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Sensory attributes of wines made from vines of differing phosphorus status

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ABSTRACT

Aim: The implications of water and nutrient deficiencies for photosynthesis, dry matter production, and yield have been well documented. However, whereas multiple studies show that water deficits affect grape and wine quality as well as wine sensory characteristics, the corresponding implications of manipulating vine nutrient status through fertilizer additions remain largely unexplored.

Methods and results: In this study, phosphorus (P) fertilizers were applied to P-deficient vineyards of Cabernet-Sauvignon, Chardonnay, and Chenin blanc growing in rhyolite, granite, and schist derived soils. Bloomtime leaf lamina P levels, basic soil chemical characteristics, juice P, and wine chemical parameters were measured after harvest. A highly sensitive protocol for sensory evaluation was used to test the wines made from the treated and untreated grapes for differences in wine appearance, flavor, aroma, and taste.

All P additions were effective in rapidly increasing both vine P status and P in the harvested juice. In Cabernet-Sauvignon vines growing on rhyolite, juice P was linearly related to vine P status compared to a non-linear accumulation of juice P in Chenin blanc vines growing on an calcium-rich schist soil. Soil CEC and Ca levels were both higher in the schist derived soil than in the rhyolite or granite soils, indicating a possible association of P release with soil parent material. Differences were detected in appearance, flavor, aroma, and taste in wines made from all three varieties on all three sites. Also, increasing vine P status increased the concentration of anthocyanins and soluble phenolics in the wines, and reduced fermentation time in the Cabernet-Sauvignon.

Conclusions: The results of this study show that vine P nutrient status can be manipulated by the grower across different soil types. Vine nutrient status has also been shown to significantly affect vine bud fertility, photosynthesis, dry matter and fruit production, and grape and wine chemical parameters. However, there is a lack of data describing the effect of manipulating vine nutrient status through fertilizer additions on the resulting wine sensory profile. Our results show that vine P status can influence the sensory attributes of wines.

Significance and impact of the study: The results of this study and earlier work with vine water status identify two vineyard sources of variation in wine sensory attributes that occur both naturally and can be manipulated by the grower across different soil types.

KEYWORDS

grapevine, plant nutrition, phosphorus, soil chemistry, wine sensory, terroir

INTRODUCTION

In the 1980s, phosphorus (P) deficiencies were discovered in vineyards of California (Cook *et al.*, 1983 ; Skinner *et al.*, 1988) and Arizona (Stroehlein *et al.*, 1990) after decades of assuming sufficient P was available for vine growth, productivity, and fruit quality (Christensen *et al.*, 1978). A rapid and sensitive assay for vine P status was developed (Skinner *et al.*, 1987) that helped establish that varieties may vary in their P requirements (Skinner *et al.*, 1988), and that P deficiency inhibits floral bud initiation, differentiation, and maintenance (Skinner and Matthews, 1989). Additionally, it was discovered that low P availability in the soil leads to Mg deficiencies in the shoots (Skinner and Matthews, 1990). From these and a few subsequent studies (Grant and Matthews, 1996a ; Grant and Matthews, 1996b ; Schreiner *et al.*, 2013), an important role of vine P status in growth and development of the grapevine has become clear. However, important questions remain regarding means for correction of P deficiency on different soil types, and whether vine P status is a factor determining wine composition and flavor.

It is clearly important to discover the factors of fruit and wine character that are manageable in the vineyard. Unfortunately, most investigations into vineyard-based differences in wine composition and flavor have been directed at variations among vineyards. Consequently, factors that are relatively "fixed" for an individual vineyard, such as variety, rootstock, mesoclimate, or soil type, have received the most attention. An exception is water deficits ; multiple studies show that managing water deficits impacts grape composition as well as wine sensory characteristics. However, the corresponding implications of manipulating vine nutrient status through fertilizer additions remain largely unexplored, particularly with respect to phosphorus.

Vine N status has been reported to have a strong impact on grape composition and organoleptic characteristics (Choné *et al.*, 2006 ; Peyrot des Gachons *et al.*, 2005). For example, Helwi *et al.* (2016) found that N additions increased the content of the precursor for grapefruit aroma of Sauvignon blanc. Coga *et al.* (2008) studied P dynamics on acid and calcareous soils in Croatia and concluded that soil pH has a significant effect on P content in leaves and that P content in leaves influences the contents of P, sugar, and TA in the must of Sauvignon blanc wines. A recent study in Argentina (Solanes *et al.*, 2017) found the calcareous content of soils in different vineyards was related to variables associated with the aroma and polyphenol content of three different wines and that the wines could be distinguished by a tasting panel. Phosphorus additions to Pinot noir and Chardonnay vines growing on low pH soils increased cane mass and reduced the yield/cane ratio on 140 Ruggeri rootstock compared to 99R, 110R, and SO4 rootstocks (Wooldridge *et al.*, 2010). The P additions increased petiole P and Mg in vines on 140 R and the N and Mg content in their must. Unfortunately, the lack of a control treatment for each scion/rootstock combination makes interpretation of the effects on wine sensory characteristics difficult.

Sensory variation among wines due to within vineyard treatments may be less than that derived from differences in variety or climate among vineyards. Therefore, sensory testing for treatments such as irrigation or fertilizer requires sensitive sensory protocols and judges. Also, most previous sensory work with wines has involved the use of multi-factored sensory scorecards. These scorecards incorporate visual, olfactory, and taste stimuli and responses into a single score (Kwan and Kowalski, 1980). This allows stimuli from one sense to bias a judge's interpretation of stimuli to another sense (Filipello, 1956), relies on judge's introspections to determine the specific of differences, and lacks sensitivity. Thus, real differences and their sources may be overlooked by scorecard testing.

