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Phosphorus Requirements of Wine Grapes: Extractable Phosphate of Leaves Indicates Phosphorus Status

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Additional index words. phosphorus, grapevines, phosphate, growth, yield, mineral nutrition, *Vitis vinifera*

Abstract. **Although premium wine grapes (*Vitis vinifera* L.) are planted in soils of low P availability, there is little knowledge of P requirements and of the appropriate methods of P analysis in grapevines. Therefore, acetic acid extractable P was investigated as a method for determining grapevine P status. Analysis of absorption spectra established that absorbance peaks at 710 or 882 nm of the phosphomolybdate complex formed in acetic acid extracts of grapevine tissues can be used for analysis of P status. Acetic acid-extractable P was independent of sample size and extraction time and was linearly related (r^2 = 0.96) to total P in four premium wine grape varieties. Lamina indicated whole plant P status better than petioles, since extractable P accumulated more in lamina than in petioles. Total and extractable P of basal lamina decreased significantly after anthesis, which diminished the differences between P-sufficient and P-deficient vines. Therefore, the best time to assess vine P status using leaves is at anthesis. When P fertilizer was applied, lamina-extractable P was positively correlated with berry weight, yield, and pruning weight in Chenin blanc vines growing under P-deficient conditions. The results indicate that acetic acid extractable P in lamina sampled at anthesis is a sensitive measure of grapevine P status.**

Phosphorus deficiency of perennial fruit crops rarely has been reported, but recently was recognized for the first time in vineyards in Arizona (11) and California (9). The conditions that give rise to and the criteria for identification of P deficiencies in grapevines are unclear. Nevertheless, P is an essential macronutrient, and deficiencies of P reduce growth of roots and shoots, development of flowers and berries, and induce premature senescence of leaves (10).

Clearly, plant nutrient status must be quantified accurately in order to ascertain and alleviate nutrient deficiencies. The determination of P status in perennial plants is difficult due to the storage of P within bark, wood, and roots; the rapid mobility of P between vegetative and reproductive organs; and the compartmentation between inorganic and organic P pools. Analysis of grapevine P status under field conditions requires a method that uses easily collected plant tissue at a recognizably distinct phenological stage. Ideally, the method would be rapid, safe, inexpensive, and would use small sample sizes, short extraction times, and standard laboratory equipment.

At present, P status in grapevines is reported as total P (TP) in oven-dried petioles sampled at anthesis (1, 4, 19, 26). This determination of TP is not sensitive to P partitioning within inorganic and organic P pools, is relatively slow, and requires the use of expensive and concentrated strong acids and nonstandard laboratory equipment [i.e., muffle furnace, digestion block (12)].

Alternatively, a method that measures only the inorganic P pool may be more sensitive to plant P status than TP, since the inorganic P pool is depleted rapidly under deficiency conditions (3, 13, 29). Acetic acid-extractable P (14) is a measure of the inorganic P in lamina of several annual crops (16), and critical P levels have been established for alfalfa (22), strawberries (27), and potatoes (30). There have been few investigations of P nutrition of grapevines and critical levels of extractable P have not been established for grapevines or perennial crops. Therefore, an acetic acid extraction procedure was investigated for speed and sensitivity in determining the P status of premium wine grape varieties.

Materials and Methods

Plant material. Mature, commercial vineyards of *Vitis vinifera* cvs. Cabernet Sauvignon and Chenin blanc (Napa County, Calif.), and Zinfandel and Chardonnay (El Dorado County, Calif.) were selected for experimentation on the basis of preliminary assays that indicated low soil or tissue P. Planting density was ≈1000 vines/ha (454 vines/acre). In order to vary P status, vines were treated with different P fertilizer applications during dormancy before the 1984 season. Leaf samples were taken within two nodes of the basal cluster at different times throughout the 1984 season. Lamina and petiole samples were separated and oven-dried at 70°C for 48 hr, ground to pass a 1-mm mesh screen, and stored at room temperature until analyzed.

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Yield and growth measurements. Cluster counts and yield were determined at harvest. Pruning weights were measured during dormancy following the 1984 season by weighing the brush produced at pruning. Vegetative and reproductive growth is reported on a per-vine basis.

Total phosphorus. For comparative purposes, TP was analyzed by a common procedure for grapevines (12). TP in tissue was determined by dry-ashing 100-mg lamina and petiole samples at 450°C for 8 hr. The ash was dissolved in 0.5 ml 2 N HCl, diluted to 10.0 ml with distilled H_2O, and digested for 1 hr. A 0.25-ml aliquot of the tissue digest was mixed with 0.5 ml ammonium molybdate sulfuric acid solution, 4.0 ml distilled H_2O, and 0.25 ml 1,2,4-amino-naptholsulfonic acid reducing agent (12). Color development proceeded for 1 hr in a 30° water bath, and then was stopped by placing the samples in a 0° water bath. Absorbance at 640 nm (Bausch and Lomb Spectronic 21) was determined within 1 hr. TP was determined from a standard curve prepared with KH_2PO_4.

