SOME EFFECTS OF ROOTSTOCKS ON SELECTED CHEMICAL COMPONENTS OF BUFFALO GRAPE BERRIES AT VARIOUS STAGES OF MATURITY

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INTRODUCTION

One of the primary objectives of grape-breeding is the development of high-quality table, or wine cultivars. The concentration of sugars and acid and their relationship are major factors influencing the quality of the grape cultivar. The importance of the total fermentable sugar in grape musts is universally recognized. The final alcohol content of unfortified wines depends primarily on the initial sugar content. For table grapes it has been shown by many workers that, above a certain minimum sugar content, the balance between sugar and acidity is more important than the sugar content itself.

The sugars present in grapes are largely glucose (dextrose) and fructose (levulose), Amerine (6). Cultivars having high fructose and glucose contents would result in maximum sweetness. This must be balanced with the proper amount of acid to provide a pleasing taste. Table cultivars high in fructose content can be harvested at a lower percentage of total sugar and be equal in sweetness to fruit of higher sugar content but with greater amounts of glucose, provided the acidity is not too high.

Most of the work in determining the sugar changes in grape berries of American species and French hybrids has been done in the past twenty years. However, little is found in the literature concerning the sugar components of grape berries of a given cultivar at different developmental stages as affected by different rootstock.

The grape is propagated easily both by sexual (seeds) and asexual methods (cuttings, layering, budding or grafting). Seeds are used only when new cultivars are sought, Hartmann (24). Grape cultivars of the American type, Vitis labrusca, are usually propagated from hardwood cuttings, Mahlstede (38). Hartmann (24) stated that cultivars difficult to root by cuttings, such as Muscadine, can be propagated by layering. Budding or grafting on rootstocks is used to increase vine life, plant vigor, and yields. Mahlstede (38) stated that vigorous rootstock improve the yield of fruits. He also found that by grafting several species of grapes gave sizeable increase over plants propagated from cuttings. Shepard (41) reported that in Missouri bud-grafted vines proved superior to whip-and-tongue bench grafts because of much higher and faster percentage of takes. Hartmann (24) stated that where noxious soil organisms, such as phylloxera, rootknot nematodes or cotton root rot are present and where varieties of susceptible species, such as Vitis vinifera are to be grown, it is necessary to graft or bud the desired variety on a resistant rootstock.

Objectives of this study were to determine the differences in chemical components: (1) glucose, (2) fructose, (3) total solids, (4) pH and (5) total acidity of the fruits of a single grape cultivar Buffalo, as affected by different rootstocks with fruit sampled during various stages of development.

REVIEW OF LITERATURE

Barrett (8) had classified dessert grapes in four different groups -

V. *vinifera*, French hybrids, American types and Muscadines. For the sake of comparison of grape types, the V. *vinifera* dessert cultivars were considered as the standard. These varieties possessed most of the desirable characteristics for quality. Among American types Buffalo has been considered as one of the favorite, Barrett (9). Brook (12) pointed out that Buffalo was the result of a cross between Herbert and Watkins which was bred by Richard Wellington and released by the New York State Agriculture Experiment Station in 1921. Slate et al. (43) have reported Buffalo as an excellent black variety in Washington and Illinois. It has also performed quite well in other states including Pennsylvania, Kentucky, Missouri and North Carolina. Fruit of this variety has been considered to be of excellent quality ripening early and retaining flavor even when harvest is delayed. Slate and et al. further mentioned that the detected flavor of Buffalo appealed to many and it kept much better in storage than Concord types. The sugar content of this variety was reported to be high and the clusters were usually loose, but moderately compact on vigorous vines.

It was reported by Bioletti (11) that American varieties were highly resistant to the attack of Phylloxera species as compared to European varieties. Breeding of resistant rootstocks was started in California in the latter part of the 19th century. The stocks selected for breeding work were partly pure American species, partly hybrids between two or more American species, and partly hybrids between American species and V. *vinifera* varieties. Bioletti (11) further mentioned only the pure American species V. *rupestris* and V. *riparia* were selected for breeding resistant rootstocks. *V. rupestris* and *V. riparia* were successfully crossed which resulted in three different hybrids, i.e. 101-14, 3306 and 3309. The *V. riparia* x *V. rupestris*, 101-14 had more of the characteristics of the *V. riparia*, they tended to increase yield, but were susceptible to injury from neglect and ill treatment than *V. rupestris*. The *V. riparia* x *V. rupestris*, 3309 had more of the characteristics of *V. rupestris*, this hybrid had vigorous stout plants with fleshy root-system; and the *V. riparia* x *V. rupestris*, 3306 was more or less intermediate of the two, Bioletti (11).

Little work had been done in grafting American grape varieties on rootstocks of other varieties and species according to Vaile (47). The most outstanding work of this type has been carried on at the New York State Experiment Station, since 1902. Gladwin and Hedrick (23, 25) found that the yield and vigor of certain varieties might be influenced by the stock used. The general tendency was for the yield and vigor of the varieties tested to be higher when grafted on stocks of highly vigorous species. At the Arkansas Experiment Station, Vaile (47) observed that low yield and uneven ripening were highly significant in limiting the production of blue American grapes in the Ozark region.

The concentration of glucose and of fructose in grapes, especially during the periods of berry ripening and fruit maturity has been studied by many investigators. The subject has been reviewed by Heide & Schmitthenner (26), Amerine (4), and Amerine and Thoukis (6). Most studies have indicated that glucose predominates in unripe grapes, the glucose/fructose ratio at berry maturity is about 1, and fructose constitutes the major sugar in over-ripe grapes, Kliewer (30). Winkler and Williams (49) observed that, in succulent green grape tissue, starch and sucrose were of relatively minor importance compared to reducing sugar content.

According to Amerine and Thoukis (6) the sugars present in grapes are largely reducing sugars, glucose (dextrose) and fructose (levulose). Fructose is much sweeter than glucose. Fifteen per cent fructose is approximately equal in sweetness to 22.8 per cent glucose and 17.8 per cent sucrose. Cameron (14) pointed out that the relative sweetness changes slightly with concentration, so that at higher concentrations the difference in sweetness is not so great but at lower concentrations the differences are larger.

According to Amerine (6) most yeast ferment glucose more rapidly than fructose but rapidly ferment both in mixtures. Other yeasts, e.g., the so-called Sauternes strain or Dubourg yeast, ferment fructose more rapidly than glucose and complete the fermentation slowly, if at all. According to Hewitt et. al. (27), many grape varieties frequently contain one or more viruses which may greatly delay maturation of fruit. These facts are of particular interest to viticulturists. In the production of grape juice from grapes grown in cool climates, Amerine (6) pointed out that use of grapes high in fructose would result in sweeter tasting musts at lower total sugar contents. On the other hand, for grapes grown in warm climatic regions where sugar production is frequently too high for balanced grape juice, high-glucose cultivars would yield less sweet and better-balanced musts. Since it is apparently the sweetness-to-acid taste which is important a wide range of fructose to titratable acidity relationships is possible.

Little data on the glucose/fructose ratio in different cultivars harvested approximately at the same maturity from the same climatic region are available. Genevois and Ribereau-Gayon (21), for example, mentioned that at maturity the two sugars are generally present in equimolar quantities. Peynaud (39) reported the usual decrease in glucose/fructose ratio during the ripening of five Bordeaux cultivars based on grams per 100 berries.