In order to make accurate and sensitive determinations among wines, a novel sensory protocol was developed. Using a similar approach, we demonstrated that wine appearance, flavor, and aroma could be altered by changes in vine water status imposed in the vineyard (Matthews *et al.*, 1990). These differences were independent of any effects of water status on yield. As with vine water status (Matthews and Anderson, 1989), vine P status plays an important role in reproductive development (Skinner and Matthews, 1989). Therefore, this study was conducted to determine whether P deficiencies also alter vine physiology such that detectable differences occur in the composition and sensory attributes of premium wines produced from grapes grown on hillside soils in California.

MATERIALS AND METHODS

1. Plant material, soil chemistry and fertilizer treatments

Three premium wine grape (*Vitis vinifera* L.) vineyards were selected for experimentation on the basis of low soil or plant P levels (Table 1) (Skinner *et al.*, 1987; Skinner *et al.*, 1988). The vineyards included red and white wine grape varieties, own-rooted and grafted vines, and three different soil types (Table 2). The Cabernet-Sauvignon (110R) vines on Forward soil were cane pruned; the Chenin blanc (St. George) vines on Sobrante soil and Chardonnay (own-rooted) vines on Musick soil were spur pruned and cordon trained to a two wire vertical trellis. All vines were drip irrigated and planted at an approximate density of 1120 vines/ha.

A randomized complete block design consisting of three (Chardonnay and Chenin blanc) or four (Cabernet-Sauvignon) replicates and ten (Chardonnay and Chenin blanc) or three (Cabernet-Sauvignon) vines per replicate was used. Five soil sub-samples were collected from the surface (0-30 cm) soil of each 10-vine replicate and composited by replicate, air-dried, and passed through a 2-mm sieve. Determinations were made of available P using a modified Bray-1 procedure (Olsen, 1982), pH using a saturated paste (McLean, 1982), EC using a saturation extract (Rhoades, 1982b), CEC using a Ba saturation and Ca replacement (Rhoades, 1982a), exchangeable K, Ca and Mg using neutral normal ammonium acetate (1.0N NH_4OAc at pH 7.0) equilibration and flame photometry or atomic absorption spectrophotometry (Doll and Lucas, 1973).

Standard disease and pest management practices were imposed as necessary. Fertilizer rates in each vineyard trial varied because of differences in initial P and pH soil levels (Table 1), the level of P deficiency symptoms present in leaf lamina, and the initial goal to establish whether P status affected vines and wines in a detectable way. The Cabernet-Sauvignon vines were treated with 0.0, 0.44, 0.88, and 1.32 kg P/vine as mono potassium phosphate, mono ammonium phosphate or triple superphosphate in the winters of 1984 (Figure 1) and again with the same rates in the winter of 1986. The Chenin blanc vines were treated with 0.0, 0.45, 0.90, and 0.135 kg P/vine in the fall of 1982 (Figure 2) and again with the same rates in the winter of 1986. The Chardonnay vines were treated with 0.0, 0.18 and 0.27 kg P/vine in the winters of 1984 and again with the same rates in the winter of 1986 (Figure 3). Fertilizers were applied on the soil surface (Cabernet-Sauvignon and Chenin blanc)

TABLE 1. Chemical analysis of three vineyard soils (0-30 cm) to which P additions were made.

Soil	pH	P mg/kg	CEC me/100g	K me/100g	Ca me/100g	Mg me/100g	Color Munsell	Fe estimated
Sobrante	5.9	3	24	0.46	11.1	1.79	7.5 YR 6/4	Med
Forward	5.3	4	13	0.33	3.9	1.47	10 YR 7/2	Low
Musick	5.5	1.9	15.5	0.15	3.5	0.4	2.5 YR 5/6	High

CEC, cation-exchange capacity

TABLE 2. Soil classification, base saturation, color and estimated Fe.

Soil Series	Parent material	Classification	Base saturation %	B2T Color	Color	Est. Fe
Sobrante	Schist	Mollic Haplo Xeralf	70-90	7.5 YR 6/4	Yellow red	Med
Forward	Rhyolitic tuff	Typic Vitrixerand	40-80	10 YR 7/2	Pale yellow	Low
Musick	Quartz diorite	Ultic Haplo Xeralf	40-70	2.5 YR 3/6	Dark red	High

or at a depth of 15 cm (Chardonnay) directly beneath the drip emitter. Fruit was harvested at maturity in 1984 and 1985. Vine P status, yield and fruit composition responses to P fertilizer treatments were recorded for several seasons in each plot. The data reported here for selected seasons is limited to those wines used to conduct sensory evaluations with trained judges.

2. Phosphorus status

Vine nutrient status was estimated from leaf samples taken opposite basal clusters at anthesis. Lamina were oven-dried at 70 °C for 48 h, ground to pass a 1-mm mesh screen, and stored at room temperature until analyzed. Lamina extractable P (EP) was determined using a modified Skinner method (Skinner *et al.*, 1987). Phosphorus status of grape juice was determined using the same extraction procedure as for leaves.

3. Statistical analysis

Data for all parameters were tested to verify if the assumptions of analysis of variance (ANOVA) were met using Shapiro-Wilk's test. Data were analyzed using a generalized linear model (GLM) procedure in SAS (version 9.3: SAS Institute Inc., Cary, NC). The significance level was set at a=0.5 and means were separated using Tukey's honestly significant difference test. Interactions between year and treatments were tested and whenever these interactions were significant ($P<0.5$) analysis was conducted separately for each year.

