the problem of excessive acidity in their grape juice products, which was also

detrimental to juice quality. They explored the feasibility of reducing the acidity of stored grape juice (after chilling and subsequent potassium bitartrate precipitation) by increasing the K concentration in the juice. Their research did not involve a field trial; instead, they analyzed a large number of samples from commercial vineyards selected on the basis of vine K nutrient status as determined by leaf petiole analysis. They found no relationship between K concentrations and the total acidity of grapes or juice before cold storage, but a significant correlation between the two factors was found after cold storage (and potassium bitartrate precipitation). Greater concentrations of K in the grapes caused greater amounts of potassium bitartrate precipitation. They concluded that under their cool climatic conditions the amount of extra K which could be incorporated into the grapes by K fertilization was insufficient to cause any significant additional reduction in juice acidity. The reason for this was the high malic acid content of the grape juices which would compete for K ions and reduce the precipitation of potassium bitartrate. The authors suggested that reduction of malate through vine management practices would be a more appropriate method of reducing juice acidity (Mattick et al., 1972)

The effect of two levels of potassium fertilization (0 and 2.2 kg K_2SO_4 per vine per year) was studied on

field-grown Carignane vines at Davis, California. The authors found that irrigation increased K uptake. K uptake rates were relatively high below 10° Brix and above 17° Brix soluble solids in the berries, and relatively low between 10° and 17° Brix. There was no apparent effect of K fertilization on grape juice pH, TA, or K levels. None of the treatments, including the unfertilized controls, showed a petiolar K deficiency (Freeman and Kliever, 1983). In an Arkansas experiment with Concord vines, which had never received prior K fertilization, fresh juice pH and K content were increased by K₂SO₄ fertilization (Morris and Cawthon, 1982). In Australian Shiraz vines, KCl fertilization increased the K content of wine from a cool, dryland vineyard but not from a hot, irrigated vineyard. However, leaf petiole K concentrations were increased in the hot vineyard but not in the cool one. The authors concluded that the application of K fertilizers to vines which were not deficient in petiolar K caused little change in yield or wine composition, and that there seemed to be little relationship between soil K content and wine K concentration and pH (Dundon et al., 1984).

Other experiments involving K fertilization will be mentioned briefly. A Swiss experiment with the cultivars Chasselas and Sylvaner, which occasionally yield wines deficient in acidity when grown in warmer climates, found

that the absence of K fertilization led to a reduction in leaf petiolar K values but found no evidence that high rates of K fertilization contributed to must acidity (Murisier et al., 1982). An Italian foliar feeding trial with 1.2% K_2SO_4 applied on four dates between fruit set and veraison showed little effect on TA or pH of the juice, although reducing sugar content was increased from 16.7 to 18.4% (Lisi et al., 1983). A third experiment involving the source of potassium (KCl vs. K_2SO_4) found that TA was little affected by the source (Edelbauer, 1979).

Crop load. The amount of fruit that the vine must ripen is a major factor affecting fruit acidity and pH. Field experiments with Tokay vines have shown that, at any given date, the malate concentration and TA are lowest and the pH highest in the lowest-cropped vines (Kliewer and Weaver, 1971). The effect of increasing crop load is generally one of delayed maturity: a lower pH and higher TA in the fruit associated with lower sugar concentrations (Winkler et al., 1974).

Rootstock. The type of rootstock to which a vine is grafted may affect the composition of the fruit. In one field study comparing many cultivars grafted onto Rupestris St. George or 99-R rootstocks, the fruit from the more vigorous St. George rootstock had a higher total acidity and a higher pH (Ough et al., 1968). A later study, under controlled conditions of temperature, found that fruit from

vines grafted onto the St. George rootstock had a higher pH and lower TA than fruit from vines growing on AXR1, SO₄, or own roots (Zelleke and Kliewer, 1979).

Canopy microclimate as affected by trellising, spacing, and cultural factors. The canopy microclimate of a grapevine as defined by Smart (1985) is the sum of the radiation, temperature, relative humidity, windspeed, and evaporation components within the canopy as affected by "the amount and distribution of leaf area in space and its interaction with above-ground climate". It can be modified by the type and height of the trellis employed, vine and row spacing and orientation, vine vigor, shoot density, fertilization, soil texture and depth, soil management, water availability, cropping level, summer (green) pruning, vine health, and many other factors (Smart, 1985). It is possible that many of the significant effects of these factors on the acidity and pH of grapes reported earlier in this literature review were in fact due indirectly to a change in canopy microclimatic equilibrium.

Researchers in Bordeaux have shown that the type of trellis and training system can significantly influence the malic and tartaric acid contents of musts. At harvest, grapes grown on a half-divided canopy with a long trunk and downward-bearing canes contained about 110 meq/l malic acid, while those grown in an upright open lyre configuration contained about 87 meq/l malic acid.

Tartaric acid concentrations ranged from about 89 meq/l for an upward-bearing, half-divided canopy planted in wide rows to about 103 meq/l for a single, vertical canopy subjected to summer pruning and planted in narrow rows. The differences were attributed to differences in the microclimatic equilibrium between leaves and bunches for the different trellising and training systems. The best systems were those which allowed partial but not complete penetration of sunlight throughout the canopy in general and on the bunches in particular (Carbonneau and Huglin, 1982).

Experiments in Australia showed that must malate levels were positively correlated with vine leaf area and negatively correlated with fruit exposure. Shiraz vines growing in a hot, dry climate were manipulated to give a variety of canopy densities. Vines with a greater proportion of "exterior" fruit and leaves (i.e., better sunlight penetration of the canopy) had significantly warmer foliage and fruit. Vines with a high ratio of leaf area to vine surface area, high shoot number, and heavy shade had increased K concentrations in their leaves, stems, and petioles at veraison (Smart et al., 1985a). The grapes from these vines produced musts with reduced sugar content and a higher pH, K and malic acid content (Smart, 1982; Smart et al., 1985b). The resulting wine was poor in quality due to higher pH and K content, and to reduced

proportions of ionized anthocyanins (Smart et al., 1985b).

Conversely, a University of California study at Davis, California, found no significant difference in pH or TA between Chenin Blanc grapes trained and trellised at two heights on a single plane vertical, T, or Y type trellis, even though the shoot density on the Y trellis was only 2/3 that of the other systems (Kasimatis et al., 1982).

Within-row vine spacings may have some effect on fruit acidity. Concord grapes grown at wide within-row vine spacings (3.05 m) in an Arkansas experiment tended to have a lower TA than those grown at narrow within-row vine spacings (Morris and Cawthon, 1981).

A cultural practice which has been reported to affect general maturity at harvest (and thus, indirectly, TA) is early summer topping. Russian experiments with three Vitis vinifera cultivars showed that early summer topping hastened maturity, while topping several times in a season produced no results (Madenov et al., 1979). This was confirmed by an Italian trial which found that topping hanging shoots 1 week after anthesis enhanced ripening, whereas topping 7 or 11 weeks after anthesis delayed ripening. The delay in ripening was attributed to competition between fruit clusters and young laterals (Intrieri et al., 1983).

Relationship between organic acid concentrations, pH, and titratable acidity in grapes and musts.

A grape must is an aqueous solution of organic and inorganic acids and their salts, sugars, proteins, and other substances derived from the crushed and pressed grapes. The pH of the must depends upon the amount of each amino acid, carboxylic acid, mineral acid, and phenolic compound present, the pK_a s of their ionizable acidic moieties, and the extent of salt formation which has occurred. Of the organic constituents, tartaric and malic acid exert the most influence on pH. Tartaric acid has lower pK_a s than malic acid (3.01 and 4.05 vs. 3.46 and 5.05) (Amerine et al., 1972) and at the pH of grape must has the greater influence on pH. The greater the relative proportion of tartrate in a must, the lower is the pH that the must is buffered to (Boulton, 1980b). Grapes also contain a high concentration of potassium, which has an even greater effect on pH than does tartaric acid (Boulton, 1980b,c).

Boulton (1980d) has shown that the number of protons expected from the acidic composition of wine (termed "total acidity") is equal to the sum of the titratable protons (titratable acidity), potassium ions, and sodium ions present. The other mineral and organic constituents of grape juice and wine contribute little to the pH or

titratable acidity. To explain the cause of this relationship, he hypothesized the existence of a membrane-bound adenosine triphosphatase (ATPase) molecule in the plasmalemma of grapevine root, leaf, and berry cells which exchanges monovalent metal cations (potassium and, to a lesser extent, sodium) for protons while hydrolyzing adenosine triphosphate (ATP) to adenosine diphosphate (ADP) (Boulton, 1980a). This leads to an increase in pH due to the formation of acid salts (Boulton, 1980b). Although the existence of such an enzyme has not yet been demonstrated in grapes, similar enzymes are found in other plants, such as oats, barley, wheat, turnips, beans, maize, sugar beets, gourds, and peas (Boulton, 1980a).

Boulton further hypothesizes that it is the ATP level in the cytoplasm of the cells containing these ATPases which influences the exchange of monovalent metal cations and protons. High ATP levels would allow greater cation import and proton export, while low ATP (high ADP) levels would allow a reverse flow to occur. Enhanced potassium uptake has been shown to occur in grapes during ripening in cool regions when sugar accumulation has begun to cease or is delayed, resulting in an increase in pH and a decrease in TA. According to the ATPase theory, this may be due to a competition for available ATP between the transporting enzymes for sugar and cations which favors sugar accumulation at higher temperatures (Boulton, 1980a).

Analytical methods for quantitating malate, tartrate, and potassium in grapes and grape juice or must.

Malate and tartrate may be quantitated by means of gas chromatography (GC) (Mattick et al., 1970), high performance liquid chromatography (HPLC) (McCord et al., 1984; Frayne, 1986), or by spectrophotometric means. The spectrophotometric method for quantitating malate (McCloskey, 1980) measures the quantity of NADH produced when the enzyme malate dehydrogenase (E.C. 1.1.1.37) dehydrogenates malate to produce oxaloacetate. The amount of NADH produced is measured at 340 nm, and is directly proportional to the amount of malate in the sample. Tartrate is quantitated spectrophotometrically by measuring the formation of a colored vanadate complex (Vidal and Blouin, 1978) at 520 nm. Potassium may be quantitated by atomic absorption (AA) (Amerine and Ough, 1980).

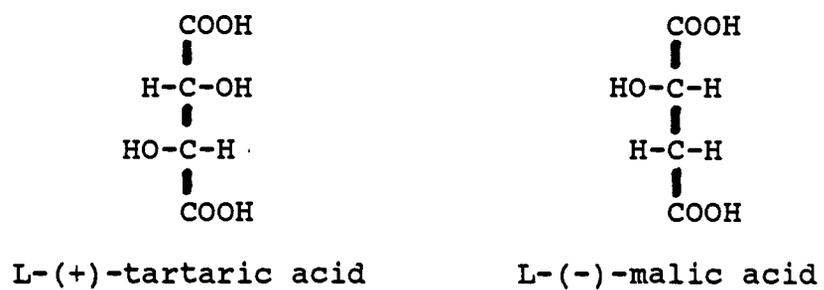


Fig. 1 Principal acids of grapes.