In an extensive study of the fructose and glucose contents of Hungarian grapes, Szabo and Rakcsanyi (44) showed that at the beginning of the ripening period glucose predominated. During ripening fructose increased more rapidly than glucose, until in over-ripe grapes it amounted to 56 per cent of the total reducing sugars. Fructose equaled or exceeded glucose when the total sugar was about 17 per cent. In unripe grapes Sidersky (42) reported a glucose/fructose ratio of above 1, but this varies with cultivar and origin. Amerine and Thoukis (6) showed the glucose to decrease from 86 per cent of the total reducing sugar to about 47 per cent during ripening. In thirty cultivars the ratio at maturity varied from 0.87 to 0.96, average 0.90. Kliewer (34) determined the concentrations of glucose, fructose and total soluble solids in the fruits of 28 table cultivars, 26 red wine cultivars and 24 white wine cultivars of <u>Vitis vinifera L</u>. at an early and a late stage of maturity. The glucose/fructose ratio of table cultivars ranged from 0.74 to 0.97 (mean 0.91 for the slightly to moderately ripe fruits, and 0.83 for the very ripe to over-ripe fruits). The ratio of the wine varieties ranged from 0.74 to 1.05 (mean 0.94 for the early harvested fruits and 0.85 for the late harvested fruits). In every cultivar there was either a decrease or no change in the glucose/fructose ratio between the early and the late-harvested fruits. Kliewer (34) showed that the glucose/fructose ratios of mature fruits from six varieties were lower during the warm 1966 seasons. Temperature has considerable effect on the amount of organic acids and sugars in grapes, Caldwell and Kliewer (13, 28). Amerine and Gerber (7, 22) in 1898 investigated the influence of organic acids in grapes and showed that the respiratory quotient changed with temperature, indicating a change in respiratory substrate. These changes were correlated with the levels of several sugars and organic acids present. He also reported that when the temperatures of grapes exceeded 35°C tartarates were respired, while malic acid was respired at 30°C and sugars were respired at much lower temperatures.

Amerine (3) and Kliewer (29, 32, 33) showed that the characteristic feature of the ripening of grapes is the simultaneous increase in sugars and decrease in total titratable acidity. Considerable practical importance is attached to these changes, as the utilization of the mature grape depends to a large extent on the percentage of sugar and acids in the fruit at harvest time. The important acids found in grape musts are tartaric, malic, tannic, phosphoric and citric, the first two constituting well over 90 per cent of the total, Amerine and Winkler (2). All of these, and other acids as well, are found in wines. The acids are chiefly important because of the acid taste which they give to the wine. But this effect, at least partially, and other effects wholly, depend on the hydrogen ion concentration rather than the concentration of acids, Amerine and Winkler (2).

They further mentioned that the importance of pH in establishing the percentage of free acid was recognized by early workers like Von der Heide and Baragiola who found over 30 per cent of the tartaric acid and over 50 per cent of the malic acid to be free in two German wines. Tarantola (45) found a somewhat lower percentage of the tartaric to be free in an Italian wine. The high pH Muscat wines of northern Italy have been noted by Tarantola (45) to give poor hydrolysis of sucrose when they are used for making sparkling wines if the sugar is not hydrolyzed prior to bottling. The extraction of coloring matter and probably of flavoring constituents are better at the lower pH. The color of the resulting wines is also more stable and of a better tint under such conditions.

Amerine and Webster (4, 48) mentioned that in the grape, of course, the total titratable acidity is almost entirely composed of fixed or nonvolatile acids. The relative and total amounts of the two important acids, tartaric and malic, vary greatly from variety to variety, from region to region, or from season to season (for the same cultivar). During ripening of the grapes three factors operate to decrease the acidity: dilution owing to an increase in the size of the fruit, continuous translocation of bases into the fruit, and respiration. Amerine (4), Kliewer and Peynand (31, 39) showed that tartaric as well as malic acid decreased during ripening. Robinson and et al. (40) showed that sugar, acid and tartarate changes in concord grapes were affected by prunings. Pruning exerted a pronounced effect on sugar content, but had little effect on the acidity of grape.

The changes in pH during ripening have not been studied so extensively. Crisci (18) however, has followed the changes in titratable acidity and pH in two varieties during ripening and has found a continuous decrease in acidity and rise in pH during the later stages of maturation. The curves of Amerine and Joslyn (1) also show this and likewise show the small rise in acidity and decrease in pH which takes place very early in the season. This early rise in acidity was also noted by Bioletti et al. and Crowther (10, 19). The fermentation of musts of high pH (above 4.0) is always hazardous, particularly when the fermentation is to be carried to complete dryness, and the resulting wines are usually of poor keeping quality. According to Castor (15, 16) amino acids may be of basic importance in the production, flavor, and quality of fermented beverages.

MATERIALS AND METHODS

The samples for the study were obtained from plants of the cultivar Buffalo growing on two rootstocks and its own roots. The following combinations constituted the experimental treatments:

(1) Buffalo on rootstock 3306

(2) Buffalo on rootstock 3309

(3) Buffalo on its own roots

These vines were planted with a spacing of 8 x 8 feet on fine sandy loam soil in the year 1959. They have been maintained under standard cultural practices since that date. The vines were trained and pruned to the 4-cane kniffin-system.

The samples for analytical work were collected at different dates as shown in Table 1 of the Results and Discussion. This table shows the visual change in fruit maturity from the unripe green stage to the ripe purple stage of grape berries.

In order to obtain representative samples, seven healthy vines of each combination were selected and all the samples were taken from these tagged vines at an interval of eleven days starting June 30, 1969. Three composite samples were made from each experimental treatment at each sampling date. The sample consisted of 2-3 grapes from each cluster, and a total of 5-7 clusters from each vine were selected. These clusters were randomly selected from all sides of the vine at eleven days intervals, similar to the method followed by Amerine (5). Berries were collected in the morning, immediately placed in an ice-chest and carried to the laboratory for analysis. In the laboratory, 70 typical berries from each sample were cut to remove seeds and crushed in a blender. After blending the pulp was squeezed out by hand through four layers of cheese cloth until no more juice could be extracted.

Total soluble solids in the juice of each sample were measured by a temperature-corrected Bausch and Lomb hand refractometer graduated in 0-1° Balling units.

The pH, read with a Coleman's glass-electrode pH meter,was obtained from 25 ml. of juice. Titratable acidity was determined from 5-10 ml. of juice diluted with 50 to 200 ml. of freshly boiled distilled water by titration with 0.0951 N. sodium hydroxide to a phenolphtalein end point, similar to the method followed by Kliewer (36).

Glucose was determined by the enzymatic glucose oxidase method developed by Teller (46), using carbohydrase-free glucostat reagent (Worthington Biochemicals Corp.). Each sample of juice was diluted with distilled water so that 1 ml. contained 10-100 μg. of glucose. The reagent mixture consisted of 2 ml. of unknown diluted sample, and 2 ml. of glucostat reagent (contents of chromogen and glucose oxidase vials dissolved in 50 ml. of distilled water). Duplicate samples were incubated at 37°C for 30 minutes and allowed to cool. A drop of 6 N HCl was added to each sample to stabilize the oxidized chromogen. Per cent absorbance was read at 400mμ on Beckman DB Spectrophotometer, with the reagent blank set at zero absorbancy. The concentration of glucose was determined by the use of standard curve. This standard curve was prepared by taking the optical density of ten different concentrations of glucose ranging from 20 μg to 200 μg with an interval of 20 μg. A set of glucose standards containing 1-80 μg of glucose was run with each glucostat mixture.

Fructose was determined by the colorimetric method described by Dische (20), using the blue color produced by diphenylamine in acid media. The same dilute sample as prepared for the glucose method was used for the determination of fructose. The reaction mixture consisted of 1 ml. unknown sample and 2 ml. of diphenylamine reagent (contents having 10 ml. of 10% diphenylamine, 90 ml. of glacial acetic acid and 100 ml. of concentrated HCl. dissolved together). Samples were heated in boiling water for 10 minutes and then allowed to cool. Per cent absorbance was then read at 635 mμ on Beckman DB Spectrophotometer, with the reagent blank set at zero absorbancy. The concentration of fructose was determined by use of the standard curve. This standard curve was established with ten different concentrations of fructose ranging from 10 μg to 100 μg with an interval of 10 μg each. According to Kliewer (34) a preliminary experiment indicated that glucose in concentrations between 0 and 200 μg per reaction mixture did not interfere with fructose determination.

RESULTS AND DISCUSSION

Table 1 indicates that the apparent changes in color of ripening grape berries made by visual observation were not distinctly affected by different rootstocks on the same sampling dates.

Table 1. Color observations of Buffalo grape berries produced on different rootstocks made at various sampling dates.