4. Fruit and wine composition

Fruit from the replicate plots of each treatment was pooled and crushed at the University of California, Davis, experimental winery. The resultant must was separated into three replicates of approximately 20 liters each and made to wine by standard practices. Replicate wines were bench tested for off-flavor or aroma. Sound replicates were blended after sparging all bottles with N_2 gas. Total phenolics in blended wines were estimated using the method by Slinkard and Singleton (1977). Residual reducing sugars and ethanol concentration were determined according to Amerine and Ough (1980). Wine color analyses and estimates of total anthocyanins were obtained by the methods of Somers and Evans (1977). Soluble solids, pH, and titratable acidity (TA) of musts and pH, TA, [alcohol], [reducing sugars], and color were determined by standard procedures (Amerine and Ough, 1980 ; Matthews *et al.*, 1990).

5. Sensory evaluation

The sensory testing was conducted using an approach developed to test potential effects of vineyard P and water status on wine sensory attributes (O'Mahony, 1988). Judges were used as analytical instruments to determine whether sensory differences could actually be detected using the visual, olfactory, or gustatory/tactile senses under laboratory controlled conditions. For the sensory experiments, judges were chosen from a pool of 19 students and staff at the University of California, Davis (11 M 6 F, ages 22-58 years) ; all had at least one year's experience in either food or wine tasting. Data are presented for those judges who exhibited the greatest success in discerning differences between wines for each sensory attribute. All difference testing for a given wine pair was performed in a single day.

Experimental details were described earlier (Matthews *et al.*, 1990). Briefly, judges were tested individually during two sessions in an isolated booth. Visual and flavor differences were tested in the first session, smell and taste differences in the second session. Each judge performed at least ten paired comparisons for each attribute (visual, flavor, aroma, and taste). All experiments were conducted double-blind. No descriptive words were supplied. For visual difference tests, judges were presented with a sample (20 mL in clear, transparent, round-stemmed glasses) of two wines for visual inspection. The judge was informed that the two wines were different and was asked to compare the two wines and then describe the visual difference ; that description was used for performing the paired comparisons that followed. Judges did not smell or sip the wine so as to eliminate interference by flavor cues. In all sensory tests, judges worked at their own speed. Testing times ranged from 7 to 41 min.

For flavor testing, judges were presented with paired 20 mL wine samples in black opaque round-stemmed glasses covered by watch glasses to eliminate color cues. Judges sampled the wine by removing the watch glass, swirling the contents, sniffing, and expectorating. To facilitate 'warm-up' (O'Mahony *et al.*, 1988), judges were first given paired wine samples (30 mL each) to sample alternately and then describe; these descriptions were used to phase the relevant question for the subsequent paired comparison tests. This testing protocol has been called the warmed-up paired comparison (Thieme and O'Mahony, 1990) and is a procedure for maintaining maximum sensitivity (Dawson *et al.*, 1963) when the changes in attributes cannot be predicted in advance. The 'warm-up' samples were kept for reference during the subsequent paired comparisons. Testing times ranged from 5 to 45 min. Judges were given the option of interstimulus water rinsing. Rinsing reduces adaptation effects (Noble, 1989; O'Mahony, 1979) yet the time taken for rinsing facilitates memory loss of the flavor. As judges varied in their rinsing efficiency and memory retention skills, preliminary experiments were used to establish each judge's optimum rinsing regime. During testing, the experimenter monitored performance and intervened by instructing the judge to rinse, when performance deteriorated.

For aroma testing, the same procedure as for flavor was used except that the wines were only sniffed so as to eliminate oral cues, the watch glasses being removed immediately prior to sniffing. The reference samples were again 20 mL samples to maintain constant headspace. Testing times ranged from 3 to 40 min. Taste differences were investigated using the same procedure as for flavor testing (stimulus volume = 30 mL) except that noseclips were worn by each judge to eliminate odor cues. Thus, judgments were made according to differences in taste or any oral tactile cues that might be available. Should judges wish for relief from having their nose clipped, they were required to rinse the mouth with water before removing the clip. This removed the stimulus from the mouth and reduced the chance of retronasal olfactory cues occurring during the testing session. Testing times ranged from 5 to 40 min.

RESULTS

All fertilizer applications increased vine P status as indicated by EP of bloomtime leaf lamina. However, the magnitude of the increase and the response over time appears to have varied with soil parent material. In Cabernet-Sauvignon growing on the Forward soil, one application of three different forms of phosphorus fertilizer applied at rates from 0.0 to 1.2 kg P/vine resulted in significant increases (100-300 %) in lamina EP (Figure 1). In Chenin blanc vines on the Sobrante soil, lamina EP was approximately 2x greater in treated vines than in untreated controls in 1983 the first year after treatment (Figure 2). Second year, 1984, effects were slightly greater. In the third year, 1985, all treatments decreased in lamina EP compared to the previous year. After a second treatment in the winter of 1986, all treated vines responded with the largest increase in lamina EP observed during the five-year experimental period (Figure 2, Figure 3, Cabernet-Sauvignon data