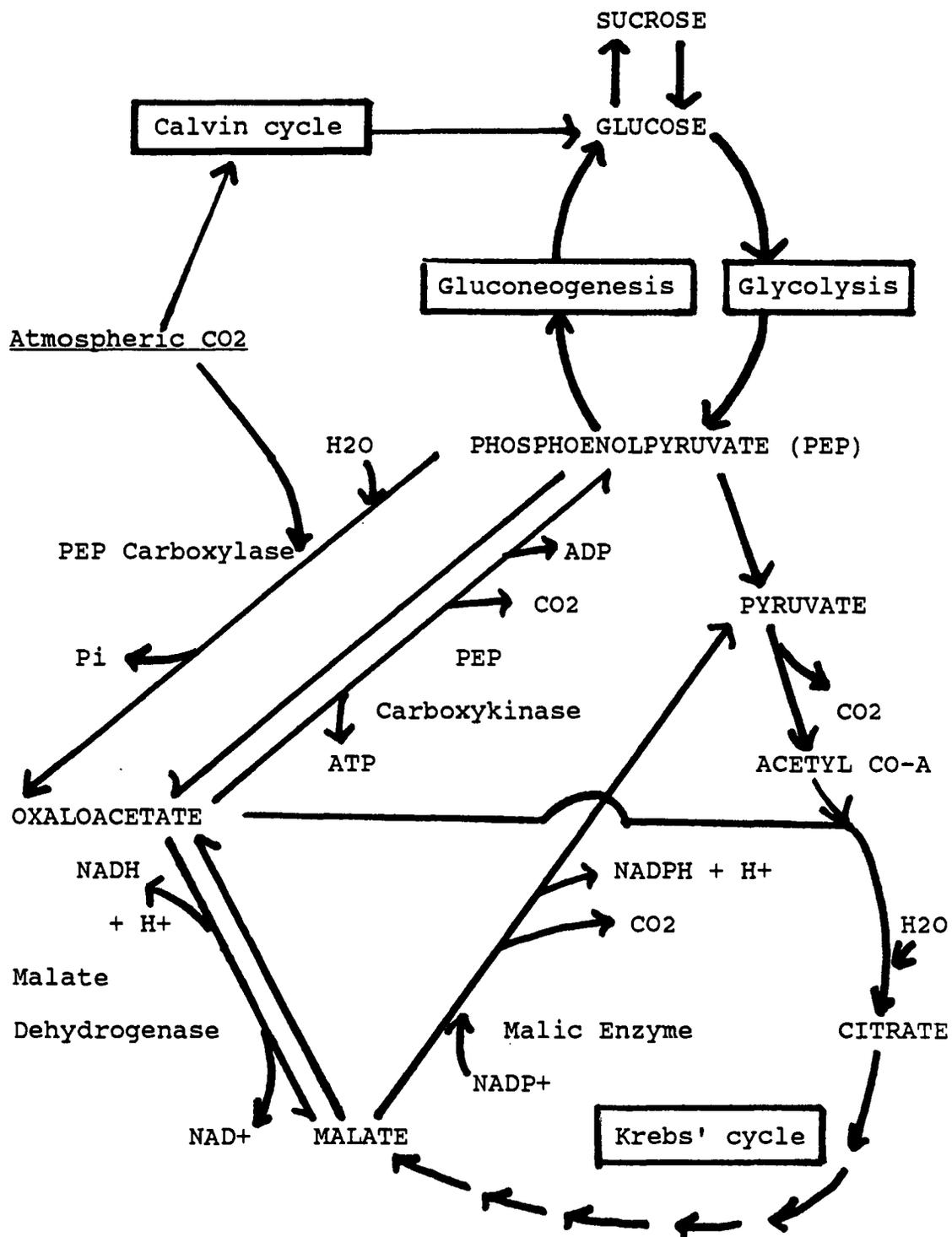


Fig. 2 Overview of malate metabolism in *Vitis*.

Effect of Cluster Exposure by Defoliation at Veraison
on Malate, Tartrate, and Potassium in Berries
of Three Cultivars of Vitis vinifera L.

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and David A. Heatherbell³

ABSTRACT

The effects of cluster exposure by basal leaf removal at veraison on the malate, tartrate, and potassium content of grapes were investigated. At veraison, clusters of Chardonnay grapes were a) exposed to the sun by removal of all leaves opposite or below the clusters, b) treated as in a) but shaded with shade cloth, c) exposed to the sun by tying back leaves opposite or below the clusters, or d) left untreated as a control. Clusters of White Riesling were exposed to the sun by similar leaf removal a) 10 days before veraison, b) 10 days after veraison, or c) untreated. Clusters of Pinot Noir were exposed a) at veraison, b) 2 weeks after veraison, or c) untreated. Exposed clusters received 3 to 3.5 times more light than shaded clusters and up to 32% more heat, with temperature differences between exposed and shaded treatments being most pronounced during cool, sunny weather. None of the treatments had any effect on juice or berry malate,

tartrate, or potassium content; however, exposed clusters of Pinot Noir had a lower pH (.03) and higher TA (.06%) than the control at harvest. Cluster exposure of Chardonnay increased sunburning of grapes, and cluster exposure of Pinot Noir at veraison caused a 1% reduction in juice soluble solids concentration at harvest. The detrimental effects of cluster exposure by basal leaf removal at veraison, as well as the lack of any major effect on the acid content of the berries, suggest that the practice has no value for acid reduction during a warm, dry maturation season in western Oregon.

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INTRODUCTION

One of the most important environmental factors affecting the titratable acidity of grapes is temperature (Kliewer, 1968; Winkler et al., 1974). Correlations have been demonstrated between the titratable acidity of the fruit and the temperature summation of the growing season (Winkler and Williams, 1939), the temperature of the root system (Zelleke and Kliewer, 1979), and the temperature of the clusters (Kliewer and Lider, 1968; Klenert et al., 1978). Generally, the titratable acidity of fruit matured in warmer seasons is lower than that of fruit matured in cooler seasons, due to increased respiration of malic acid during maturation (Kliewer, 1968; Klenert et al., 1978). The mechanism is two-fold: 1) warmer temperatures between bloom and veraison tend to advance the onset of veraison (Klenert et al., 1978), 2) allowing maturation and malate respiration to occur more rapidly and to a further extent under the warmer climatic conditions usually found in early autumn (Alleweldt et al., 1984). Since the utilization of malate as a respiratory substrate (Gerber, 1898) and the rate of malate respiration (Winkler et al., 1974; Kliewer, 1964) are affected by temperature, any conditions which lead to higher cluster temperatures, such as warmer phototemperatures (Kliewer, 1968; Buttrose et al., 1971)

and nyctotemperatures (Kliewer, 1973), cluster exposure to the sun (Kliewer and Lider, 1968), and higher light intensities (Kliewer and Lider, 1970) might be expected to increase malate respiration rates.

Although the macroclimate of a specific vineyard cannot be altered, the cluster microclimate may be optimized by selecting a canopy configuration that allows sunlight illumination of interior leaves and bunches (Carbonneau and Huglin, 1980; Koblet, 1984). 'Open' canopies (i.e., greater fruit exposure to the sun) have been associated with lower malate and higher tartrate levels in the fruit at harvest (Reynolds et al., 1985; 1986). Vines with a lower ratio of leaf surface area to canopy surface area have significantly warmer foliage and fruit during sunny weather, and the grapes may have a lower malate content (Smart, 1980).

To control bunch rot, some western Oregon winegrowers have adopted the European practice of exposing clusters by removing basal leaves during maturation. This practice has also been reported to reduce the titratable acidity of grapes in European vineyards (Carbonneau and Huglin, 1980; Koblet, 1984). A reduction in titratable acidity due to increased malate respiration in a cool year would be desirable in western Oregon wine grapes because the finished wine would have a lower titratable acidity and would undergo a smaller pH rise after malolactic fermentation. The purpose of this investigation was to

determine the effects of cluster exposure by basal leaf removal at veraison on the malate, tartrate, and potassium concentrations and their dependent measurements, pH and titratable acidity, of grapes under western Oregon maturation conditions.

MATERIALS AND METHODS

Site One

This trial was conducted during the 1985 growing season with three vine cultivars at two sites. The first site was on a flat Chehalis silty clay loam soil at the Oregon State University Lewis-Brown Horticultural Farm, Corvallis, Oregon. Fifteen year old own-rooted, cane-pruned Chardonnay and White Riesling vines trained by the Guyot method to a vertical 7-foot trellis in north-south rows and arranged into four 5-vine blocks were used.

Chardonnay trial: The Chardonnay trial contained four treatments, all applied on August 30: A) Cluster exposure by basal leaf removal, in which all leaves at or below the uppermost cluster were removed at veraison; B) cluster shading with basal leaf removal, identical to A) except that the exposed clusters were covered with black shade cloth; C) cluster exposure without basal leaf removal, in which basal leaves were tied back to expose the clusters to sunlight; and D) cluster shading without basal leaf removal, or no treatment, which was used as a control. Because the number of vines was limited, the treatments were split between canes, so that each vine contained two treatments. This doubled the number of vines receiving any one treatment, and was considered justifiable since the effects of cluster temperature on the malate content of the

berries are probably localized within the affected cluster (Klenert et al., 1978). All treatments were randomly distributed throughout each block and the canes at the ends of each block were left untreated to serve as buffers.

The internal cluster temperatures of two selected clusters (one on the east side of the row, and one on the west) within each treatment in one block, as well as internal and external canopy air temperatures, were measured hourly by thermocouple leads and automatically recorded to the nearest 0.1°C by a CR5 Digital Recorder (Campbell Scientific, Inc., Logan, Utah). Light penetration of the canopy at cluster level was measured on the west side of the canopy between 10 and 11:30 am and on the east side between 3 and 4:30 pm (pdt) during clear weather on September 3 using a LI-1915B Line Quantum Sensor (Li-Cor, Lincoln, Neb.) held in a horizontal position. The average photosynthetic photon flux density (in $\mu\text{Es}^{-1}\text{m}^{-2}$) for three consecutive ten second intervals each at two positions per treatment, describing the amount of sunlight present on the shady side of the clusters, was used as a measure of canopy density.

The canopy composition at the cluster level was determined after the modified point quadrant method of Smart (1980). A thin metal rod was inserted into the canopy at the cluster level at an angle of 45° to the vertical (this being the average morning or afternoon sun

angle) and the nature of the first four contacts (leaf, bunch, wood, or ground) was recorded. Forty insertions, twenty from each side of the canopy, were made for each block and treatment combination.