Sampling dates	Buffalo on 3306	Buffalo on 3309	Buffalo on own roots
June 30	Green	Green	Green
July 11	Green	Green	Green
July 22	Red & Green	Red & Green	Red & Green
August 2	Purple, red & Green	Purple, red & Green	Purple, red & Green
August 13	Purple	Purple	Purple
August 24	Purple	Purple	Purple

Influence of Sampling Dates on Glucose Content

Figure 1 shows the general trend of glucose content of fruits of Buffalo grafted on different rootstocks. The terms R_1, R_2 and R_3 were designated to differentiate the fruits from a single cultivar Buffalo grafted on rootstocks 3306, 3309 and Buffalo on its own roots, respectively. For the first two sampling dates the rise in glucose content of berries from R_1, R_2 and R_3 vines was slow except for the berries from R_3 vines which tended to increase rapidly from July 11 to August 13 sampling. In case of fruits from R_2 vines there was a rapid increase in glucose content from July 22 to the August 13 harvest. Whereas for fruits from R_1 vines the rise in glucose content was slow and steady till August 2 harvest, after which there was a sharp increase noted. The trend in glucose increase decreased with the August 13 harvest in all the fruits from R_1, R_2 and R_3 vines.

The analysis of variance on glucose (Table 2) showed highly significant interaction between rootstocks and sampling dates. Glucose content of fruits from the vines of certain rootstocks increased more rapidly than others. Table 3 was constructed to compare the means of the different rootstocks at various sampling dates. The June 30 sampling showed a higher glucose content of fruits from the R_1 vines than from the R_2 and R_3 but fruits from the latter two combinations did not differ significantly. Combinations of the July 11 sampling revealed that, fruits from R_1 vines had significantly higher glucose content than that from vines of R_2, but at the same time there were

F I G U R E I

Influence of sampling dates on glucose content of Buffalo grape berries as affected by different rootstocks.

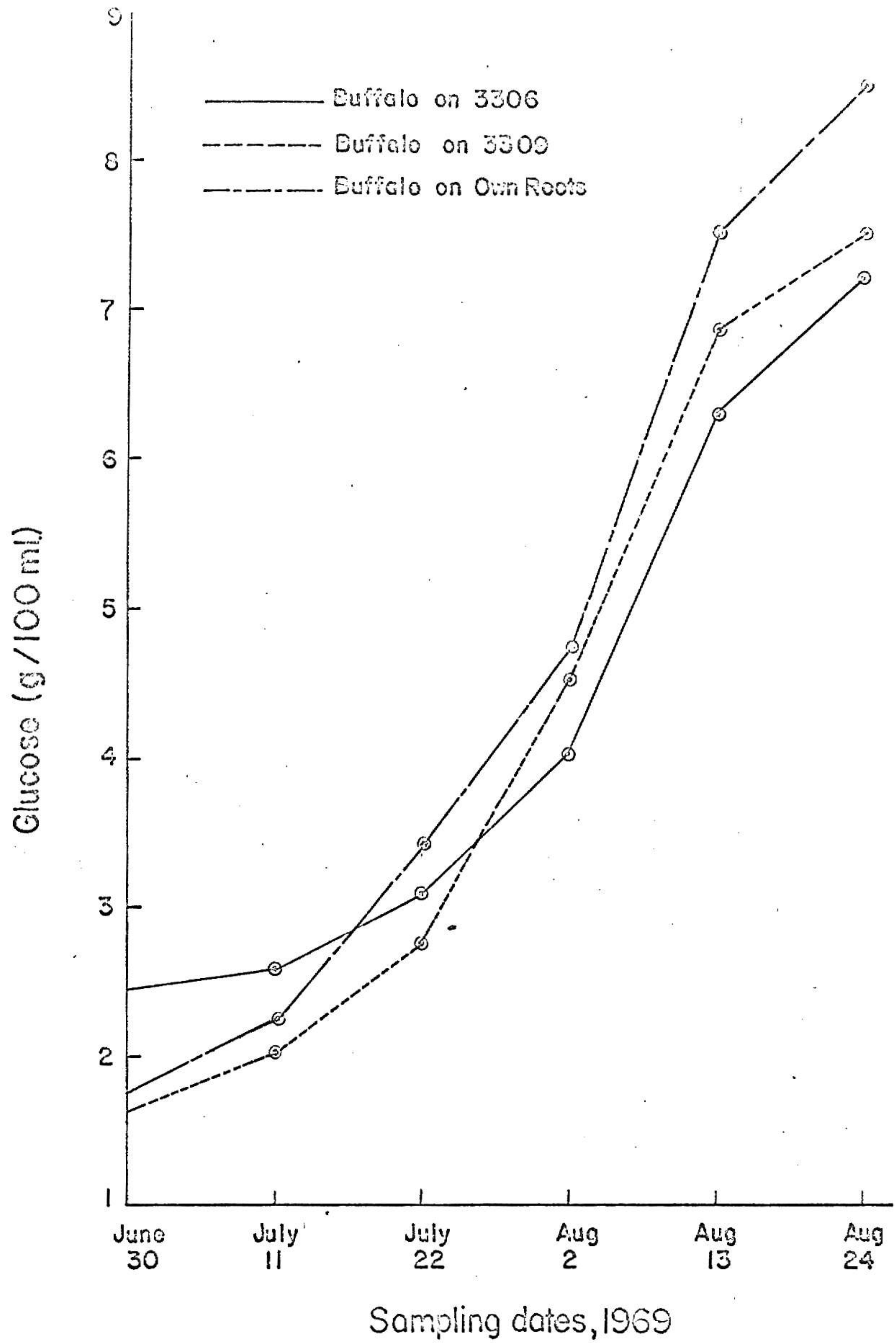

Buffalo on 3306
Buffalo on 3309
Buffalo on Own Roots
Glucose (g/100 ml)
9
8
7
6
5
4
3
2
1
June 30
July 11
July 22
Aug 2
Aug 13
Aug 24
Sampling dates, 1969

Table 2. Analysis of variance for the effect of sampling dates on glucose contents of grape berries on different rootstocks

Factor	df	Ss	Ms	F
Rootstocks	2	2.43	1.215	13.65**
Dates	5	270.19	54.038	607.17**
Rootstock x Dates	10	5.41	0.541	6.08**
Error	36	3.19	0.089	
Total	53	281.22		

* Significant at 5% level of significance.
**Significant at 1% level of significance

Table 3. Means of rootstocks x sampling dates interaction on glucose content of grape berries. Values expressed in g/100 ml. of juice.

	Buffalo on 3306 (R_1)	Buffalo on 3309 (R_2)	Buffalo on own roots (R_3)	Probable ranking of combinations
June 30	2.43	1.64	1.76	$R_1 > R_2 = R_3$
July 11	2.57	1.99	2.23	$R_1 > R_2$, $R_2 = R_3$, $R_1 = R_3$
July 22	3.08	2.75	3.41	$R_3 > R_2$, $R_1 = R_2$, $R_1 = R_3$
Aug. 2	4.08	4.52	4.73	$R_3 > R_1$, $R_2 = R_3$, $R_1 = R_2$
Aug. 13	6.33	6.86	7.53	$R_3 > R_2 > R_1$
Aug. 24	7.25	7.53	8.53	$R_3 > R_2 = R_1$

LSD at 5% level of significance for row or column was 0.49.

no significant differences in glucose content between the fruits from R_2 and R_3, and R_1 and R_3 vines. Fruits collected on July 22 showed higher glucose content from the vines designated R_3 and R_2, but there were no significant differences in this sugar when comparisons were made between R_1 and R_2, and R_1 and R_3 vines. Fruits grown on the vines of R_3 gave higher glucose content at the August 2 harvest than the fruits from R_1 but no significant differences were noted when comparing values of fruits from R_2 and R_3, and R_1 and R_2 vines. There were significant differences in amounts of glucose in fruits from all cultivar-rootstock combinations on August 13. Fruits from R_3 vines were the highest in glucose content followed by R_2 and R_1 vines, respectively. The same relationship was observed for August 24 sampling date when the fruit was considered to be mature.