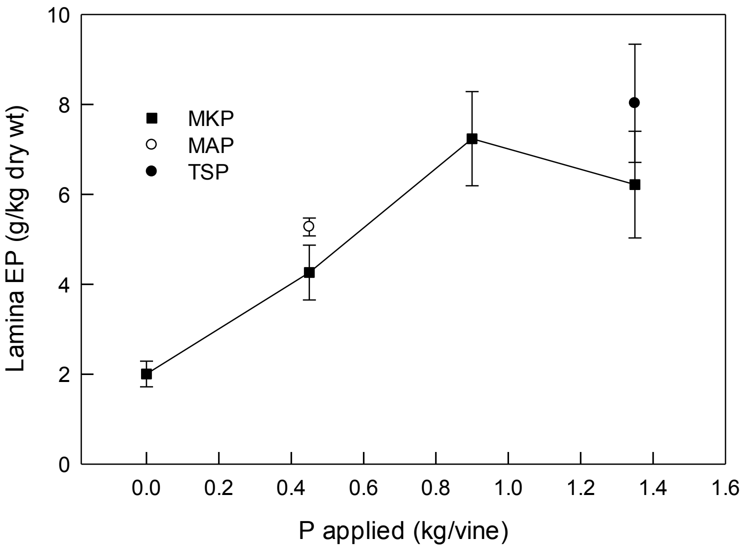

FIGURE 1. The concentration of extractable phosphorus at bloom 1984 in basal leaf lamina of Cabernet-Sauvignon vines growing on Forward soil series that had received different phosphorus fertilizer applications: MKP (mono potassium phosphate), MAP (mono ammonium phosphate), TSP (triple superphosphate) in January 1984. Vertical bars represent s.e.m. n=4.

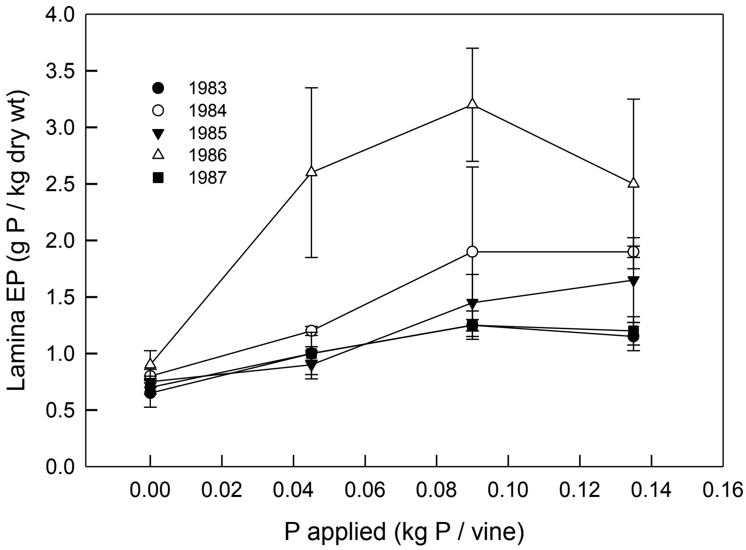

FIGURE 2. The concentration of extractable phosphorus at bloom in basal leaf lamina of Chenin blanc vines growing on Sobrante soil series that had received two similar phosphorus fertilizer applications in 1982 and 1986 over a five-year period. Vertical bars represent s.e.m. n=3.

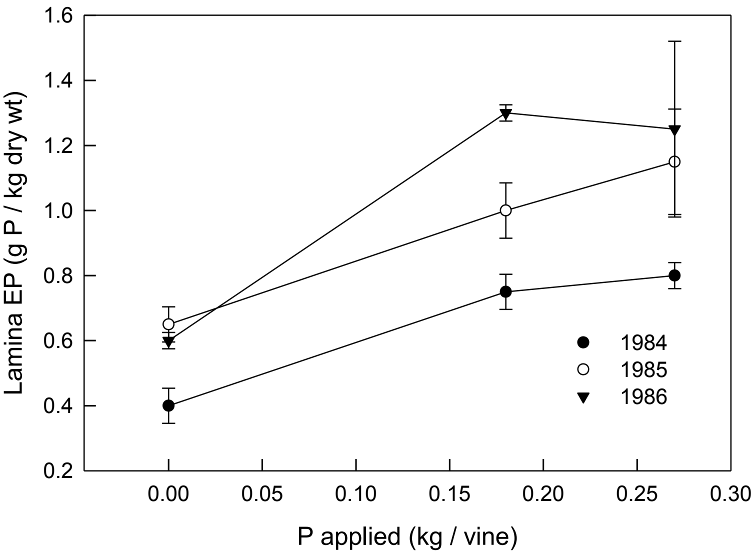

FIGURE 3. The concentration of extractable phosphorus at bloom in basal leaf lamina of Chardonnay vines growing on Musick soil series that had received two similar phosphorus fertilizer applications in 1984 and 1986 over a three-year period. Vertical bars represent s.e.m. n=3.

not shown). In Chardonnay vines growing on the Musick soil, applications of applied P ranging from 0.0 to 0.27 kg P/vine led to a maximum increase in lamina EP of approximately 1.2 g P/kg dry wt. compared to approximately 7.0 g P/kg dry wt. in the Cabernet-Sauvignon (Figure 1) or 3.0 g P/kg dry wt. in the Chenin blanc vines (Figure 3).

The increases in vine P status following P applications resulted in increases in yield in each variety but the magnitude differed significantly (Table 3). Yield of Cabernet-Sauvignon followed a pattern similar to that of vine P status with a large initial increase (ca 60 % in 1984) followed by a smaller increase in 1985. In Chardonnay and Chenin blanc, treated vines had yields 25 % and 200 % greater, respectively, than in untreated vines (Table 3).

1. Fruit and wine composition

TABLE 3. Yield and leaf P status of premium wine grape varieties to which different P fertilizer applications were made (Cabernet-Sauvignon: control or 1.32 kg P/vine, Chardonnay: control or 0.27 kg P/vine, Chenin blanc: control or 0.135 kg P/vine).