At harvest on October 12 the yields per vine and treatment were recorded, along with the percentage of sunburned berries on each cluster.

White Riesling trial: The White Riesling trial was conducted in the same manner as the Chardonnay trial except that the treatments were as follows: A) cluster exposure by basal leaf removal on August 30 (10 days pre-veraison), B) cluster exposure on September 19 (10 days post-veraison), and C) no treatment. At harvest on October 14 the percentage of raisined grapes on each cluster was measured rather than the percentage of sunburned clusters. No light, temperature, or canopy composition measurements were made.

Site Two

Pinot Noir trial: The second trial site was a 7 year old Pinot Noir vineyard located on an Olympic clay loam soil in the Eola Hills near Salem, Oregon. This vineyard was trained on a single wire trellis in north-south rows to a 125 cm high bilateral cordon with hanging shoots. Treatments were similar to the White Riesling trial except that there were four 12-vine blocks, each block containing

three treatments of four vines each. Treatments were applied on September 4 (veraison) and 18 (post-veraison), and final samples were taken on September 30. The photosynthetic photon flux measurements were made on September 30 during clear weather between 1:00 and 3:15 pm (pdt) by holding the line quantum sensor in a horizontal position inside the canopy at the cluster level. Measurements were adjusted for the increasing sun angle of the early afternoon by comparing them to similar measurements taken above the canopy.

Sampling

All trials were sampled for both juice and whole berry analysis. Sampling for juice analysis was at harvest, and consisted of a random 150 berry sample taken from each block and treatment combination. Samples for whole berry analysis were taken both at the beginning of the trial (100 berries) and at harvest (150 berries), and were frozen at -12° C in airtight plastic bags (Ziploc, Dow Chemical Co., Indianapolis, Indiana) for later analysis.

Sample preparation and analysis

Juice samples: The berries were weighed and then gently crushed with a mortar and pestle and the juice expressed through cheesecloth by hand after the method of Amerine and Ough (1980). The settled juice was analyzed

for soluble solids, pH, and titratable acidity (Amerine and Ough, 1980), then frozen at -12° C in airtight plastic bottles (VWR Scientific, Inc., San Francisco, Calif.). Frozen juice samples were heated to 60° C for 6 hours with periodic shaking to redissolve any precipitated potassium bitartrate salts, centrifuged to remove solids, and analyzed for tartrate by the ammonium meta-vanadate method (Vidal and Blouin, 1978), for malate by an enzymatic method (McCloskey, 1980), and for potassium by flame atomic absorption (Amerine and Ough, 1980) on a Perkin-Elmer 4000 atomic absorption spectrophotometer (Perkin-Elmer, Norwalk, CT).

Whole berry samples: The frozen berry samples for whole berry analysis were made into an extract by blending, boiling, and dilution to volume according to the method of Mattick (1983), and analyzed in the same manner as the juice samples (with the exception of total soluble solids, which was not determined). Titratable acidity, tartrate, malate, and potassium were expressed as grams per berry.

RESULTS AND DISCUSSION

Temperatures

The average daily heat summation above 10°C (in °C-hrs) for the east and west sides of each treatment in the Chardonnay trial during different weather conditions was calculated as a ratio of the heat summation of the air temperature outside of the canopy (Table I.1). Heat summations were calculated with 10°C as a reference because photosynthetic rates in grapevines decrease rapidly below that temperature. This is also the standard reference temperature used in the literature to relate the effects of climate on grape growing (Winkler *et al.*, 1974). Treatment A (cluster exposure x basal leaf removal) always showed the highest heat summation, and treatment D (control) usually showed the lowest heat summation, with the exception of windy weather, when treatment C (cluster exposure with leaves tied back) was lowest. The differences in heat summation between exposed and shaded treatments were most marked on cool and sunny or partly cloudy days. Under these conditions treatment A accumulated as many as 32% more degree-hours of heat than treatment D. Clusters exposed to direct or diffuse sunlight quickly warmed up to temperatures several degrees in excess of the ambient air temperature; however, once they had passed into shade they quickly cooled down to air

temperature (Fig. I.1). Treatment B (artificial shading x basal leaf removal) showed a greater heat summation than did treatment D. Apparently the black shade cloth, being an efficient solar energy absorber (and re-radiator), failed to effectively prevent solar heating of the clusters. The leaves shading the clusters in treatments C and D were very efficient at blocking solar heating of the clusters; treatment C, in which the leaves were tied back to reduce cluster shading, received less heat than either treatment A or B. During periods of heavy cloud cover, none of the treatments showed a heat summation greater than that of the air temperature.

The heat summations above 25°C and 30°C for the entire maturation period were also calculated for each treatment (Figs. I.2 and I.3), because references in the literature (Bremond, 1937; Winkler *et al.*, 1974) suggested that malate respiration would occur more readily at or above those temperatures. Clusters which were exposed to the sun (treatments A and C) showed the greatest heat summation above either temperature, and clusters on the west side of the canopy (with the exception of treatment B) received more heat than those on the east side. However, when the heat summation was calculated with 10°C as a reference, there was little difference in heat summation between the east or west sides of the canopy. This was probably due to the fact that the heat summation above

10°C was influenced more by the air temperature, while the heat summation above 25°C or 30°C was influenced more by direct solar heating.

Effects on Cluster Quality

In the Chardonnay trial, both cluster exposure treatments significantly decreased the percent of clusters with <5% sunburned berries, and the cluster exposure x basal leaf removal treatment resulted in a significant increase in the percent of clusters with >25% sunburned berries as compared to the control (Table I.2). An increase in sunburned berries was an undesirable but expected side effect of cluster exposure early in maturation. Carbonneau and Huglin (1980) have cautioned against 'exposing the bunches to too warm a climate'. Cluster exposure in the White Riesling trial did not significantly affect the incidence of raisined berries. Since the weather during the final 3 weeks before harvest was generally warm and dry, it was not possible to determine whether the raisining of the White Riesling berries was due to Botrytis infection or not; the grey mold growth typical of Botrytis was absent.

Effects on Juice Composition

Although the different combinations of cluster exposure and leaf removal treatments significantly affected sunlight penetration into the canopies at the cluster level (Tables I.3 and I.4), there was no significant effect on juice tartrate, malate, or potassium concentrations in any of the trials (Table I.5). Cluster exposure of Pinot Noir significantly increased the titratable acidity and lowered the pH of the juice at harvest as compared to the control. The reasons for this are not understood, as the malate, tartrate, and potassium concentrations of the juice from all treatments were similar. Possibly the exposed treatments contained greater concentrations of other buffer components, such as phosphoric acid. Hrazdina et al. (1984) have shown that phosphate is the third most abundant anion in grapes and its concentration increases during the latter stages of maturity.

In addition, cluster exposure by basal leaf removal at veraison in Pinot Noir resulted in a significantly lower juice soluble solids content at harvest. This may have been due to a loss in photosynthetic capacity of the vine due to the removal of the basal leaves, which in this vineyard (bilateral cordon with hanging shoots) were well exposed to sunlight. The fact that a similar trend did not occur in the White Riesling and Chardonnay trials may be due to (1) the higher canopy density and shading of the

basal leaves in the Corvallis vineyard, (2) the training of the Corvallis vineyard to a vertical upright trellis, which places the basal leaves in the lowest and most shaded position, or (3) the fact that the treatments were applied to single canes rather than to whole vines. May et al. (1969) have shown that translocation of sugars from non-defoliated shoots to defoliated shoots within a vine can occur.

Effects on Berry Composition

None of the treatments had any significant effect on the titratable acidity or malate, tartrate, and potassium concentrations per berry at harvest (data not shown). However, when the differences in titratable acidity, tartrate, malate, and potassium between veraison and harvest were expressed as a percent of the amount at veraison, some differences became apparent (Table I.6). Cluster exposure of Chardonnay grapes by basal leaf removal at veraison significantly increased the percent reduction of malate and titratable acidity per berry as compared to the control, and significantly reduced the percent uptake of potassium per berry. In the White Riesling and Pinot Noir trials there was no significant effect of the treatments on the percent change in the whole berry composition, although a similar nonsignificant trend for exposed clusters to have a lower percent potassium uptake

was observed in White Riesling.

On a whole berry basis, all three cultivars behaved similarly with regard to percent change in titratable acidity, malate, and tartrate per berry, but differently with regard to percent change in potassium per berry. There was a 23 to 40% decrease in titratable acidity associated with a 32 to 60% decrease in malate between veraison and maturity, and either a slight increase or no change in berry tartrate content. Both Chardonnay and White Riesling accumulated potassium during this time, but Pinot Noir did not.

The lack of potassium accumulation apparent in the Pinot Noir trial is unusual, because grape berries normally experience a large influx of potassium during the latter stages of maturation (Boulton, 1980; Freeman and Kliever, 1983; Hrazdina et al., 1984). It may be a result of water stress, as the vineyard is a hillside site with limited soil depth and water holding capacity. 1985 was a very dry growing season in western Oregon and other blocks of vines within this vineyard showed symptoms of severe water stress. Freeman and Kliever (1983) found that water stress reduced the concentration of potassium in Carignane berries. Boulton (1986) believes that potassium uptake is affected by the size and activity of the vine's root mass. Vines rooted in deep, moist soil should have a larger number of roots and consequently should be able to take up

more potassium during the growing season than vines with fewer roots growing in shallow, dry soil. The low uptake of potassium in the Pinot Noir trial may also be due to the low potassium content of the soil.

Since significant differences between treatments in the malate and potassium concentrations of the Chardonnay berries became apparent only when considering the percent change from veraison, it is suggested that the relationship between the concentrations of these substances in the berries and the amount of sunlight or heat reaching the clusters during maturation was very slight. Although sunlight is the ultimate source of heat in the vineyard, the majority of the heat absorbed even by a cluster exposed to sunlight seems to derive from other sources, such as secondary radiation from sun-warmed earth and conductive heat transfer from warm air. The heat gains obtained through cluster exposure did not translate into lower malate or potassium levels at harvest.

Other published works showing a relationship between cluster microclimate and malate and titratable acidity levels at harvest have been conducted either in growing areas with more intense sunlight than western Oregon or over a longer portion of the growing season, or both (Carbonneau and Huglin, 1980; Kliewer and Lider, 1968; Radler, 1965; Reynolds *et al.*, 1985, 1986; Smart, 1980; Wolf *et al.*, 1985; Wolpert *et al.*, 1983). Those conducted

in regions of intense sunlight utilized trellis systems, such as the tee trellis, which caused heavy shade upon the fruit. This would be expected to result in a much greater heat summation differential between exposed and shaded clusters than would be found in a trial conducted in relatively weak sunlight on trellises designed to minimize fruit shading. Similarly, trials extending over a longer period of the growing season would also result in a greater heat summation differential between treatments. Thus, the failure of this experiment to obtain significant differences between treatments may be due to too small a heat summation differential.