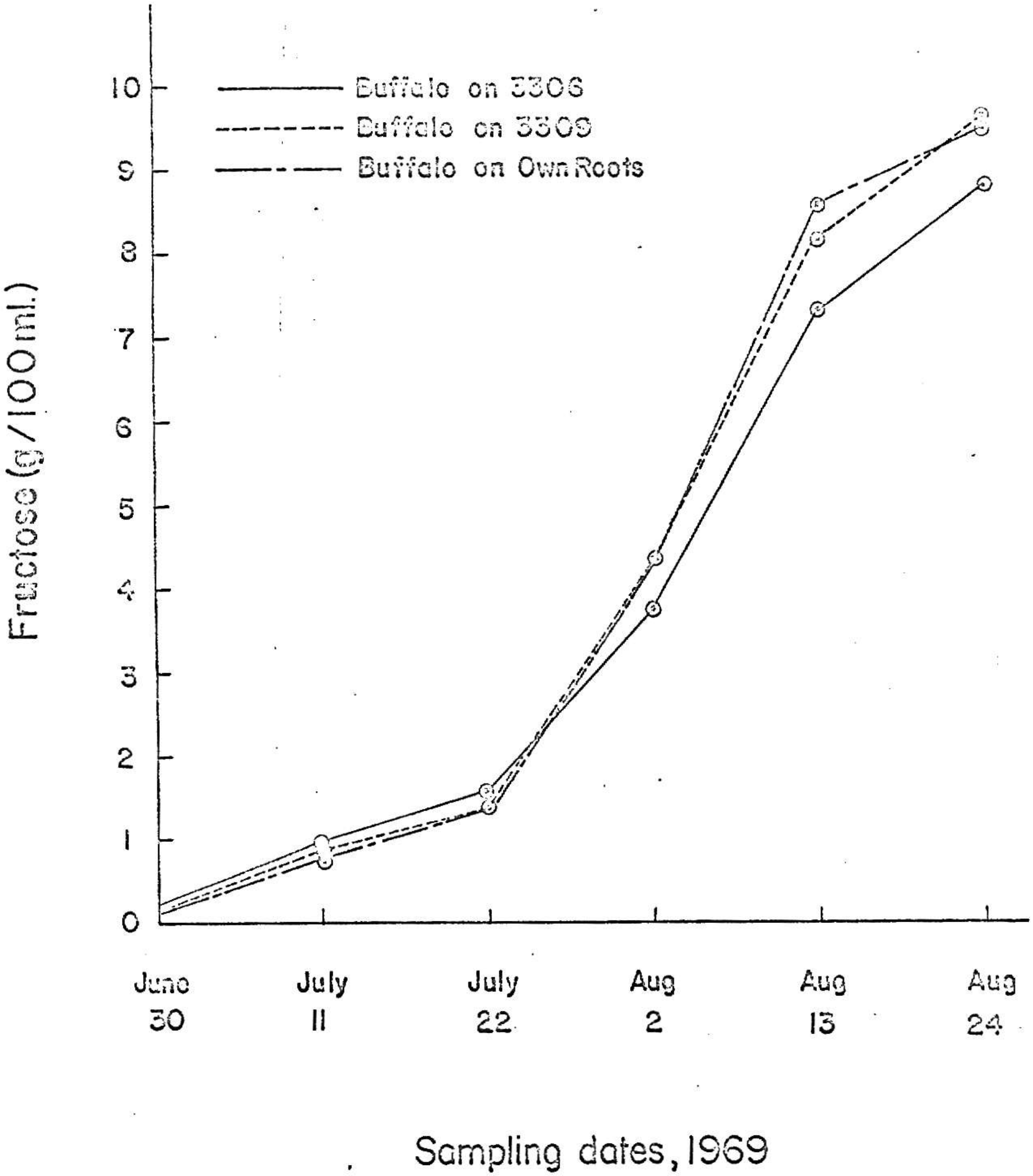
The rootstocks 3306 and 3309 are known for their vigor, however, the fruits of these combinations were lower in glucose content as compared with Buffalo on its own rootstock. This might be governed by some environmental factor. Kliewer (37) observed a consistently lower content of both glucose and fructose in shaded fruits.

Influence of Sampling Dates on Fructose Content

A comparison of the effect of the various rootstocks on fructose content of fruits are depicted in Figure 11. It was clear from the trend curve of each rootstock for various dates of harvest that for the first three sampling dates, there was a gradual rise in fructose content. The differences in fructose content of fruits on different

F I G U R E II

Influence of sampling dates on fructose content of Buffalo grape berries as affected by different rootstocks.



Buffalo on 3306
Buffalo on 3309
Buffalo on Own Roots
Fructose (g/100ml.)
0
1
2
3
4
5
6
7
8
9
10
June 30
July 11
July 22
Aug 2
Aug 13
Aug 24
Sampling dates, 1969

rootstock were not great. There was a sharp increase in fructose content after July 22 and this trend was maintained until August 13. After August 13 the increase in observed fructose content of fruits slowed down for all the vines.

The analysis of variance on fructose (Table 4) showed a significant interaction between rootstocks and dates of harvest. This implied that the increase in fructose content of the fruits for different rootstocks was not the same at various sampling dates. Table 5 shows no significant difference in fructose content of fruits taken from the various rootstocks from June 30 to August 2. The rate of increase in fructose content was almost the same for all rootstocks under study from June 30 to August 2 (Figure 11). But on August 13 and August 24, fruit from vines designated R_2 and R_3 had higher fructose content than found for vines R_1 and the difference between R_2 and R_3 vines in fructose content of the fruit was not significant.

Kliewer (30) observed the rapid increase in reducing sugars (fructose and glucose) during the maturity of fruits. The mechanism of this sudden change in the metabolism of the vine is not known. In several grape cultivars, Coombe (17) had shown a rise in auxin in the berries that coincided with the beginning of sugaring. According to Kliewer (30) large accumulations of carbohydrate reserves were observed during the green stage of berry development in several parts of the vine, especially in the permanent wood of the trunk and arms. These reserves are probably available for rapid movement to the fruits at the beginning of ripening.

Table 4. Analysis of variance for the effect of sampling dates on fructose contents of grape berries produced on different rootstock.

Factor	df	Ss	Ms	F
Rootstocks	2	1.48	0.740	5.83**
Dates	5	677.26	135.452	1066.55**
Rootstocks x Dates	10	3.32	0.332	2.61*
Error	36	4.57	0.127	
Total	53	686.63		

* Significant at 5% level of significance
**Significant at 1% level of significance.

Table 5. Means of rootstocks x sampling dates interaction on fructose content of grape berries. Values expressed in g/ml. of juice.

	Buffalo on 3306 (R_1)	Buffalo on 3309 (R_2)	Buffalo on own roots (R_3)	Probable ranking of combinations
June 30	0.22	0.20	0.14	$R_1 = R_2 = R_3$
July 11	0.99	0.91	0.80	$R_1 = R_2 = R_3$
July 22	1.58	1.37	1.37	$R_1 = R_2 = R_3$
Aug. 2	3.75	4.33	4.33	$R_1 = R_2 = R_3$
Aug. 13	7.30	8.20	8.57	$R_3 = R_2 > R_1$
Aug. 24	8.80	9.60	9.50	$R_2 = R_3 > R_1$

LSD at 5% level of significance for row or column was 0.59.