Variety	Year	Treatment	Lamina EP (g P/kg)	Yield (kg/vine)
Cabernet-Sauvignon (n=4)	1984	- P	2.19b	5.63b
		+ P	6.60a	9.l0a
			P<0.0001	P<0.001
	1985	- P	2.82b	7.48
		+ P	5.19a	7.9
			P<0.0001	ns
Chardonnay (n=3)	1985	- P	0.64b	3.32
		+ P	1.01a	4.2
			P<0.0001	ns
Chenin blanc (n=3)	1986	- P	0.89b	2.20b
		+ P	2.55a	6.37a
			P<0.0001	P<0.0001

TABLE 4. Fruit composition of the musts from wine grape varieties to which different P fertilizer applications were made (Cabernet-Sauvignon: control or 1.32 kg P/vine, Chardonnay: control or 0.27 kg P/vine, Chenin blanc: control or 0.135 kg P/vine). The musts were used to make wines for sensory analysis presented in Tables 5 through 9.

Variety	Year	Treatment	°Brix	pH	TA (g tartaric acid /L)	EP (ppm)
Cabernet-Sauvignon	1984	- P	23.5a	3.3b	0.72b	95b
		+ P	22.5b	3.37a	0.84a	135a
			P<0.05	P<0.05	P<0.05	P<0.05
	1985	- P	21.3	3.1b	1.02	18b
		+ P	21.5	3.19a	1.02	38a
			ns	P<0.05	ns	P<0.05
Chardonnay	1985	- P	20.6	3.20a	0.81b	16b
		+ P	20.5	3.1b	0.87a	32a
			ns	P<0.05	P<0.05	P<0.05
Chenin blanc	1986	- P	20.5	3.08	0.99a	24b
		+ P	20.5	3.05	0.93b	45a
			ns	ns	P<0.05	P<0.05

Means within columns designated by different letters are significantly different at P<0.0% (Tukey's test). ns, non-significantly different.

The concentration of EP in the juice of harvested grapes was increased by P applications. In Cabernet-Sauvignon juice EP increased from 90 mg/L to 145 mg/L as lamina EP increased from 2.0 g/kg to 8.0 g/kg (Figure 4A). Although the concentration of lamina EP decreased the following year, juice EP was still higher in treated vines than untreated controls (Table 4). In Chenin blanc juice, EP varied from approximately 10 to 50 mg/L (Figure 4C) and Chardonnay was lower yet varying from 7 to 48 ppm (Figure 4B). There were no consistent treatment differences in sugar accumulation, or TA for all three varieties investigated (Table 4).

In the blended wines used for sensory analyses, the pH and alcohol concentration also showed no consistent differences between treatments in all three varieties, but titratable acidity increased slightly with P applications for each wine

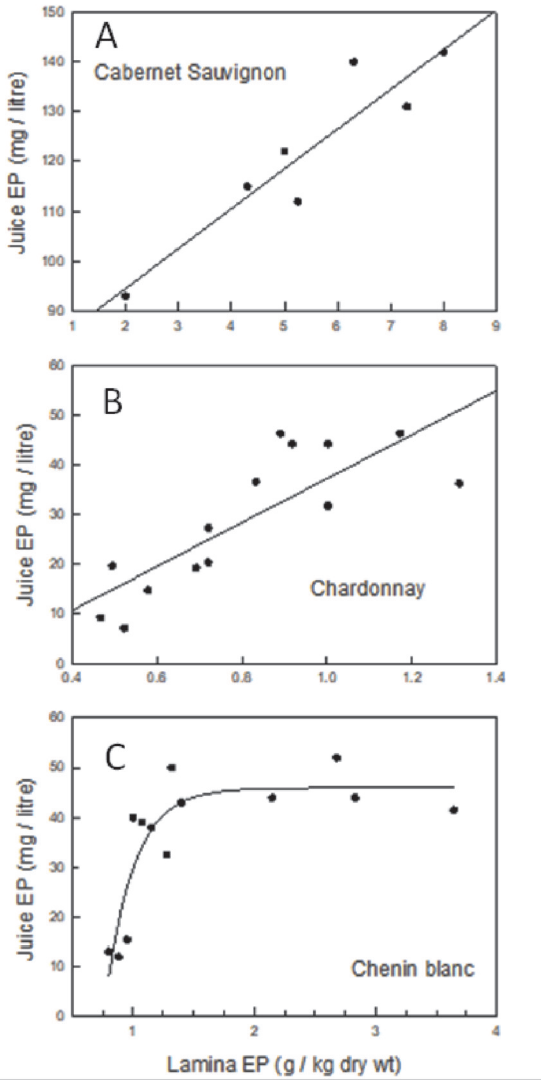

FIGURE 4. The concentration of extractable phosphorus in the harvested juice at various vine phosphorus statuses (lamina EP) for (A) Cabernet-Sauvignon, $r^2 = 0.86$; (B) Chardonnay, $r^2 = 0.67$; and (C) Chenin blanc vines. The relationship between Chenin blanc lamina EP and juice EP was described by an equation of the form: y=ab(x-c)/ sq rt ($b^2+a^2(x-c)^2$) where a, b, and c are constants and x=lamina EP.

(Table 5). Reducing sugars increased slightly in Cabernet-Sauvignon and decreased slightly in the white wines from treated vines compared to the wines from untreated vines. For both 1984 and 1985, the color density and the concentrations of anthocyanins and total soluble phenolics in Cabernet-Sauvignon was greater in wines made following P applications than in wines made from untreated controls (Table 6).