The possibility also exists that the microclimate of the entire canopy may be more important in determining the rate of malate respiration than the microclimate of the clusters alone. Malate is respired during maturation to provide the energy necessary for the import and concentration of sugars within the berries, as well as the import of potassium, the biosynthesis of color and flavor compounds, and other energy requiring processes. The enzymic complex which controls the rate of malate catabolism in the berries is apparently directly regulated by the NADP⁺/NADPH ratio in the grape berry cells, not by temperature (Ruffner, 1982; Ruffner *et al.*, 1984). Presumably, a naturally 'open' canopy which allows good fruit exposure to the sun would also allow good leaf

exposure (Smart, 1980; Reynolds et al., 1986). This might result in the production of more photosynthates by leaves in an 'open' canopy as compared to a 'dense' one, which would lead to a greater energy demand for the purposes of sugar storage within the berries, resulting in an increase in malate respiration rates.

CONCLUSIONS

For the purpose of acid reduction, there appears to be no value in the practice of cluster exposure by basal leaf removal during a warm, dry maturation season in western Oregon on either of the trellis systems investigated in this study. Rather, the practice may harm fruit quality by sunburning the grapes or reducing sugar uptake. Since cluster exposure is used primarily for botrytis control, it would be unnecessary during dry weather anyway. Exposed clusters show a greater proportional difference in heat summation over shaded clusters during cool and sunny or partly cloudy weather rather than during warm, sunny weather. Assuming that the previously reported relationship between malate respiration rates and cluster temperature during maturation does exist, then cluster exposure after veraison during cool conditions might cause the greater reduction in malate.

Table I.1 Effect of exposure or shading of clusters with or without basal leaf removal on the ratios between the cluster and air heat summations. Chardonnay trial. Corvallis, Oregon. 1985.

Treatments	Date Conditions	Ratios			
		9/5 cloudy	9/25 sunny	10/9 sunny, windy	10/10 partly cloudy
	Max. air temp (°C)	21	31	17	19
A: Exposed cluster x basal leaf removal		.99	.99	1.12	1.25
B: Shaded cluster x basal leaf removal		.97	.98	1.06	1.08
C: Exposed cluster x no basal leaf removal		.97	.97	.94	.98
D: Shaded cluster x no basal leaf removal		.97	.94	.98	.95
A/D		1.02	1.05	1.14	1.32

Fig. I.1 Effect of side of canopy during clear weather on the internal temperature of clusters exposed by basal leaf removal. Chardonnay trial, Corvallis, Or. Sept. 25, 1985.

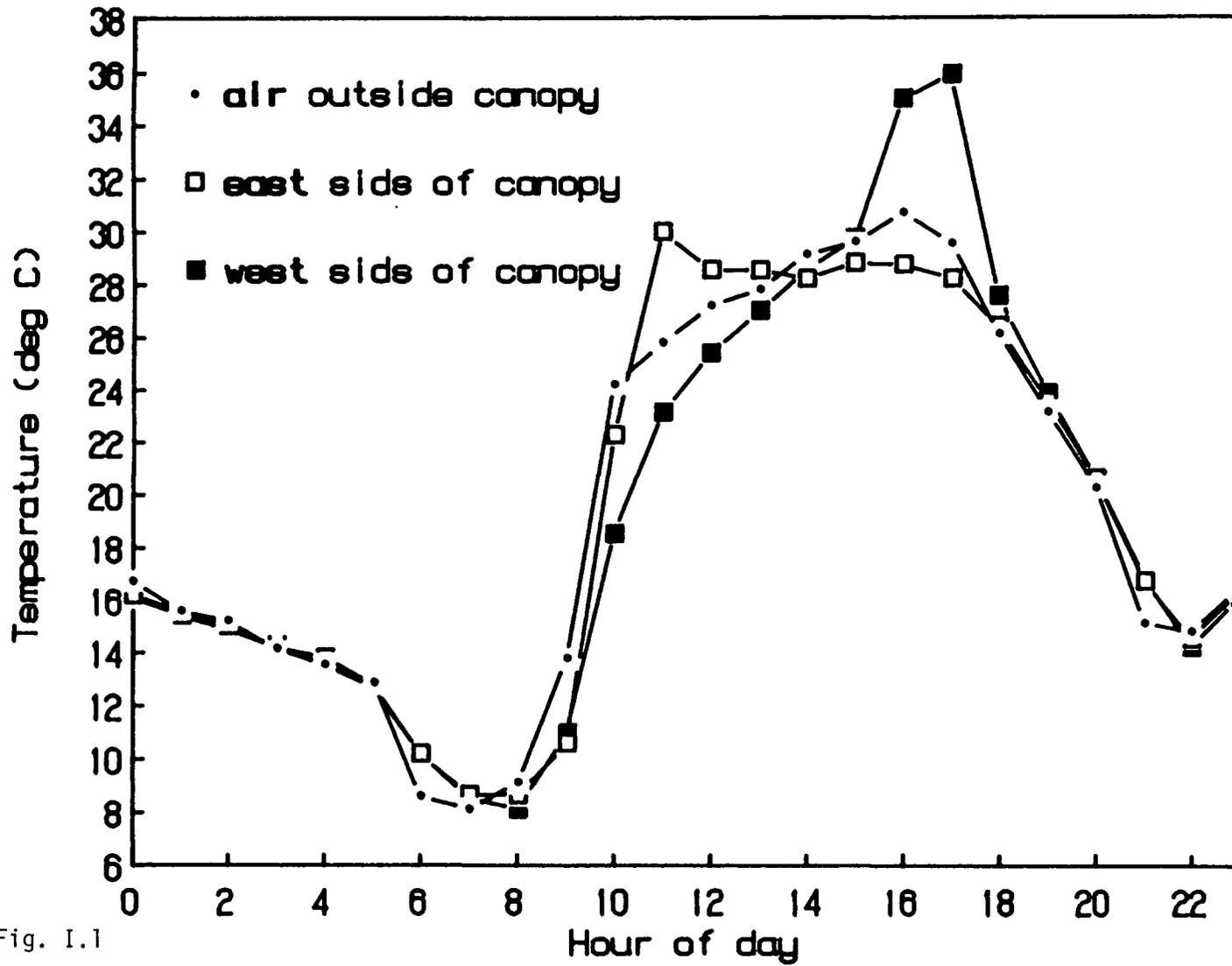


Fig. I.1

Fig. I.2 Heat summations (number of deg C-hrs over 25 deg C) between veraison and harvest for exposed and shaded clusters with or without basal leaf removal on the east and west sides of the canopy. Chardonnay trial. Corvallis, Or. 1985.

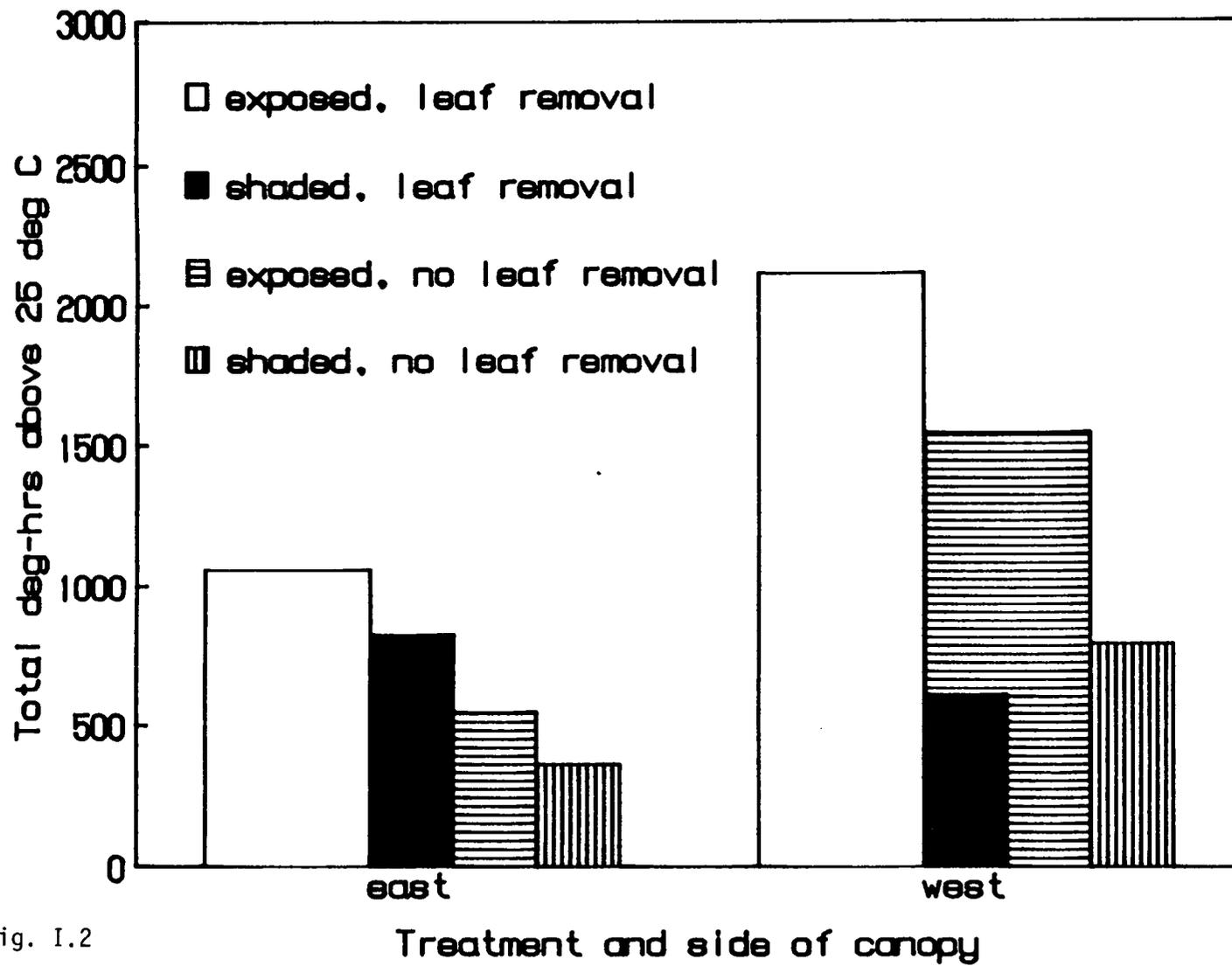


Fig. I.2

Fig. I.3 Heat summations (number of deg C-hrs over 30 deg C) between veraison and harvest for exposed and shaded clusters with or without basal leaf removal on the east and west sides of the canopy. Chardonnay trial. Corvallis, Oregon. 1985.