Influence of Sampling Dates on pH

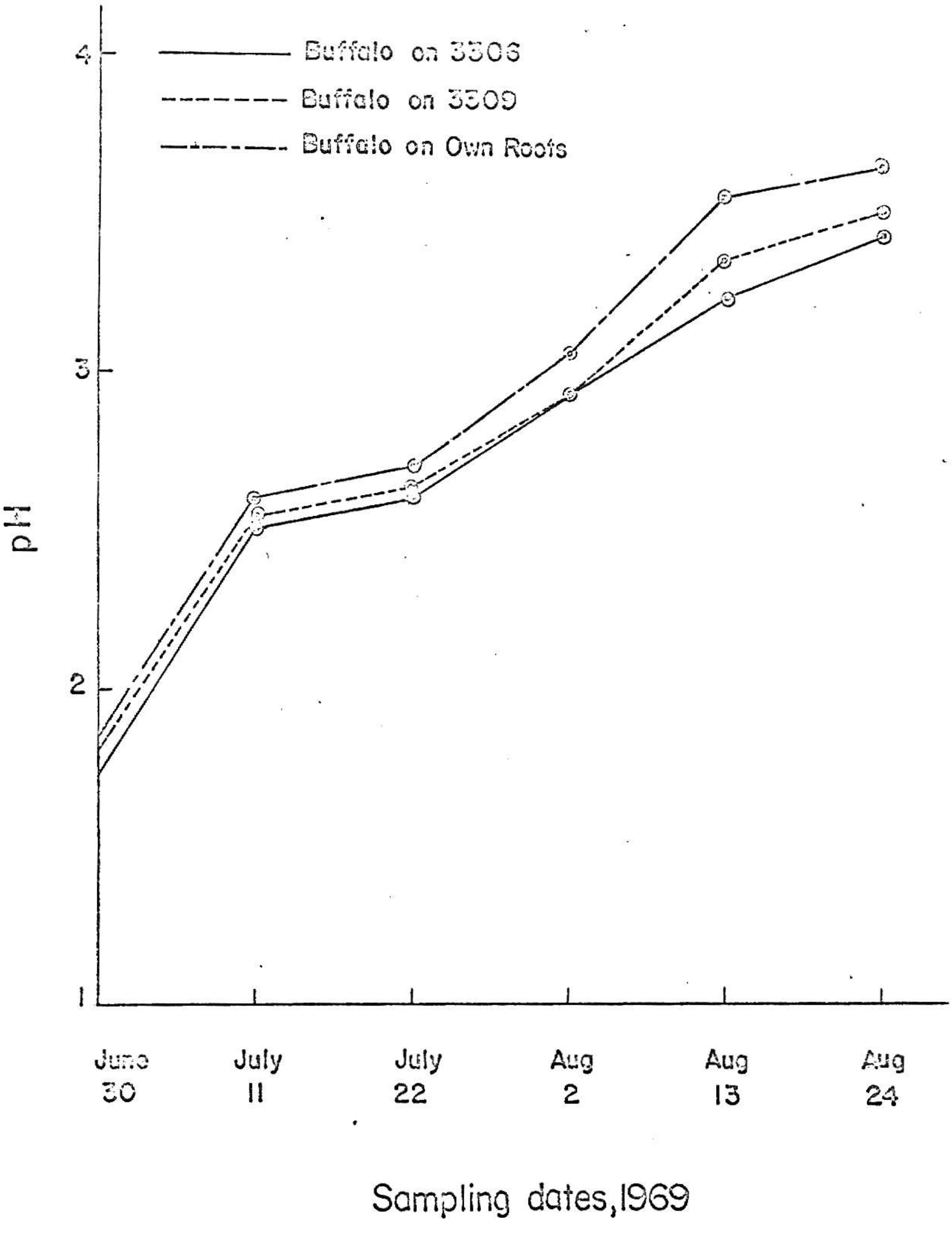
The effect of the different rootstocks on the pH of juice of the fruit is shown in Fig. III. Similar trends are observed regardless of rootstock. From June 30 to July 11 harvest the pH increased rapidly and then slowed down after the July 11 sampling date. This retarded increase in pH was followed by a moderately rapid increase in pH from July 22 to August 13. Fruits from vines grown on their own roots consistently had the highest pH values followed in order by fruits from 3309 and 3306.

Amerine (2) has pointed out that the steady increase in pH during maturation reflected the continuous change in the ratio of acid salts to free acids. During the unripe green stage of berries 50 to 80 per cent of the tartrates are present as free acid, these amounts are reduced to 10-20 per cent by the time the fruit is mature.

The analysis of variance, Table 6, showed a highly significant F-value for the pH interaction between rootstocks and sampling dates. The pH value of the grapes was not independent of sampling dates, but there were rootstock differences in pH levels for the same date of sampling. In Table 7 and for the fruit samples collected June 30, there was no significant difference observed between R_2 and R_3 vines in pH level but fruit from these two vines had significantly higher pH than the fruits from R_1 vines. From July 11 to August 2 collected fruits of vines designated R_3 have a significantly higher pH than fruit from the other two rootstocks and there was no significant

F I G U R E III

Influence of sampling dates on pH of Buffalo grape berries as affected by different rootstocks.



Buffalo on 3306
Buffalo on 3309
Buffalo on Own Roots
4
3
2
1
pH
June 30
July 11
July 22
Aug 2
Aug 13
Aug 24
Sampling dates,1969

Table 6. Analysis of variance for the effect of sampling dates on pH of grape berries produced on different rootstocks.

Factor	df	Ss	Ms	F
Rootstocks	2	0.26	0.130	130.00**
Dates	5	18.03	3.606	3606.00**
Rootstocks x Dates	10	0.06	0.006	6.00**
Error	36	0.05	0.001	
Total	53	18.40		

* Significant at 5% level of significance.
**Significant at 1% level of significance.

Table 7. Means of rootstocks x sampling dates interaction on pH levels of grape berries.

	Buffalo on 3306 (R_1)	Buffalo on 3309 (R_2)	Buffalo on own roots (R_3)	Probable ranking of combinations
June 30	1.73	1.80	1.83	$R_3 = R_2 > R_1$
July 11	2.50	2.53	2.60	$R_3 > R_2 = R_1$
July 22	2.60	2.63	2.70	$R_3 > R_2 = R_1$
Aug. 2	2.92	2.92	3.06	$R_3 > R_1 = R_2$
Aug. 13	3.23	3.35	3.55	$R_3 > R_2 > R_1$
Aug. 24	3.43	3.50	3.65	$R_3 > R_2 > R_1$

LSD at 5% level of significance for row or column was 0.05.

difference between these later two rootstocks. But in fruit samples taken from August 13 to August 24 dates, R_3 vines gave significantly higher level of pH followed by R_2 and R_1 vines, respectively. It was interesting to note that the fruits from R_3 vines had a consistently higher pH than from the other two rootstocks for all the sampling dates.

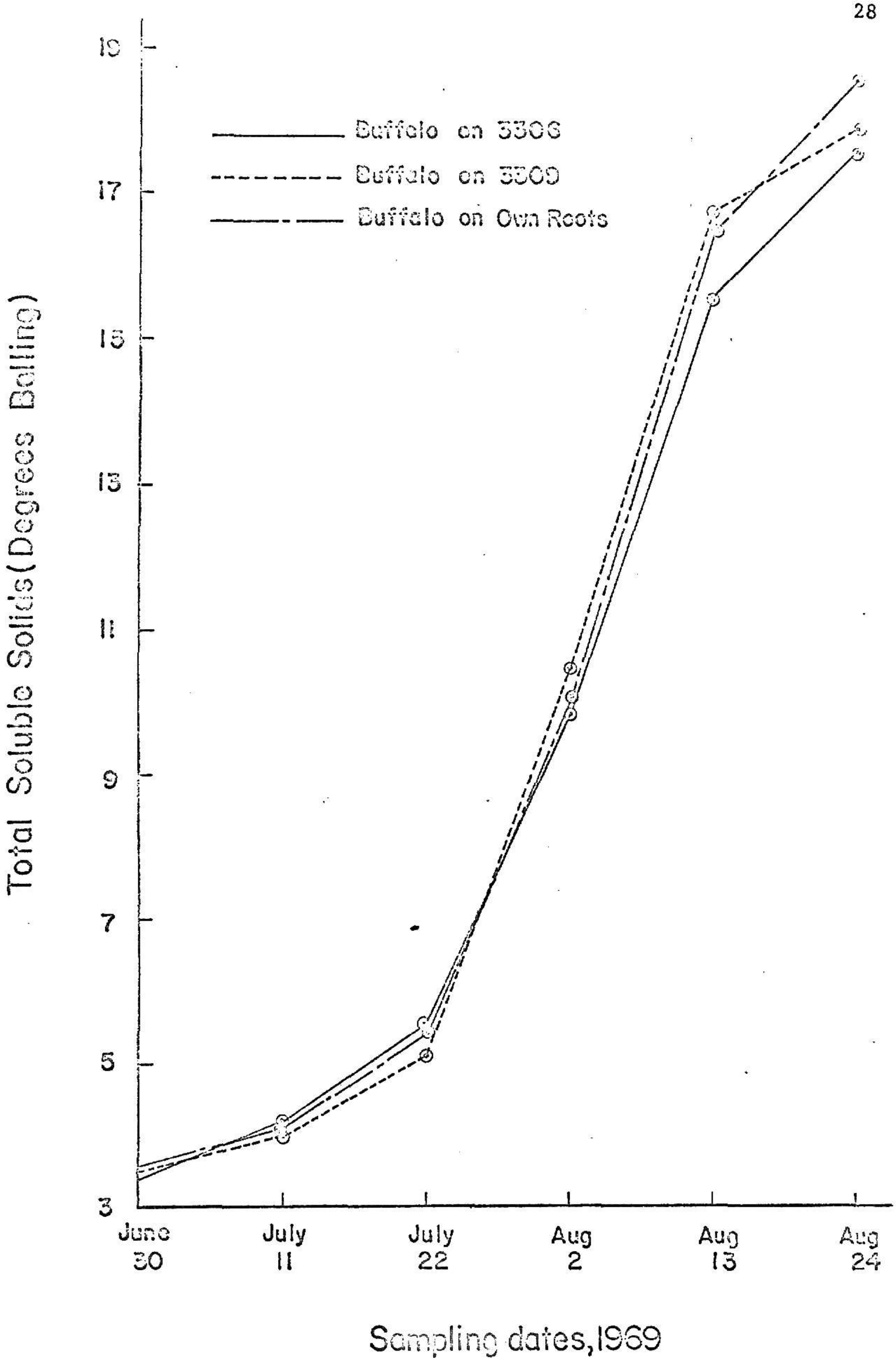
Influence of Sampling Dates on Total Soluble Solids