2. Fermentation

In 1984, the must of Cabernet-Sauvignon from P-treated vines fermented more rapidly and steadily than the control must (Figure 5A). The fermentation of the must from P-treated vines was complete three or more days earlier than the controls. In 1985, the fermentation of P-treated musts was again faster than the controls, though only slightly (Figure 5B). The fermentations of the Chenin blanc and Chardonnay showed no clear differences among treatments (data not shown). Although not yet clearly defined, a conservative estimate of the minimum P levels needed to prevent sluggish fermentations has been reported to be 100 ppm (Schreiner *et al.*, 2013). This level is supported by both the data for the Cabernet-Sauvignon fermentations in our study which had less than 100 ppm in the control treatment and more than 100 ppm in the treated vines (Figure 4A) and also in the Chenin blanc and Chardonnay musts where all treatments had must P levels less than 100 ppm (Figure 4B, and 4C).

3. Sensory analyses of wines

For the first stage of the sensory testing, judges were used as analytical instruments to determine whether sensory differences existed for the visual, olfactory, or mouthfeel. Only the data from those judges who could generally distinguish on several tests are presented. The data in Tables 7 through 10 indicate the number of correct paired comparison tests that each judge performed with corresponding levels of significance (one-tailed binomial tests: 24).

Visual and flavor tests were conducted with both 1984 and 1985 Cabernet-Sauvignon wines. Some judges could consistently detect visual and flavor differences between 1984 wines from treated and untreated vines (Table 7). For the 1985 wines, more judges could consistently establish differences in flavor, and some could detect a difference in wine appearance (Table 8).

The contributions of taste and aroma to flavor differences were investigated by separate tests. For 1984 Cabernet-Sauvignon, differences in aroma and taste were detected consistently by some judges but not others (Table 7). For the 1985 wines a difference in aroma between P-treated and controls was readily detected by most judges (Table 8).

TABLE 5. Composition of wines made from three premium wine grape varieties to which different P fertilizer applications were made (Cabernet Sauvignon: control or 1.32 kg P/vine, Chardonnay: control or 0.27 kg P/vine, Chenin blanc: control or 0.135 kg P/vine). Wines were used for sensory analysis presented in Tables 5 through 9.

Variety	Year	Treatment	ETOH (vol %)	pH	TA (g tartaric acid/L)
Cabernet-Sauvignon	1984	- P	13.9a	3.70b	0.73
		+ P	13.5b	3.79a	0.75
			P<0.05	P<0.05	ns
	1985	- P	12.2a	3.48b	0.76
		+ P	11.9b	3.56a	0.79
			P<0.05	P<0.05	ns
Chardonnay	1985	- P	12.3	3.03	0.73b
		+ P	12.3	3	0.9a
			ns	ns	P<0.05
Chenin blanc	1986	- P	11.8	2.88	0.83
		+ P	12	2.94	0.86
			ns	ns	ns

Means within columns designated by different letters are significantly different at P<0.0% (Tukey's test). ns, non-significantly different.

TABLE 6. The concentration of total soluble phenolics and total anthocyanins and the color density of +P and -P Cabernet-Sauvignon wines used for sensory analysis (control or 1.32 kg P/vine).

Treatment		Color density (A420 + A520)	Total anthocyanins (mg/L)	Total phenolics* (mg/L)
1984	+ P	9.59a	305a	2111a
1984	- P	9.35b	216b	1742b
		P<0.05	P<0.05	P<0.05
1985	+ P	9.41a	460a	2861a
1985	- P	9.31b	392b	2485b
		P<0.05	P<0.05	P<0.05

* Gallic acid equivalents
Means within columns designated by different letters are significantly different at P<0.0% (Tukey's test). ns, non-significantly different.

For the Chardonnay wines, differences in appearance, flavor, and aroma were consistently detected by four judges (Table 9). Visual and flavor differences were also readily detected in the Chenin blanc wines (Table 10), but the flavor differences were less readily detected as aroma or taste components.

DISCUSSION

Much about the physiology of phosphorus within grapevines is not known (Skinner and Matthews, 1990). Further investigations are needed to establish the physiological relationships between P deficiency and the production of anthocyanins, aroma and flavor precursors, and the phenolic content of grapes and wines. This study was limited to the practical question of whether vine P status affected wines in a detectable way.

The primary goals of this research were to establish whether differences in vine P status resulted in changes in the aroma, flavor, taste, and visual characteristics in wines that could be detected consistently and whether soil type played any role. When soil and vine P deficiencies were corrected by P fertilizer additions, sensory differences were detected between wines made from P-sufficient and P-deficient vines for both red and white wines made from three varieties (Cabernet-Sauvignon, Chardonnay, and Chenin blanc), and from vines growing on three different sites and soil types (Forward, Sobrante and Musick soil series).

A secondary goal was to demonstrate sensory methodology for detecting small differences among wines (O'Mahony, 1988). Sensory difference tests were used because the anticipated sensory differences were small. There was no precedent for phosphorus-determined wine sensory attributes, but a similar approach was successful in discriminating among wines made from vines with different water status (Matthews *et al.* 1990). The use of this modified paired comparison protocol proved effective in detecting sensory differences among wines caused by differences in vine P status. The testing made it possible to elucidate the nature of the difference (visual, olfactory, etc.) without having to depend on judges' introspections. The flavor differences between P treatments arose from both aroma and taste differences. Where among-judge comparisons could be made, the differences in aroma were often easier to detect than those in taste, suggesting that changes in volatile constituents tended to be greater than changes in soluble constituents.