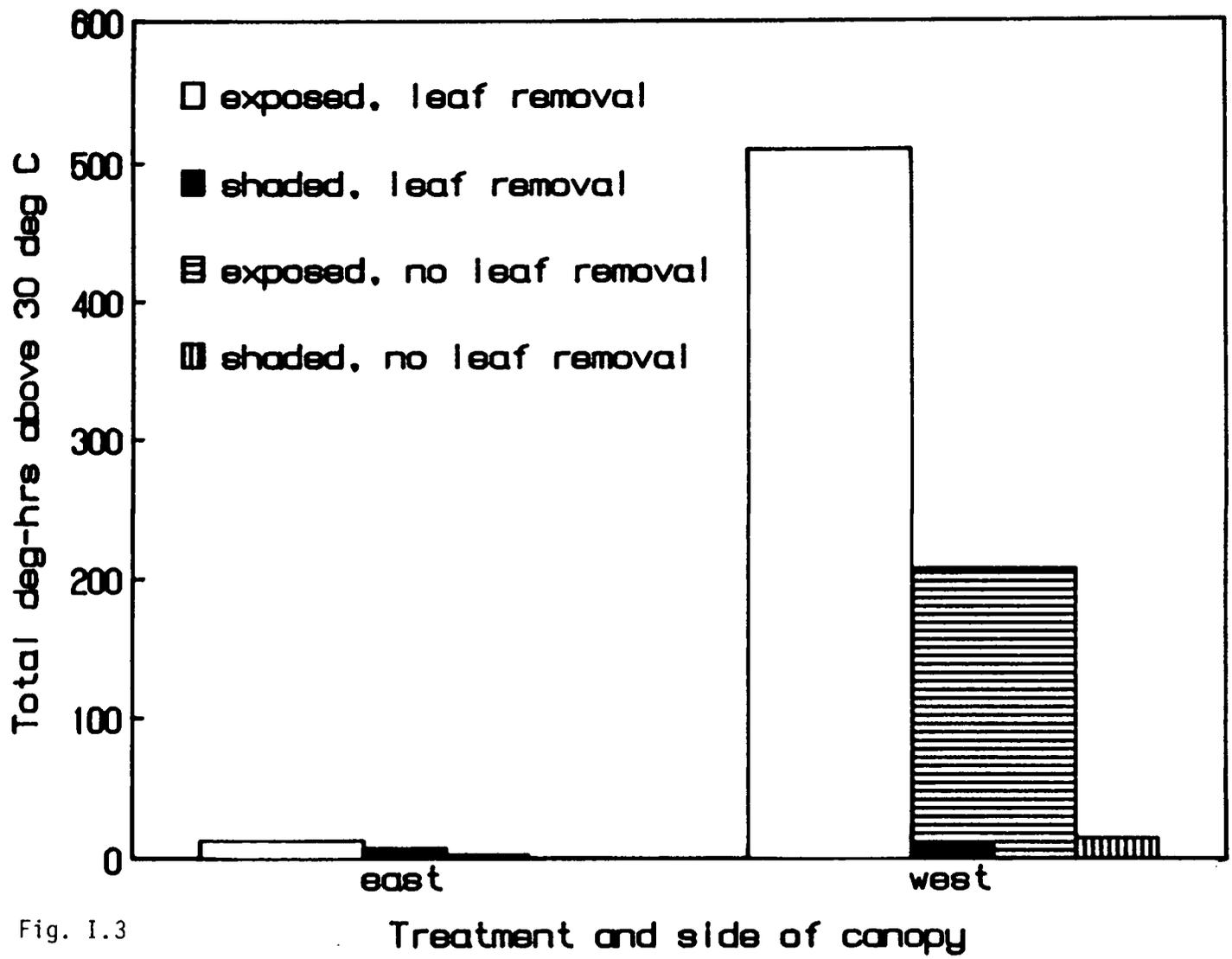


Fig. 1.3

Table I.2 Effect of cluster exposure and basal leaf removal treatments on percent of sunburned clusters of Chardonnay grapes. Corvallis, Oregon. 1985.^z

Treatment	% of clusters with		
	<5% sunburned berries	5-25% sunburned berries	>25% sunburned berries
A: Cluster exposure x basal leaf removal	25a	40a	36b
B: Cluster shading x basal leaf removal	48b	38a	14ab
C: Cluster exposure x no basal leaf removal	32a	36a	33ab
D: Cluster shading x no basal leaf removal	55b	35a	10a

^z Means separated within columns at p=.05 by Duncan's multiple range test

Table I.3 Effects of cluster exposure and basal leaf removal treatments on amount of fruit shading and light penetration of canopy at cluster level of vines trained to a single curtain vertical trellis. Chardonnay trial. Corvallis, Oregon. 1985.²

Treatment	Fruit shading			Average light level on shady side of bunches ($\mu\text{Es}^{-1}\text{m}^{-2} \times 10^3$)	
	Percent of fruit shaded by			Time	
	<u>0</u>	<u>1</u>	<u>2</u>	10-11:30 am	3-4:30 pm
A: Clus. exp. x basal leaf removal	46.5c	40.8ab	12.7a	5.15b	2.73c
B: Clus. shade x basal leaf removal	0a	57.2c	42.8c	1.73a	0.82a
C: Clus. exp. x no basal leaf removal	48.8c	33.0a	18.2a	4.92b	2.77c
D: Clus. shade x no basal leaf removal	18.8b	51.5bc	29.7b	2.32a	1.72b

z Means separated within columns at $p=.05$ by Duncan's multiple range test.

Table I.4 Effect of basal leaf removal on light penetration of canopy at cluster level of vines trained to a bilateral cordon with hanging shoots. Pinot Noir trial, Salem, Or. 1985^z.

Treatment	% light penetration ^y
A. Cluster exposure on Sept. 4 (50% veraison)	49b
B. Cluster exposure on Sept. 18 (post-veraison)	56b
C. Control - no cluster exposure	30a

z Means separated at $p=.05$ by Duncan's multiple range test

y Expressed as a percent ratio of the photosynthetic photon flux above the canopy.

Table I.5 Effects of basal leaf removal and cluster exposure treatments during maturation on juice composition of three Oregon *Vitis vinifera* grape cultivars. 1985.^z

Cultivar	Treatment	Sol. solids (deg. Brix)	pH	TA (g/l)	Tartrate (g/l)	Malate (g/l)	K (mg/l)
Chardonnay	A	22.3a	2.93a	12.5a	7.8a	7.0a	1365a
	B	22.3a	2.92a	12.7a	7.8a	7.3a	1385a
	C	22.0a	2.94a	12.3a	7.7a	7.1a	1410a
	D	22.2a	2.97a	12.4a	7.7a	7.3a	1475a
White Riesling	A	18.9a	2.87a	10.0a	7.9a	3.8a	1040a
	B	18.9a	2.89a	10.2a	7.8a	3.8a	1060a
	C	19.3a	2.89a	9.7a	7.8a	4.1a	1030a
Pinot Noir	A	23.2a	3.14a	8.9b	6.5a	4.5a	1215a
	B	24.1b	3.15a	9.0b	6.5a	4.8a	1185a
	C	24.0b	3.18b	8.3a	6.7a	4.7a	1100a

^z Means separated within columns and cultivars at $p=.05$ by Duncan's multiple range test.

Table I.6 Effects of cluster exposure and basal leaf removal treatments on the percent change between veraison and harvest in the amounts of potassium, titratable acidity, malate, and tartrate per berry in Oregon *Vitis vinifera* grapes. 1985.^z

Cultivar	Treatment	% dec in in TA/ berry	% inc in tartrate/ berry	% dec in malate/ berry	% inc in K/berry
Chardonnay	A	39.8b	3.0a	49.4b	9.4a
	B	36.8ab	4.5a	46.6b	18.5ab
	C	37.2ab	6.6a	46.5b	18.1ab
	D	34.7a	7.3a	41.8a	21.6b
White Riesling	A	39.6a	10.8a	60.0a	20.1a
	B	39.8a	8.3a	59.9a	15.9a
	C	38.5a	6.9a	56.9a	27.0a
Pinot Noir	A	23.3a	1.7a	32.1a	-0.3a
	B	23.0a	-2.7a	31.5a	5.0a
	C	24.5a	-3.2a	36.3a	-0.4a

^z Means separated within columns and cultivars at p=.05 by Duncan's multiple range test.

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A Rapid, Simple Method for the Estimation
of Malate and Tartrate in Grape Juice

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ABSTRACT

A rapid, simple procedure for the estimation of the malate and tartrate content of grape juice is described. The procedure, which requires only a pH meter for instrumentation, does not directly measure malate and tartrate but instead measures their buffering effect. Samples are titrated between pH 2.70-3.00 and pH 4.50-4.80 and the titrant volumes required are compared to two sets of empirically derived standard curves. The malate and tartrate composition of the sample may be determined by a graphical or algebraic method. The use of the estimation method, its advantages, and its limitations are illustrated with different viticultural trials. The estimation error (estimated value - measured value) was influenced by many factors including maturity, season, vineyard location, and cultivar. Standard deviations of the estimation error for malate and tartrate were equal to 9% and 15%, respectively,

of the mean malate and tartrate concentrations in pooled samples of mature Pinot Noir and Chardonnay grapes grown in different vineyards over a 4 year period. The estimation error is probably due to interference from other buffers present in juice. Although not as accurate as existing analytical methods, the estimation method appears potentially useful for determining relative effects on malate and tartrate of treatments in vineyard trials where analytical equipment is unavailable or for monitoring malate decline during maturation of grapes.

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INTRODUCTION

Winemakers have long used three simple objective laboratory tests as aids for determining the suitability of wine grapes for harvest - soluble solids, pH, and titratable acidity. These three measurements provide an approximate prediction of the alcoholic and acidic composition of the finished wine and, when taken into consideration with subjective evaluations such as flavor, allow the winemaker to create a desired style of wine by harvesting the grapes at the appropriate maturity.

An exact prediction of wine acidic composition is not possible, partly due to the complexity of wine, and partly due to the fact that pH and titratable acidity are each dependent upon a combination of many independent and variable constituents of the must. The values of titratable acidity and pH are affected by the types, amounts, and relative proportions of the organic and inorganic acids in the must, the pK_a s of their acidic moieties, and the amount of salt formation (proton substitution by metallic cations) which has occurred (Boulton, 1980; Mattick *et al.*, 1980).

In grape musts, pH and titratable acidity are principally affected by malic and tartaric acids, which together constitute at least 90% of the total acids

(Winkler et al., 1974), and by the potassium content, which accounts for most of the salt formation (Boulton, 1980). It is not practical to determine the concentrations of malate, tartrate, and potassium in a must from examination of the pH and titratable acidity values alone, because different combinations of the three constituents may cause a similar pH and titratable acidity. For example, a must containing 8 g/l tartrate, 4 g/l malate, and 1320 mg/l potassium and a must containing 5.6 g/l tartrate, 5.6 g/l malate, and 1275 mg/l potassium would both have a pH of 3.25 and a titratable acidity of 0.86% (Boulton, 1980). After malolactic fermentation and potassium bitartrate precipitation, though, a wine made from the latter must would experience a larger, less desirable pH rise than a wine made from the former must, because the organic acid pool in the wine would be dominated more by lactic acid ($pK_a=3.79$) and would be exchanged to a greater extent by the remaining potassium ions. Clearly, knowledge of the acidic composition of the must may assist the winemaker in producing a better wine.