The trend lines of the fruits from three different rootstocks in their responses to the changes in total soluble solids were presented in graphic form in Figure IV. In fruit from all rootstocks there was a slow rise in total soluble solids from June 30 samples to July 22 samples. After July 22 the increase in total soluble solids was very rapid in the fruits from all the three rootstocks and this trend continued up to August 13. Again there was a less rapid increase in total soluble solids content after August 13.

The analysis of variance of total soluble solids (Table 8) showed a highly significant rootstocks x sampling dates interaction. The amount of total soluble solids content of fruits on different root-stocks did not increase at the same rate during the experiment. The two way Table 9 of means comparison of interaction showed that samples collected June 30 and July 11 were not significantly different in total soluble solids content. Whereas as a result of the July 22 sampling no difference in total soluble solids content between the berries from vines R_1 and R_3 but the berries from these two vines had

F I G U R E IV

Influence of sampling dates on total soluble solids of Buffalo grape berries as affected by different rootstocks.



Buffalo on 3306
Buffalo on 3309
Buffalo on Own Roots
Total Soluble Solids (Degrees Balling)
19
17
15
13
11
9
7
5
3
June 30
July 11
July 22
Aug 2
Aug 13
Aug 24
Sampling dates, 1969

Table 8. Analysis of variance for the effect of sampling dates on total soluble solids content of grape berries produced on different rootstocks.

Factor	df	Ss	Ms	F
Rootstocks	2	1.80	0.900	40.91**
Dates	5	1813.91	367.782	16490.09**
Rootstocks x Dates	10	3.55	0.355	16.14**
Error	36	0.80	0.022	
Total	53	1820.06		

* Significant at 5% level of significance
**Significant at 1% level of significance

Table 9. Means of rootstocks x sampling dates interaction on total soluble solids of grape berries. Values expressed in degree Balling.

	Buffalo on 3306 (R_1)	Buffalo on 3309 (R_2)	Buffalo on own roots (R_3)	Probable ranking of combinations
June 30	3.46	3.50	3.60	$R_1 = R_2 = R_3$
July 11	4.20	4.00	4.10	$R_1 = R_2 = R_3$
July 22	5.53	5.10	5.40	$R_1 = R_3 > R_2$
Aug. 2	9.86	10.50	10.50	$R_2 = R_3 > R_1$
Aug. 13	15.60	16.70	16.60	$R_2 = R_3 > R_1$
Aug. 24	17.60	17.90	18.60	$R_3 > R_2 > R_1$

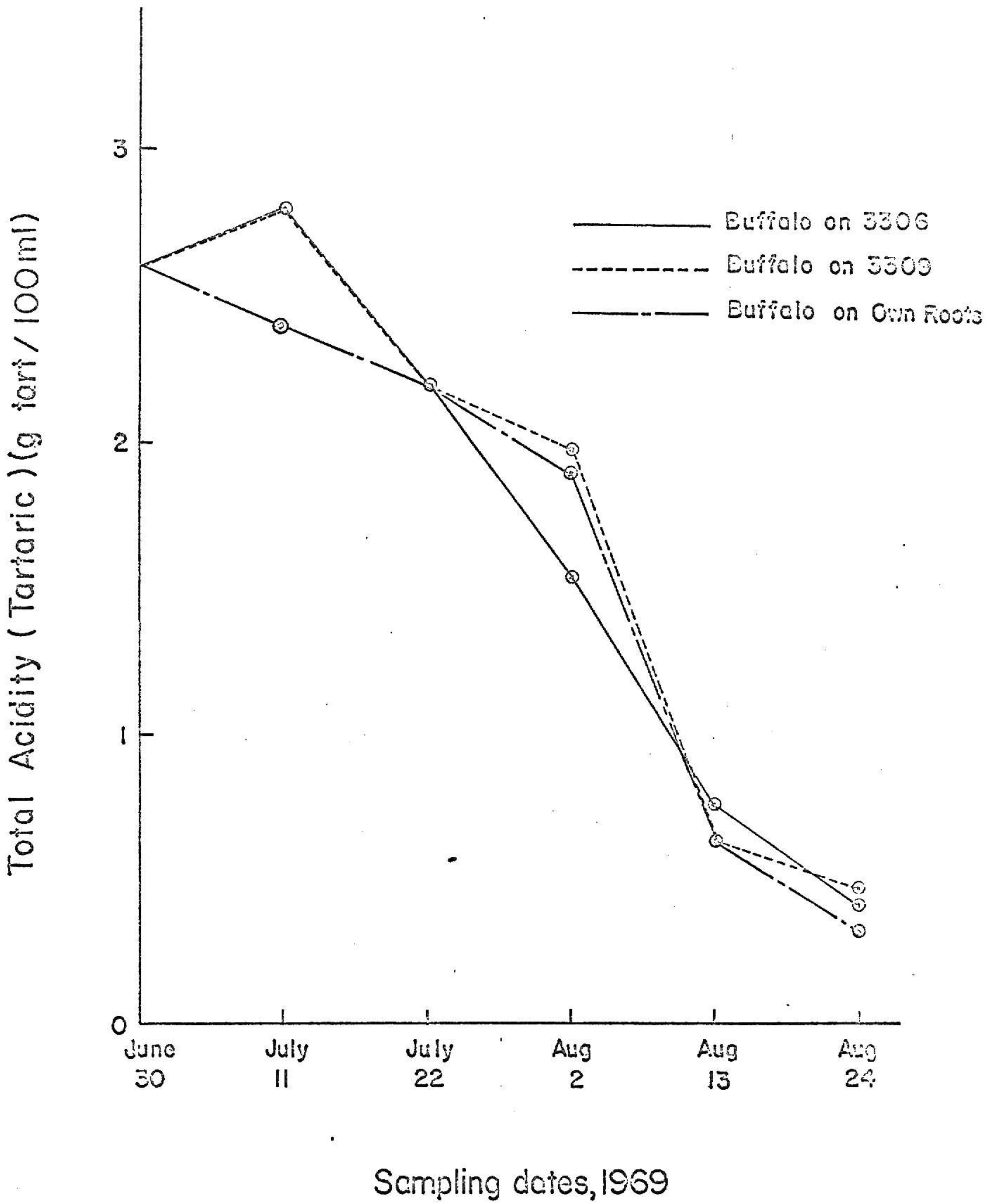
LSD at 5% level of significance for row or column was 0.25

a significantly greater content of total soluble solids as compared to R_2 vines. On the August 2 and August 13 samples, fruits from R_2 vines equalled in total solids content with those from R_3 vines but these two had significantly higher total solids content than R_1 vines. The fruits of August 24 collected from R_3 vines gave significantly higher total solids followed by fruits from R_2 and R_1 vines, respectively.