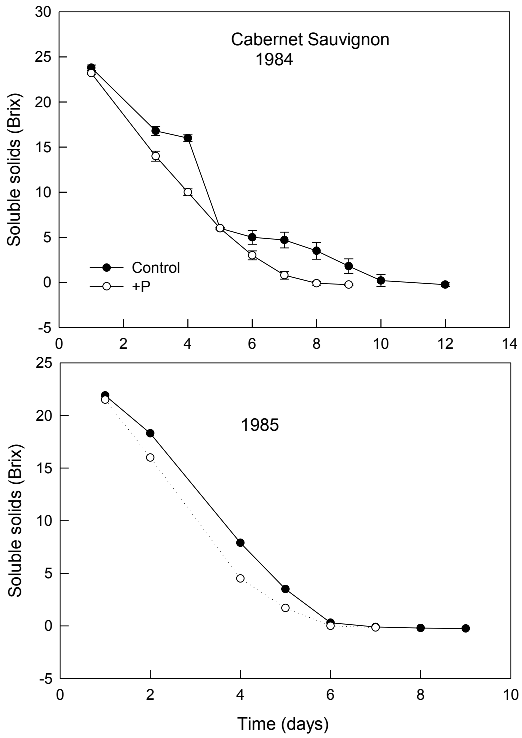

FIGURE 5. The concentration of sugars as soluble solids in fermenting musts of Cabernet-Sauvignon in 1984 and 1985 that were obtained from vines that did or did not receive P applications in 1984 (control or 1.32 kg P/vine). Vertical bars represent s.e.m. n=3. S.E. is smaller than symbol when error is not shown.

The pH, TA, and sugar content of the wines and the absence of "off" aroma or taste indicated that sensory differences were not an artifact of unsound wines. Differences among the wines in pH, TA, and sugar content were inadequate to be reproducibly detected in sensory tests (Amerine and Roessler, 1980). The differences in juice and wine composition are not attributable to the smaller fruit or lower yields caused by P deficits (Skinner *et al.*, 1988 ; Skinner and Matthews, 1989). Indeed, the concentration of phenolics in wines was greater when P-deficient vines received supplemental P. When P status was increased by soil application of fertilizer reproductive growth and yield increased, but this did not result in delayed sugar accumulation in the treated vines (Table 4). Thus, differences among treatments in Brix levels cannot be the cause of sensory differences because the fruit sources were, in general, of the same sugar status

TABLE 7. Results of difference tests for visual, flavor, aroma, and taste characteristics of 1984 wines from Cabernet-Sauvignon vines that did and did not receive P fertilizer treatments in 1984 (control or 1.32 kg P/vine). Data are presented for selected judges (see text) at the ratio of correct responses to tests.

Judge	Visual	Flavor	Aroma	Taste
1	10/10 (0.001)	10/12 (0.019)	9/11 (0.033)	
2			10/10 (0.001)	8/10 (0.055)
3	10/10 (0.001)	10/12 (0.019)		
4	10/10 (0.001)		10/11 (0.006)	8/10 (0.055)

( ) = Level of significance for one-tailed binomial test.

TABLE 8. Results of difference tests for visual, flavor, aroma, and taste characteristics of 1985 wines from Cabernet-Sauvignon vines that did and did not receive P fertilizer treatments in 1984 (control or 1.32 kg P/vine). Data are presented for selected judges (see text) at the ratio of correct responses to tests.

Judge	Visual	Flavor	Aroma	Taste
1	9/10 (0.011)	9/10 (0.011)	10/11 (0.006)	9/11 (0.033)
2	10/10 (0.001)	8/10 (0.055)	9/11 (0.033)	9/11 (0.033)
3		9/11 (0.033)	9/11 (0.033)	10/12 (0.019)
4		10/13 (0.046)	10/13 (0.036)	
5		10/10 (0.001)	10/10 (0.001)	

( ) = Level of significance for one-tailed binomial test.

TABLE 9. Results of difference tests for visual, flavor, aroma, and taste characteristics of 1985 wines from Chardonnay vines that did and did not receive P fertilizer treatments in 1984 (control or 0.27 kg P/vine). Data are presented for selected judges (see text) at the ratio of correct responses to tests.

Judge	Visual	Flavor	Aroma	Taste
1	10/10 (0.001)	9/11 (0.033)	9/11 (0.033)	8/10 (0.05S)
2	9/10 (0.011)	8/10 (0.055)	10/11 (0.006)	9/10 (0.011)
3	10/11 (0.006)	8/10 (0.055)	10/10 (0.001)	
4	10/12 (0.019)	8/10 (0.055)	10/10 (0.001)	

( ) = Level of significance for one-tailed binomial test.

TABLE 10. Results of difference tests for visual, flavor, aroma, and taste characteristics of 1986 wines from Chenin blanc vines that did and did not receive P fertilizer treatments in 1982 and again in 1986 (control or 0.135 kg P/vine). Data are presented for selected judges (see text) at the ratio of correct responses to tests.

Judge	Visual	Flavor	Aroma	Taste
1	10/10 (0.001)	9/11 (0.033)	9/11 (0.033)	9/11 (0.033)
2	10/10 (0.011)	8/10 (0.055)		
3	10/10 (0.001)	9/10 (0.011)		
4				9/11 (0.033)

( ) = Level of significance for one-tailed binomial test.

at harvest. The sensory protocol also eliminated visual bias in the flavor, aroma, and taste tests.