Since the malate and, to a lesser extent, the tartrate and potassium concentrations in grapes can be manipulated by cultural methods in the vineyard (Carbonneau and Huglin, 1982; Kliever, 1982; Smart, 1982; Freeman and Kliever, 1983; Reynolds et al., 1985; Smart et al., 1985; Wolf et al., 1986; Bledsoe et al., 1987), many winemakers are

interested in measuring the concentrations of these must components so that those methods which minimize malate and potassium content may be employed. Large wineries may quantitate malate and tartrate analytically by means of HPLC (McCord et al., 1984; Frayne, 1986), GC (Mattick et al., 1970), or by spectrophotometric means (Vidal and Blouin, 1978; McCloskey, 1980). Potassium may be quantitated by flame atomic absorption (Amerine and Ough, 1980). These methods require expensive equipment which is unavailable to most smaller vineyards and wineries, many of which own no laboratory equipment more complicated than a pH meter.

Unlike analytical methods, the estimation method described in this paper does not directly measure the malate and tartrate content of the juice sample. Rather, the combined buffering effect of all the organic and inorganic acids in the sample is measured in a manner similar to a buffer capacity titration (Mattick et al., 1980; Boulton, 1985) and compared to a standard juice sample containing known amounts of malate and tartrate. The estimation method was developed in order to allow smaller wineries the opportunity to derive more winemaking information from the limited laboratory equipment which they already possess.

MATERIALS AND METHODS

Preparation of the standard curves: The standard juice sample was prepared from a mixture of frozen grape juice samples (primarily White Riesling) grown at the Oregon State University (OSU) experimental vineyard during 1984. After thawing, the clear, settled juice was chemically deacidified using the double-salt procedure (Munz, 1961; Steele and Kunkee, 1978; 1979). This deacidified base juice was divided into 200 ml aliquots which were spiked with known amounts of tartaric and malic acids covering the concentration range of 0-10 g/l for tartaric acid and 0-12.5 g/l for malic acid. The aliquots were assayed in duplicate for malate using the enzymatic method (McCloskey, 1980), and for tartrate using the ammonium meta-vanadate method (Vidal and Blouin, 1978). The aliquots were also titrated in triplicate using the estimation method. The data obtained were used to create two sets of curves relating titrant volume to the malate and tartrate content of the juice.

Materials: A pH meter accurate to two decimal places was found to be necessary (a Corning 125 pH meter with a Corning X-EL bulb combination electrode (Corning Glass Works, Medfield, MA) was used in this study). A magnetic stirrer was used and is recommended. A 50 ml volumetric

pipet was used to measure the juice samples and a 25 ml buret calibrated to 0.1 ml was used to measure the titrant.

Reagents: All titrations were with 0.1N NaOH. The pH adjustments to the juice samples were made with concentrated (6N) HCl and 8N KOH. 8N NaOH could be used instead of KOH.

Buffers: Standardization of the pH meter was accomplished using pH 2 and pH 7 buffer preparations purchased from a commercial supplier (pHydrion, Micro Essential Laboratory, Brooklyn, NY).

Procedure: The grape juice sample to be tested should be allowed to settle at room temperature. If potassium bitartrate precipitation has occurred in the sample and one wishes to measure the total tartrate, then the sample should be placed in an airtight container, heated at 60° C in a water bath, shaken periodically until the precipitate has redissolved (about 6 hours), then allowed to cool down to air temperature again. Otherwise a low tartrate reading will be obtained.

Standardize the pH meter using the pH 2 and pH 7 buffers. These buffers should also be at room temperature. Standardization should be done while the buffer is being stirred, as the titrations will also occur

with stirring. If a magnetic stirrer is being used it is advisable to let the pH electrode equilibrate for several minutes in each buffer without stirring before attempting to standardize. Magnetic stirrers generate heat, which warms the solution being stirred and changes the pH reading; therefore, the sample or buffer should be stirred only while one is actively standardizing or performing a titration. An option is to place a styrofoam block between the beaker and the magnetic stirrer in order to prevent warming of the sample or buffer.

Using the volumetric pipet, pipet 50 ml of clear, settled juice into a 150 ml beaker. Using the 6N HCl, lower the pH of the juice until it is slightly below pH 2.70. Now slowly add some 0.1 N NaOH titrant dropwise until the pH of the juice just changes from 2.69 to 2.70. This is the starting point of the first titration. Titrate the juice with the 0.1 N NaOH from the buret until the pH just changes from 2.99 to 3.00, and record the volume of titrant used for the pH 2.70 - 3.00 titration. It is important to titrate slowly when approaching the endpoint of the titration as there is usually a time lag between titrant addition and electrode response, depending on the type of electrode being used.

The pH of the sample is next raised to just below 4.50 with the 8N KOH or NaOH. If the 4.50 point is overshoot the pH may be lowered again by adding a small amount of the 6N

HCl, then raised using the 0.1 N NaOH until the 4.49-4.50 transition point is reached. The sample is then titrated between pH 4.50 and pH 4.80 in a manner identical to that of the first titration, and the volume of titrant recorded. After this the sample may be discarded.

Calculations: Quantitation of the malate and tartrate in the sample is accomplished by comparing the titrant volumes to standard curves. There are two sets of standard curves, one for each titration.

Fig. II.1 contains the standard curves for the pH 2.70-3.00 titration. The titrant volume obtained from the pH 2.70-3.00 titration is used to enter the chart on the left-hand side (y-axis, Fig. II.1). It can be seen that for any given titrant volume there exists a set of many possible combinations of malate and tartrate values. The standard curves for the pH 4.50-4.80 titration (Fig. II.2) and the titrant volume obtained from the pH 4.50-4.80 titration may be used to generate another set of possible malate and tartrate values. The single combination of malate and tartrate values in which the two sets intersect is the sample composition, and may be solved for graphically or algebraically.

Graphical method: Figs. II.1 and II.2 contain the linear equations for the standard curves so that they may be replotted for use with the graphical method. Select two

points on the pH 2.70-3.00 standard curves where the horizontal line representing the titrant volume intersects lines of equal tartrate concentration. Plot these points, in terms of malate and tartrate values, on a separate sheet of graph paper and connect them with a line. Repeat this procedure using the pH 4.50-4.80 titrant volume and standard curves, and plot the second line on the same sheet of paper as the first. The two lines will intersect at a point representing the malate and tartrate concentrations in the juice sample.

Algebraic method: Obtain the two points from the pH 2.70-3.00 standard curves as in the graphical method. Letting x represent tartrate concentration and y represent malate concentration, the points may be used to form the equation for a line:

$$y=mx+b, \text{ where } m=y_2-y_1/x_2-x_1.$$

The value of b is equivalent to the concentration of malate when the tartrate concentration equals 0 g/l at the experimentally derived titrant volume. The 0 g/l tartrate line may be extended if necessary to obtain values of b at higher titrant volumes than shown on the chart.

An equation for a second line may be obtained in the same manner using the pH 4.50-4.80 titrant volume and standard curves. If the two equations are set equal to each other, the values of x and y representing the sample concentrations of tartrate and malate may be solved for.

RESULTS AND DISCUSSION

Grape juice is strongly buffered by its organic acid content, principally malate and tartrate, from approximately pH 2.5 to 5.0 (Amerine and Winkler, 1958; Mattick *et al.*, 1980). Because tartrate buffers the juice to a lower pH range than malate (pK_a s of 2.98 and 4.34 vs. 3.40 and 5.11, respectively) (Beelman, 1984), proportionately more of the protons titrated at the lower end of the buffered pH range will be recovered from tartrate. Titrations in the upper part of the buffered pH range recover proportionately more protons from malate. The proportion of the protons recovered from either acid within a given pH range is a function of the pK_a s of the acids, and is reflected in the slope of the lines of equal tartrate concentration in the standard curves. Each titration is over a short (0.3 unit) pH range to minimize pH changes due to sample dilution by the titrant, and the pH ranges of the titrations are spread as far apart as possible over the buffered region in order to minimize the error of the graphical solution.

Grape juice contains other buffers besides malate and tartrate. This may be inferred from the position of the 0 g/l tartrate, 0 g/l malate point on the standard curves (Figs. II.1 and II.2). The standard juice sample contained enough 'other' buffers to titrate 2.15 ml of 0.1 N NaOH in

the pH 2.70-3.00 range and 0.5 ml of 0.1N NaOH in the pH 4.50-4.80 range. An assumption is made when using the estimation method that both the sample and the standard juices are identical in that portion of their buffering content not attributable to either tartrate or malate. Since different grape juice samples will contain varying amounts of all buffers (Mattick et al., 1980; Boulton, 1985), this assumption is false, and an error is introduced into the measurement.

To determine the nature, magnitude, and possible sources of this error, a selection of frozen grape juice samples with known malate and tartrate content were tested by the estimation method. The juice samples tested were predominately Chardonnay and Pinot Noir derived from various studies conducted at western Oregon growing sites between 1982 and 1985. The estimated malate and tartrate values obtained were compared to the analytically determined values. The estimation error was found to vary with many factors, including the growing season, vineyard location, and cultivar (Table II.1).

The estimation error also varied with the relative maturity of the fruit. Fig. II.3 shows the relationship during the 1984 maturation period between the estimated and analytically determined malate concentrations of Chardonnay grapes grown at Sokol Blosser Vineyard, Dundee, Oregon. The estimation method tended to overpredict malate

concentrations early during maturation, but this tendency decreased with time. By harvest, the estimated and measured concentrations were similar. This pattern was typical of those noted during other maturation studies of malate concentrations at this and other sites. The error for tartrate predictions did not change appreciably with fruit maturity.

Blocks of vines within a vineyard were also tested to determine how the estimation error varied. Table II.2 shows the estimation error for mature grapes from blocks of Chardonnay vines at the OSU Lewis-Brown Horticultural Research Farm in 1985. Blocks 1 through 4 are located sequentially from one end of the vineyard to the other. There was a noticeable decrease in vine vigor from block 1 to block 4; the clusters of grapes in block 4 were naturally much more exposed to the sun during the entire growing season due to the lower amount of shoot growth and leaf shading. These grapes were also more mature in terms of soluble solids content and titratable acidity. The lower pH of the exposed grapes may have been due to their greater tartrate:malate ratio. The shift toward underprediction of tartrate and overprediction of malate associated with a more open canopy and greater fruit maturity may indicate a qualitative and/or quantitative change in the non-tartrate and non-malate buffer content of the grapes. Grapes contain trace amounts of more than 20

non-nitrogenous organic acids (Winkler et al., 1974) which may reasonably be expected to vary with grape maturity. They also contain phosphoric acid, which is a strong acid and which varies with maturity (Hrazdina et al., 1984). The proportionate effect of these 'other' buffers on the buffer capacity of the grapes may be expected to increase with advancing maturity since the buffer capacity decreases as malic acid is respired (Boulton, 1985).