Influence of Sampling Dates on Total Titratable Acidity

The changes in total acidity (tartaric) in grape berries as affected by different rootstocks at various sampling dates is shown in Figure V. The total acidity of the fruits of Buffalo on its own rootstock decreased slowly and steadily until August 2, after which there was a sharp decrease in total acidity until August 13. The decrease in total acidity continued after August 13. In fruits from 3306 and 3309, there was a rise in total acidity between the first and second sampling dates. After July 11, the fruits from Buffalo on 3306 showed a rapid decrease in total acidity through August 13, and then the decrease in total acidity slowed down. The fruits of Buffalo on 3309, showed the same trend as those on 3306 through July 22 and continued to decrease in total acidity until August 13 and again the decrease in acidity slowed down. The three rootstocks had the same effect on the amounts of total acidity in fruits at the first and third sampling date.

According to Amerine (7) tartaric and malic acids are the two


Total Acidity (Tartaric)(g tart / 100ml)
3
2
1
0
Buffalo on 3306
Buffalo on 3309
Buffalo on Own Roots
June 30
July 11
July 22
Aug 2
Aug 13
Aug 24
Sampling dates, 1969

main organic acids that accumulate in fruits, leaves and stems of grape vines. Kliewer (35) stated that tartaric acid was synthesised most rapidly by young developing leaves and immature fruits. This explains the higher amounts of total acidity found early in the season in immature fruits. Earlier Kliewer (30) observed that during the green berry stage, berries generally contained 2 - 5 times more total titratable acidity than reducing sugars.

The analysis of variance (Table 10) showed a significant interaction between rootstocks x sampling dates for data on total acidity. It was evident from means of rootstocks x sampling dates interaction Table 11 that there were no significant differences among rootstocks in total acidity for all sampling dates except the fruits sampled on July 11 and August 2. July 11 sampled fruits from R_3 vines had significantly lower amounts of total acidity than fruits from R_1 and R_2 vines. Whereas, in fruit samples collected August 2 from R_1 vines had significantly lower level of total acidity than the fruits from other two vines. This tendency of R_1 vines to have lower total acidity on August 2 fruit samples was altered in samples collected August 13 and August 24 when no significant differences were observed.

Seasonal Change in the Glucose/Fructose Ratios of Grapes

Tables 12 and 13 show the seasonal changes in concentrations of glucose and fructose in berries from three different rootstocks and the changes in the glucose/fructose ratios with time of harvest. It was

Table 10. Analysis of variance for the effect of sampling dates on total titratable acidity of grape berries produced on different rootstocks.

Factor	df	Ss	Ms	F
Rootstocks	2	0.09	0.045	4.09*
Dates	5	42.60	8.520	774.55**
Rootstocks x Dates	10	0.62	0.062	5.64**
Error	36	0.41	0.011	
Total	53	43.72		

* Significant at 5% level of significance.
**Significant at 1% level of significance

Table 11. Means of rootstocks x sampling dates interaction on total acidity (tartaric) of grape berries. Values expressed in g/100 ml. of juice.

	Buffalo on 3306 (R_1)	Buffalo on 3309 (R_2)	Buffalo on own roots (R_3)	Probable ranking of combinations
June 30	2.60	2.60	2.60	$R_1 = R_2 = R_3$
July 11	2.80	2.80	2.40	$R_1 = R_2 > R_3$
July 22	2.20	2.20	2.20	$R_1 = R_2 = R_3$
Aug. 2	1.54	1.98	1.90	$R_2 = R_3 > R_1$
Aug. 13	0.76	0.64	0.64	$R_1 = R_2 = R_3$
Aug. 24	0.41	0.46	0.32	$R_1 = R_2 = R_3$

LSD at 5% level of significance for row or column was 0.17.

Table 12. Seasonal changes in concentrations of glucose and fructose in grape berries from three different rootstocks. Values expressed in g/100 ml. of juice.

	June 30		July 11		July 22		Aug. 2		Aug. 13		Aug. 24	
	Gluco.	Fruc.	Gluco.	Fruc.	Gluco.	Fruc.	Gluco.	Fruc.	Gluco.	Fruc.	Gluco.	Fruc.
Buffalo on 3306	2.43	0.22	2.57	0.99	3.08	1.58	4.08	3.75	6.33	7.30	7.25	8.80
Buffalo on 3309	1.64	0.20	1.99	0.91	2.75	1.37	4.52	4.33	6.86	8.20	7.53	9.60
Buffalo on own roots	1.76	0.14	2.23	0.80	3.41	1.37	4.73	4.33	7.53	8.57	8.53	9.50

Table 13. Seasonal changes in the glucose/fructose ratio of grape berries from three different rootstocks.

	June 30	July 11	July 22	Aug. 2	Aug. 13	Aug. 24
Buffalo on 3306	11.05	2.60	1.94	1.09	0.87	0.82
Buffalo on 3309	8.20	2.19	2.00	1.04	0.84	0.78
Buffalo on own roots	12.57	2.79	2.49	1.09	0.88	0.90

clear from Table 12 that the concentrations of glucose were much higher than fructose on June 30 collected samples. The rate of change of fructose from June 30 to July 11 was much faster than that of glucose for the same period. Note the rapidly decreasing ratio (Table 13) of glucose to fructose. By the time the berries approached maturity all ratios approached unity. For all rootstocks, the ratios were below unity after August 2 and thus, the concentration of fructose was more than glucose. At the initial harvest date the three rootstocks exhibited wide differences in the ratios. These differences tended to level off from August 2 to August 24 harvest when the grapes were considered to be mature.

Changes in Amounts of Glucose, Fructose, Total Soluble Solids, pH and Total Titratable Acidity With Sampling Date

The periodic changes in amount of glucose, fructose, total acidity, pH and total soluble solids from one sampling date to another are presented in Table 14. The periodic changes of these characters were determined by subtracting the actual amount at one sampling date of that character from the next immediate sampling date. Whereas, in the average change (Table 15) was calculated by finding the difference of each character between the first and last sampling date; this difference was divided by the number of time intervals between every two successive dates. Table 14 showed initially a small increase in glucose amounts for the first time interval, then an increase in amounts were more rapid for the second, third and fourth intervals and again the amount

Table 14. Periodic change in amount of chemical components in berries from one sampling date to another

Character	June 30 - July 11			July 11-July 22			July 22-Aug. 2			Aug. 2-Aug. 13			Aug. 13-Aug. 24		
	R_1	R_2	R_3	R_1	R_2	R_3	R_1	R_2	R_3	R_1	R_2	R_3	R_1	R_2	R_3
Glucose (g/100 ml)	0.14	0.35	0.47	0.50	0.76	1.18	1.00	1.77	1.32	2.25	2.34	2.80	0.92	0.67	1.00
Fructose (g/100 ml)	0.77	0.71	0.66	0.59	0.46	0.57	2.17	2.96	2.96	3.55	3.87	4.24	1.50	1.40	0.93
Total Soluble Solids	0.74	0.50	0.50	1.33	1.10	1.30	4.33	5.40	5.10	5.74	6.20	6.10	1.00	1.20	2.00
pH	0.77	0.73	0.77	0.10	0.10	0.10	0.32	0.29	0.36	0.31	0.43	0.49	0.20	0.15	0.10
Total acidity (g/100 ml)	0.20	0.20	-0.20	-0.60	-0.60	-0.20	-0.66	-0.22	-0.30	-0.78	-1.34	-1.26	-0.35	-0.18	-0.32

R_1 = Buffalo on 3306; R_2 = Buffalo on 3309; R_3 = Buffalo on own roots.

Table 15. Average rate of change in amount of chemical components in berries from one date of harvest to another.

Character	R_1	R_2	R_3
Glucose	0.96	1.18	1.35
Fructose	1.72	1.88	1.87
Total Soluble Solids	2.83	2.88	3.00
pH	0.34	0.34	0.36
Total Acidity	-0.44	-0.43	-0.46

slowed down at the fifth time interval. For fructose the change was more rapid at the first interval than followed with rapid changes at the third and fourth intervals, finally a slowdown at the fifth interval was observed. With regards to differential increase in amounts of total soluble solids, during the first time interval the increase was small. During the second, third and fourth time intervals, the soluble solids increased more rapidly. The differential increase during the final time interval was less than the previous two sampling dates. There was a sharp increase in pH at the first interval, a smaller increase observed at the second interval, with the increase being more rapid at the third and fourth time intervals and finally during the last interval the increase in pH was small. The differential decrease in total acidity was not consistent as it was with other characters. In addition, the fruits of different rootstocks did not behave in the same way in decreasing the acidity form one sampling date to another.