It is of interest to note that wine color has frequently been related to wine preference scores by experienced wine judges (Somers and Evans, 1977; Cozzolino *et al.*, 2008). The concentration of anthocyanins and wine color were increased when the P status of Cabernet-Sauvignon wines was increased. Consequently, the changes caused by eliminating P deficiencies may be construed as positive. Withholding P from nutrient solutions used to supply potted Pinot noir vines had a large impact on must P concentration (Schreiner *et al.*, 2013) similar to previously reported effects on potted Carignane vines (Skinner and Matthews, 1989). The effects of a reduced P supply over a three-year period in the Pinot noir vines, however, did not appear to reduce the P levels in petioles or leaves below the critical levels previously reported for other varieties (Skinner *et al.*, 1987; Skinner *et al.*, 1988). Not withstanding the P levels in the leaves, the juice P levels in the must established in the Pinot noir study were significantly correlated with the volatile C-6 compounds, free and bound terpenoids, and C 13-norisoprenoids including B-damascenone in the musts in at least two of the three years of the study (Yuan *et al.*, 2018). These effects on must volatile aroma compounds support our sensory analysis findings, where increasing must P levels resulted in differences in aroma in both red and white varietals (Tables 7 through 10).

Differences in wine sensory characteristics have often and correctly been attributed to genetic differences among vines or to differences in the aerial and edaphic environments (see e.g. Matthews *et al.*, 1990). The only alternative factors are those associated with the fermentation and wine-handling processes. For these investigations, variation in the latter factors were minimized to isolate any differences inherent in the fruit. The fertilizer treatments increased the concentration of phosphorus in the harvested juice and in some cases increased the rate of fermentation. For white wines, the fermentation process is clearly important in determining wine flavor characteristics. However, vine P status affected fermentation in musts of red but not white wine grapes. Consequently, it is likely that differences in fruit composition were the primary factors in creating sensory differences and possible differences in fermentation play a secondary role.

This indication of a potential influence of vine P status on wine sensory attributes may have implications for production practices in several premium wine grape varietals. First, earlier work has shown that fruiting varieties (Skinner *et al.*, 1988) and rootstocks (Grant, 1987 ; Grant and Matthews, 1996a ; Grant and Matthews, 1996b) vary significantly in P uptake and P requirements. Thus, the P status of vines is likely to vary among varieties and rootstocks even when growing on the same soil. This may cause differences in wine composition and sensory characteristics. Clearly, once a vineyard is established, the genotypes are not a variable determining the variation in wine sensory characteristics among regions or vintages. As with any environmental factor, putative edaphic effects on wine sensory characteristics must arise as a consequence of altered reproductive physiology of the vine. Accordingly, there should be identifiable changes in aspects of vine growth and physiology when “soils are responsible for differences in wine flavor. The results of this study and of our earlier work with vine water status (Matthews *et al.*, 1990) identify two vineyard sources of variation in wine sensory attributes that occur both naturally and can be manipulated by the grower.

The results also showed differences in vine P status following soil amendment application were dependent on existing vineyard soil conditions. A recent study of P dynamics in volcanic soils of northern California established that soil parent materials have a significant effect on maintaining adequate P levels in the soil solution after P additions from different source materials (Wilson *et al.*, 2016). Soils derived from low Fe parent materials such as rhyolite had less adsorption and occlusion of added P, and thus P availability remained high following P additions. The results showed that Ca-Phosphates keep P in solution more readily than less soluble Fe-rich P compounds. They concluded the response to P fertilization they observed was more significantly influenced by initial parent material composition in highly weathered soils than is commonly recognized.

Our P uptake results show a correlation between bloomtime leaf lamina EP with the soil analysis from the five soil sub-samples/replicate of the control treatments at each of the three sites. Leaf lamina EP was correlated with both soil Ca and estimated Fe content (Table 1) from soil series color data (Torrent *et al.*, 1983 ; Schwertmann, 1993). Applications of P to Cabernet-Sauvignon vines on the Forward soil with low levels of both Ca and Fe resulted in the largest increases in lamina EP (Figure 1). Applications of P to vines growing on the high Ca and medium high Fe Sobrante soil led to sustained increases in lamina EP over a five-year period (Figure 2). The least responsive vines to two P treatments were the Chardonnay vines growing on the Musick soil (Figure 3) which has low Ca levels (Table 1) and the reddest color and therefore the most oxidized Fe content (Schwertmann, 1993). Thus, our results are consistent with the implications of Wilson *et al.* (2016), and they precipitated significantly different composition, appearance, and flavor of the subsequent wines.

It is also interesting to note that the non-linear relationship between lamina EP and juice EP shown in the Chenin blanc vines on the Sobrante soil (Figure 4C) was obtained from the same vines which exhibited a P x Mg interaction over a three-year period. This P x Mg interaction included significant effects on K concentrations in the xylem sap (Skinner and Matthews, 1990). Evidence that lamina EP and consequently juice EP are sensitive to other nutrients (Mg) which are below their critical levels may imply that P has an additional role in controlling an important genotype x environment interaction that can impact berry chemistry and ultimately wine composition and sensory attributes.

In summary, our results show that three different premium wine grape varietals growing on three different P-deficient soil types all responded to P fertilizer additions with increased lamina and juice P levels which were significantly correlated with detectable differences in the sensory characteristics, including the aroma, appearance, flavor and taste, of their associated wines. While our results show that similar fertilizer additions across different soil types can lead to detectable changes in wine sensory characteristics in the short term, the extent to which soil parent material impacts the length of time the sensory characteristics of the wines produced are influenced by P additions remains unanswered.

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