Due to the large standard deviation associated with the varying buffer content of unrelated samples, the estimation method is probably of little practical use in comparing grape samples from different vineyards. However, the standard deviations of the estimation error for malate and tartrate in samples from the same cultivar, vineyard, and year were as low as 0.14 and 0.12 g/l, respectively. Potentially, the estimation method could be very useful for monitoring malate decline during maturation within a block of vines in a vineyard. Fig. II.4 shows the relationship between malate and titratable acidity in a block of Pinot Noir vines during a very cool maturation period in western Oregon (1984). By October 9 malate respiration had slowed down but the titratable acidity continued to decrease steadily, due to proton replacement by potassium ions. From October 9 to October 22 the pH increased from 3.20 to 3.24 and the potassium concentration increased from 1670 to 1930 ppm. It would be difficult to determine from pH and

titratable acidity measurements alone how much of the loss in titratable acidity was due to malate respiration and how much was due to potassium uptake.

In general, the standard deviation of the error for tartrate estimations was almost twice that of malate estimations (Table II.1). This may indicate a difficulty in resolubilization of the precipitated potassium bitartrate salts during sample preparation, or there may be a minor and variable buffer component of the juice which is buffered in the same pH range as tartrate and which exerts a masking effect. Although the estimation method is not as accurate as the analytical methods, it may still be used to determine relative effects of treatments in vineyard trials where analytical equipment is unavailable. Table II.3 demonstrates that the trends in data for the blocking differences in the OSU Chardonnay plots are preserved when using the estimation method even though the resolution is diminished.

When all of the mature Pinot Noir and Chardonnay grape samples were considered together, it was found that the standard deviations of the estimation error for malate and tartrate were equal to 9% and 15%, respectively, of the mean malate and tartrate concentrations (Table II.4). The estimation method tended to overpredict tartrate by about 0.1 g/l and overpredict malate by about 0.45 g/l, probably because the average juice sample contained more 'other'

buffers than the standard juice sample. The standard curves can be adjusted to eliminate the overprediction and used to estimate the malate and tartrate content of Pinot Noir and Chardonnay grapes from western Oregon vineyards. They could also be used with grapes grown in other winegrowing regions, but the results might not be as accurate since the non-malate and -tartrate buffer content of those grapes could be radically different due to varietal or climatic differences. To obtain the greatest accuracy, it is recommended that growers in other regions test representative samples of their grapes and adjust the standard curves to reflect the average buffer content of their grapes.

Fig. II.1 Standard curves for the pH 2.70-3.00 titration.

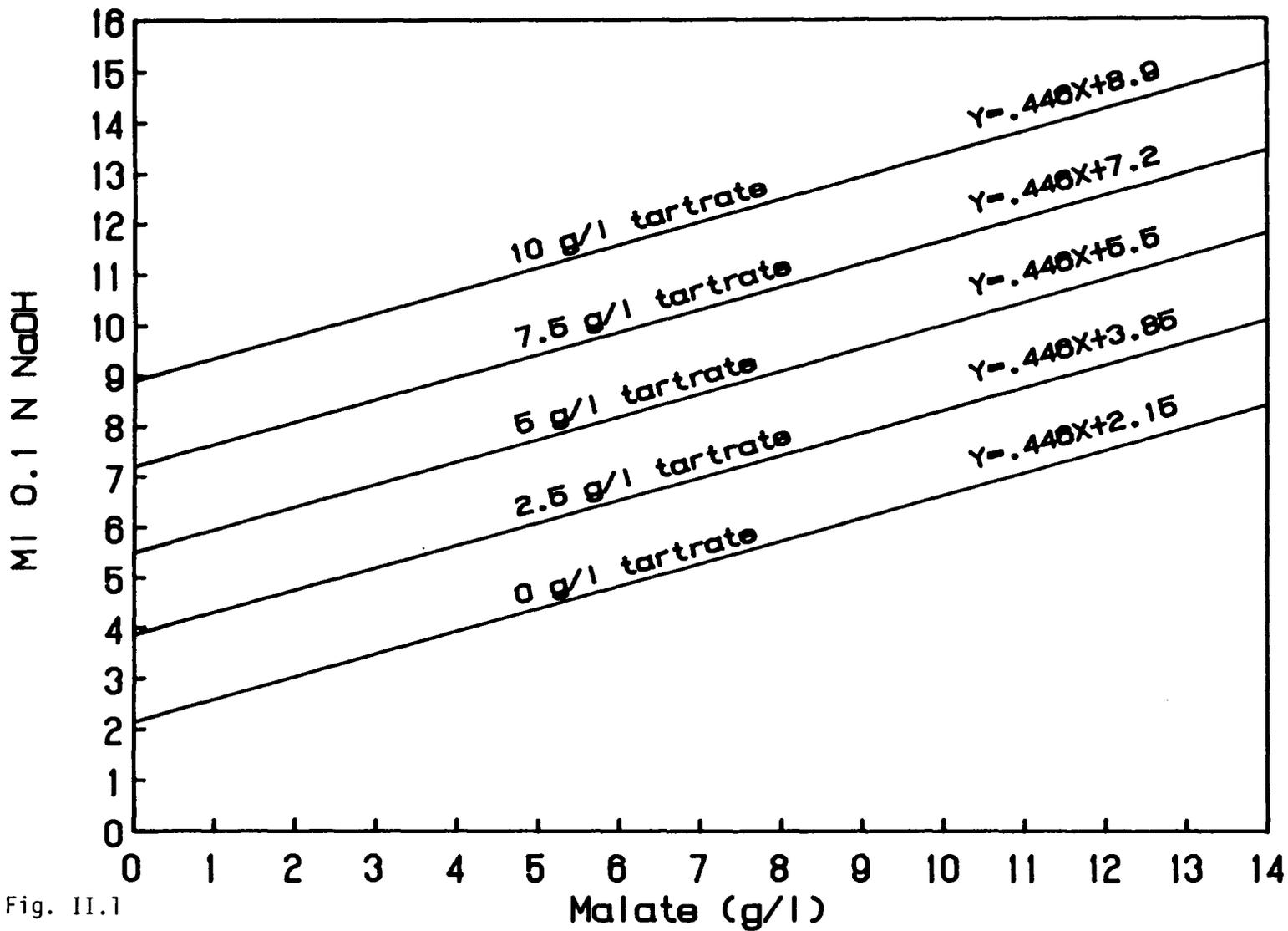


Fig. II.1

Fig. II.2 Standard curves for the pH 4.50-4.80 titration.

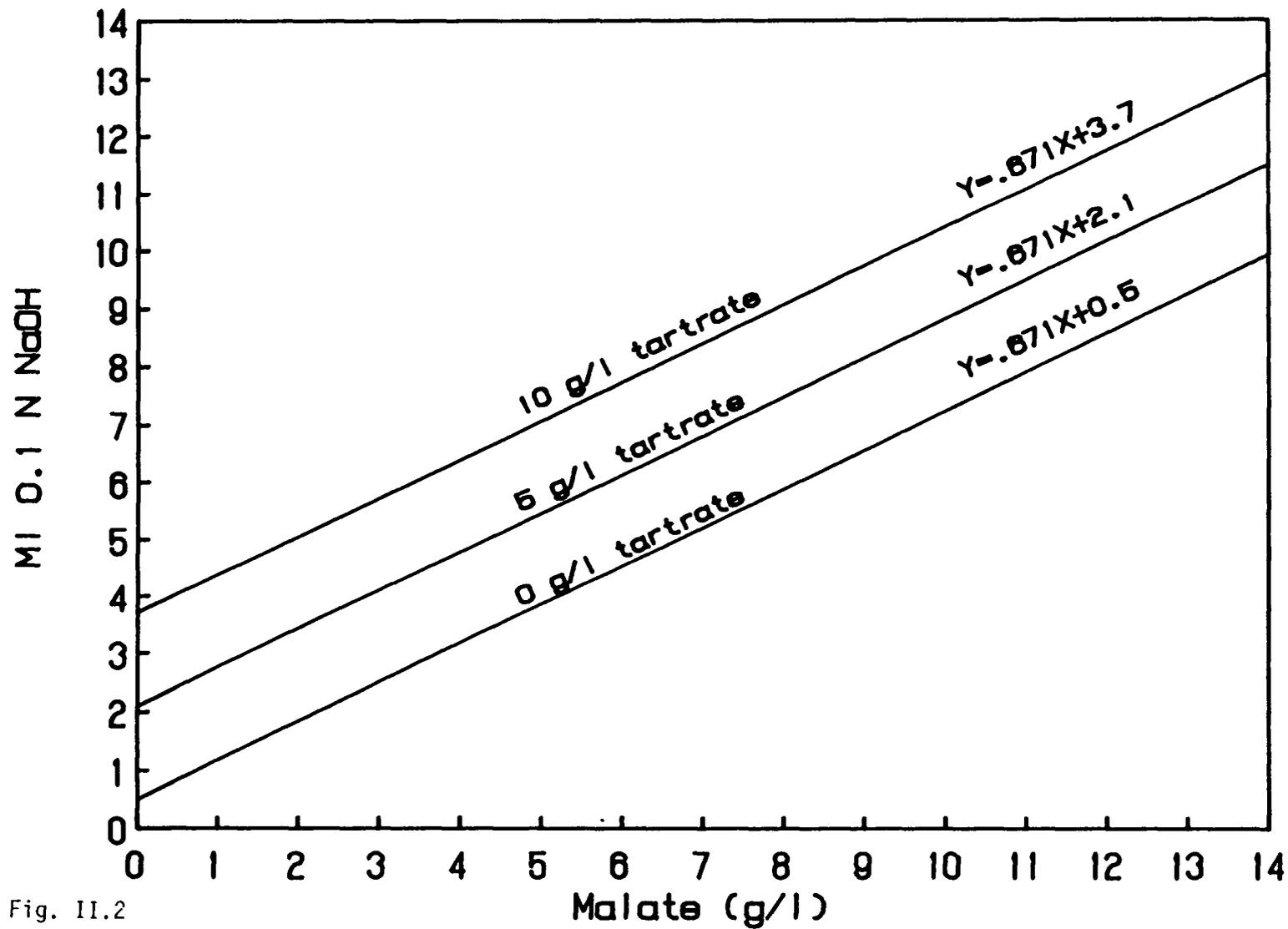


Fig. II.2

Table II.1. Measured error of acid estimation method for selected grape juice samples from various western Oregon vineyards and growing seasons.