Table 15 lists the overall average change in the amounts of these characters studied. The average rate of change for glucose was greatest in fruits from R_3 vines followed by R_2 and R_1 vines. Fruits from R_2 and R_3 vines had almost the same rate of change of fructose and their rates of change were slightly larger than those of R_1 vines. Rootstock varied little in the average rate of change for pH and total acidity. The average rate of change in the fruits of R_3 vines was greater than the fruits of R_2 and R_1 vines for total soluble solids but the difference in rates between the fruits of R_1 and R_2 was small.

Simple Correlations Among Glucose, Fructose, Total Acidity, pH and Total Soluble Solids

The correlation coefficients among glucose, fructose, total titratable acidity, total soluble solids and pH are presented in Table 16. This table shows a highly significant positive correlation between glucose and fructose, the r- value being 0.98. This indicates that as the concentration of glucose increased at successive sampling dates, the fructose content also increased. It was quite clear from Table 12 that at the initial sampling dates the glucose content was higher than fructose but subsequently fructose increased more rapidly than glucose. The positive relationship between glucose and fructose did not indicate that they increased at the same rate, but simply showed that both increased with time. There were negative associations between glucose and total titratable acidity, and fructose and total titratable acidity, the r- values being -0.96 and -0.97, respectively. These negative relationships of glucose and fructose with total titratable acidity demonstrated the decrease in acidity from one sampling date to next and increase of glucose and fructose contents in grapes. This can be visualized from Table 14. Both glucose and fructose showed positive associations with total soluble solids. As these two carbohydrates were components of total soluble solids, they were likely to have positive relationships because as the contents of glucose and fructose increased, the content of total soluble solids also increased. Thus the contribution of these hexoses to the total soluble solids cannot be overemphasized. These two hexoses also showed positive correlations

Table 16. Simple correlations among various chemical characters in grapes.

	Fructose	Total Titratable Acidity	Total Soluble Solids	pH
Glucose	0.98	-0.96	0.98	0.91
Fructose		-0.97	0.99	0.91
Total Titratable Acidity			-0.98	-0.88
Total Soluble Solids				0.91

r - value required to be significant at 5% level of significance and 1% level of significance were 0.26 and 0.34 respectively.

with pH, the r-values were 0.91 and 0.91, respectively, between glucose and pH and fructose and pH. In the ripening process, free acids like tartaric were respired and there was a constant increase in sugars and pH. These two hexoses seemed to promote the decrease in concentrations of hydrogen ions because with the increase in contents of glucose and fructose the pH also increased from one date of harvest to another. Total solids showed a highly significant negative association with total titratable acidity. Glucose, fructose, sucrose and other organic compounds constitute the total solids and as the amount of these compounds increased from one sampling date to another, the total titratable acidity tended to decrease. Kliewer (35) had earlier shown that most of the tartrate in grape berries is present as free acid until veraison, the time at which the fruits change color and then start to accumulate sugars rapidly. There was also a positive relationship between total soluble solids and pH. It has already been pointed out that as the contents of glucose and fructose, components of total solids, increased with time, the total titratable acidity tended to decrease with the increase of pH level. In other words total soluble solids had a tendency to decrease the concentration of hydrogen ions.

SUMMARY

The results of the study indicate that the glucose, fructose, total solids, pH and total acidity contents of berries at various

sampling dates were influenced by the rootstock on which the cultivar was growing. There were rootstocks x sampling dates interactions for all quantitative chemical characters studied. This implied that the different rootstocks behaved differently at various sampling dates with respect to the content of chemical constituents. The amount of increase or decrease in the chemical constituents was not consistent for all the rootstocks from one sampling date to another.

The last experimental samples were collected on August 24. At this date the majority of the grape berries were mature as indicated by the purple color of all the grape berries. At this stage of maturity (August 24) berries from Buffalo on its own roots were the highest in glucose content followed by Buffalo on 3309 and Buffalo on 3306. There was no significant difference in fructose content of grape berries of Buffalo on its own roots and Buffalo on 3309 but they contained significantly more fructose than the grape berries of Buffalo on 3306. Also, on August 24, fruits of Buffalo on its own roots had the greatest amount of total soluble solids as well as the highest pH followed by Buffalo on 3309 and on 3306, respectively. However, there was no significant difference in total titratable acidity in the fruits from the vines of different rootstocks on this date.

The glucose/fructose ratios of berries of all the rootstocks were highest at the first harvest date. There was a very rapid narrowing of the ratios in July 11 collected fruits. The ratios were only slightly higher than unity in the August 2 collected berries from all the rootstocks which indicated the presence of similar amounts of glucose and fructose. The ratios were lower than unity during the last two harvest dates. There were differences among glucose/fructose ratios of berries from different rootstocks.

The differential changes in amounts of chemical constituents of berries from one harvest date to another showed a marked difference among the rootstocks. There existed differences in the overall average rate of change of chemical constituents of berries among the different rootstocks except for pH and total acidity in which the average rate of change was almost the same for all the rootstocks.

The simple correlation analysis showed a highly significant association of glucose and fructose with total solids and pH separately, and a negative significant correlation with total acidity. The total acidity had a negative relationship with total solids and pH, but there was a positive correlation between pH and total solids.

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SOME EFFECTS OF ROOTSTOCKS ON SELECTED CHEMICAL COMPONENTS OF BUFFALO GRAPE BERRIES AT VARIOUS STAGES OF MATURITY

by

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ABSTRACT

One of the primary objectives of grape breeding research is the development of high quality table, or wine cultivars. It has been pointed out by many workers that a vigorous rootstock contributes greatly to the general performance of a particular cultivar. The main objective of this study was to investigate the differences in chemical constituents (glucose, fructose, total solids, pH and total acidity) of grape berries of one cultivar (Buffalo) as influenced by rootstocks with fruit sampled at different stages of development.

The fruit samples of this study were obtained from the following scion and stock combinations.

1. Buffalo on rootstock 3306
2. Buffalo on rootstock 3309
3. Buffalo on its own roots

These grafts were space planted at the Horticulture farm near Manhattan, Kansas, in the year 1959. The vines were trained and pruned to 4-cane kniffin system.

Three composite samples were made from each experimental treatment at selected dates. Starting from June 30, 1969, there were six sampling dates at intervals of eleven days.

The data on the chemical constitutents (glucose, fructose, total solids, pH and total acidity) of grape berries from different rootstocks was subjected to statistical analysis, and for all the chemical constituents studied there were significant rootstock x sampling date interactions.

The trend of the increase of glucose, fructose and total soluble solids content of fruits from different rootstocks was slow for the first two sampling dates. The changes were very rapid during the subsequent sampling dates followed by a slow increase in the final time interval. This trend was true for all the experimental treatments, but at the same time the rate of increase was not consistent for different treatments at each sampling date. The pH values of the fruits of different rootstocks, increased rapidly between first two sampling dates, then it increased at a slower pace between second and third sampling dates. The pace of increase was again rapid from the third to fifth sampling dates. Again the increase in pH level declined between fifth and sixth sampling dates. The total acidity in the fruits from 3306 and 3309 rootstocks increased between first and second sampling dates, however, it decreased after second sampling date. The fruits of Buffalo on its own rootstock declined in acidity from the first sampling date. The decrease in total acidity for all the fruits of different rootstocks was not as rapid between fifth and sixth sampling dates.

At the final sampling date (August 24) when berries were considered to be ripe, fruit from Buffalo on its own roots was the highest in glucose followed by Buffalo on 3309 and Buffalo on 3306. There was no significant difference in fructose content of grape berries of Buffalo on its own roots and Buffalo on 3309 but both had a significantly higher fructose content than berries produced on 3306. Also on August 24, fruits of Buffalo on its own rootstock had the greatest amount of total soluble solids as well as highest pH, followed by fruits of Buffalo on 3309 and

3306, respectively. However, there was no significant difference in total titratable acidity in the fruits from the vines of different rootstocks on this date.

The glucose/fructose ratios of all the fruits produced on different rootstocks were very high at the first sampling date and decreased rapidly by the second sampling date. By the fourth sampling date the ratio was nearly one and decreased slowly.

The experimental evidences indicated that the different rootstocks influenced amounts of selected chemical constituents of the berry of the cultivar Buffalo at various sampling dates.