Cultivar	Site	Year	n	Error (est.-meas. values)			
				Tart. (g/l)		Mal. (g/l)	
				mean ^z	s.d.	mean ^z	s.d.
Chardonnay	Corvallis	1983	3	.63	.12	.43	.31
		1984	5	-.64	.71	.32	.41
		1985	16	-.06	1.15	.16	.39
Pinot Noir	Sheridan	1983	7	.90	.79	-.13	.50
		1984	5	.20	.29	.56	.86
		1985	7	-1.0	.61	.59	.30
	Cave Junction	1983	10	-.58	.99	.83	.27
		1984	7	-.09	.34	.84	.14

^z Positive values indicate overprediction; negative values indicate underprediction.

Fig. II.3 Estimated and measured malate concentrations in Chardonnay grapes during maturation. Sokol-Blosser Vineyard, Dundee, Oregon. 1984.

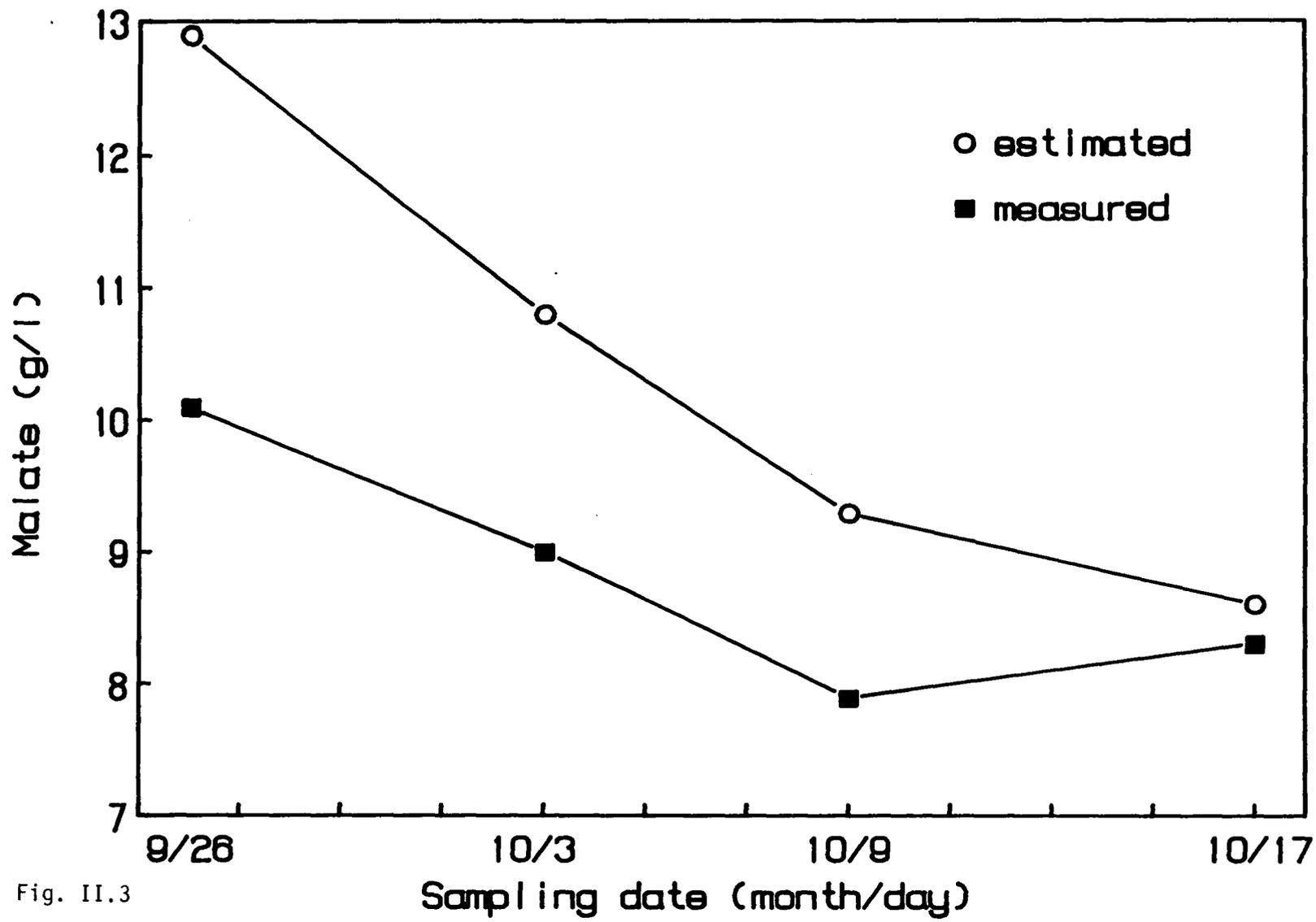


Fig. II.3

Table II.2. Analysis of variance (ANOVA) of estimation error among blocks of Chardonnay vines within a vineyard. Corvallis, Oregon. 1985.^z

Block	Estimation error ^y		Grape Maturity Data			
	Tartrate (g/l)	Malate (g/l)	pH	TA (g/l)	S.S. (g/100 ml)	K (mg/l)
1	1.33c	-.25a	2.99c	1.31c	21.4a	1120a
2	.57b	.08ab	2.96bc	1.39d	22.0b	1495b
3	-.83a	.30b	2.91ab	1.19b	22.4bc	1540b
4	-1.33a	.53b	2.89a	1.11a	22.9c	1480b

^z Means separated within columns at $p=.05$ by Duncan's multiple range test.

^y Positive values indicate overprediction; negative values indicate underprediction.

Fig. II.4 Estimated and measured acidities in Pinot Noir grapes during maturation. Sokol-Blosser Vineyard, Dundee, Oregon. 1984.

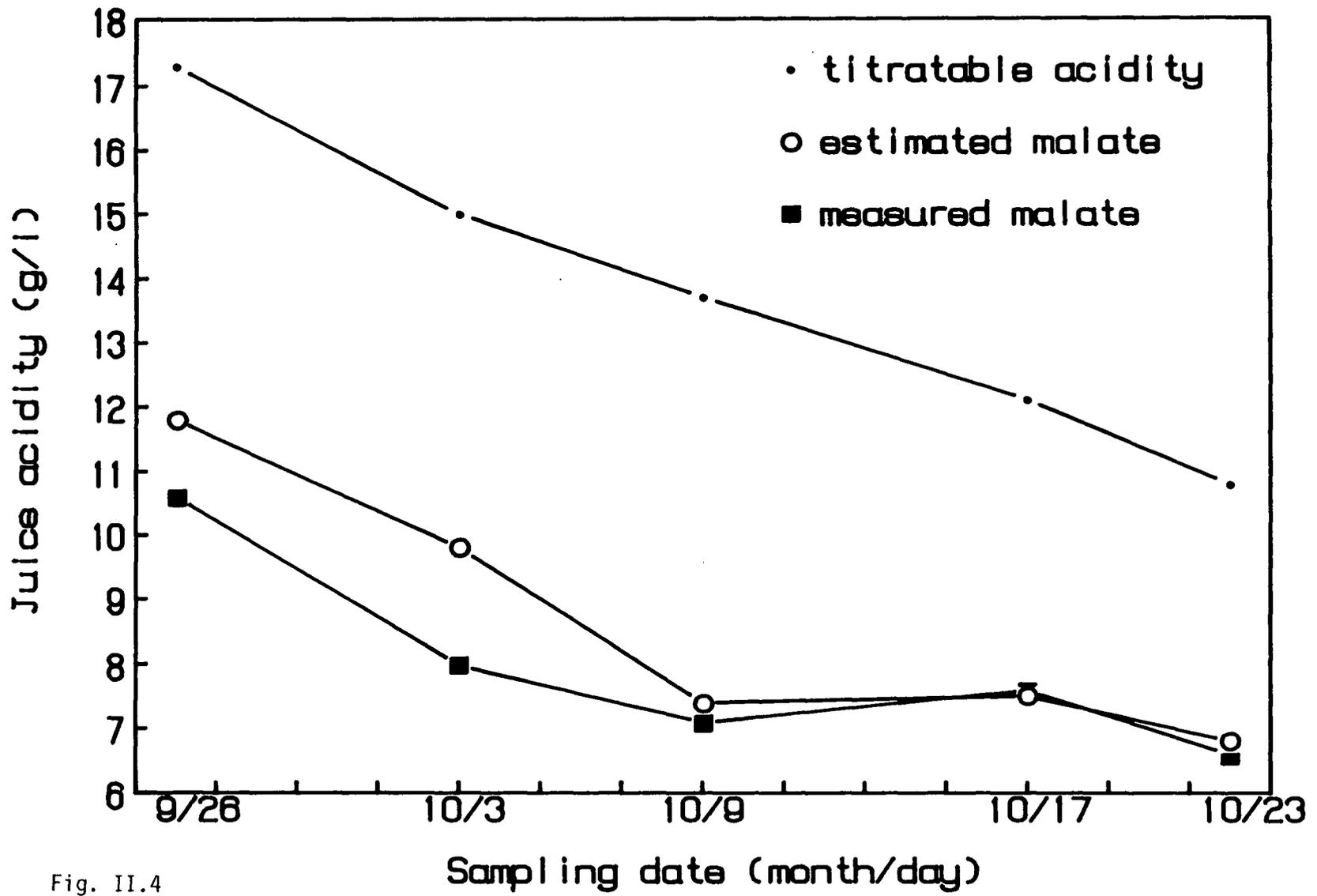


Fig. II.4

Table II.3. ANOVA of the estimated and measured tartrate and malate content of Chardonnay grapes among blocks of vines within a vineyard. Corvallis, Oregon. 1985.^z

Block	Tartrate (g/l)		Malate (g/l)	
	Est.	Meas.	Est.	Meas.
1	9.2b	7.8b	7.2a	7.5b
2	8.7b	8.2c	8.3b	8.2c
3	6.6a	7.5a	7.2a	6.9b
4	6.3a	7.6a	6.7a	6.1a

^z Means separated within columns at $p=.05$ by Duncan's multiple range test.

Table II.4. Measured error of acid estimation method for juice samples of mature Pinot Noir and Chardonnay grapes grown in various western Oregon vineyards. 1982 - 1985.

Cultivar	n	Mean sample concentrations (g/l)		Error (estimated-measured values)			
		Tartrate	Malate	Tartrate (g/l)		Malate (g/l)	
				mean ^z	s.d.	mean ^z	s.d.
Pinot Noir	51	6.8	5.3	-.03	.95	.49	.61
Chardonnay	34	7.2	7.6	.21	1.15	.40	.56
Combined	85	7.0	6.3	.07	1.03	.45	.59

z Positive values indicate overprediction; negative values indicate underprediction.

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