Extractable phosphorus (EP). One hundred-milligram lamina and petiole tissue samples (except where noted otherwise) were extracted with 50 ml 2% (v/v) acetic acid in 250-ml Erlenmeyer flasks by shaking at 120 opm for 5 min (except where noted otherwise) and filtered through No. 2V Whatman filter paper into dry, acid-washed beakers. Extracted P was determined by a modified procedure of Murphy and Riley (18). A 2.0-ml aliquot of the extract was diluted with 30 ml distilled H_2O, mixed with 8.0 ml of reagent containing 30 mM ascorbic acid, 0.4 mM antimony potassium tartrate, 4.9 mM ammonium molybdate, and 1.25 mM H_2SO_4, and brought to volume with distilled H_2O in a 50-ml volumetric flask. The reduction reaction,

$$H_3PO_4 + 4H_2MoO_4(MoO_3)_2 \rightarrow H_3P(Mo_3O_{10})_4 + 4H_2O,$$

forms a heteropolyphosphomolybdate (HPM) complex that goes to completion in <10 min (25°C) and is stable for ≈24 hr (28). Absorbance at 710 or 882 nm (Bausch and Lomb Spectronic 21) of the HPM complex was measured within 4 hr and EP determined from a standard curve prepared with KH_2PO_4. Absorbance spectra (400–900 nm, Perkin Elmer 3840) of standard solutions and of tissue extracts were obtained to test for interferences and for the optimum wavelength for analysis.

Results and Discussion

In aqueous solutions, the HPM complex exhibits absorbance peaks at 710 and 882 nm and is essentially free of interferences from metals (18). The absorbance maximum of the HPM complex and the presence of interferences in acetic acid extracts of plant tissues has not been defined, although the absorbance of such extracts has been used to estimate the P status of several annual crops (22, 30). Therefore, the absorbance spectra between 400 and 900 nm were determined for solutions of the HPM complex developed from standards and from leaf extracts. Leaf tissue samples were chosen to represent high and low P status in black and white grape varieties. The absorbance spectra of leaf extracts and standard solutions were very similar, with peaks at 710 and 882 nm (Fig. 1 A and B). The similarity of absorbance spectra between standard samples and plant tissue extracts indicates there were no significant interferences due to compounds present in plant tissues regardless of P content or of variety.

The maximum absorbance for the HPM complex in standard solutions and plant tissue extracts was at 882 nm (Fig. 1 A and B). There may be greater resolution of differences in phosphate concentration at 882 nm than at 710 nm, since the difference in

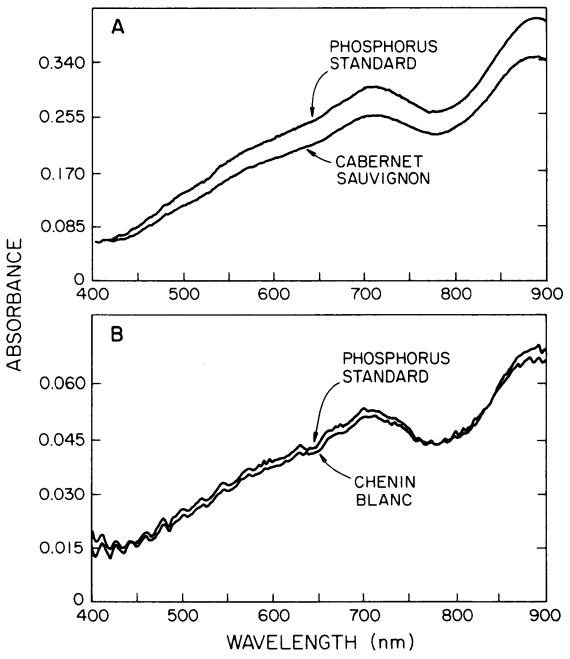

Fig. 1. Absorbance spectra of KH_2PO_4 standard solutions and acetic acid extracts of 'Cabernet Sauvignon' lamina (**A**) and 'Chenin blanc' lamina (**B**).

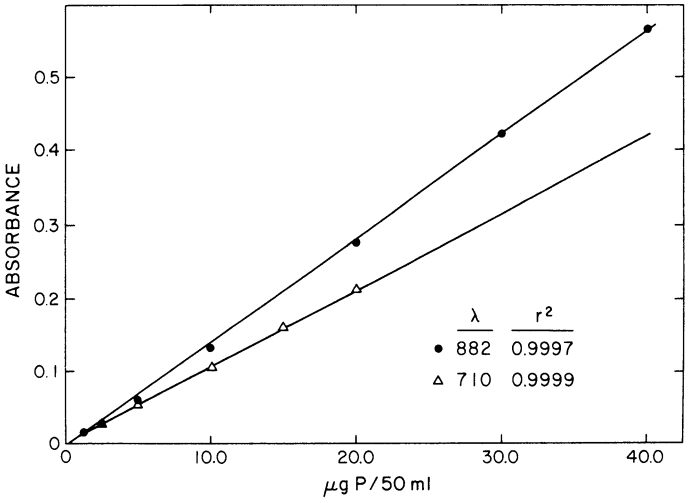

Fig. 2. Absorbance at 710 and 882 nm of KH_2PO_4 standard solutions used for determination of tissue phosphate levels.

absorbance for leaf samples with high and low P contents at 882 and 710 nm was 0.3 and 0.2 absorbance units, respectively (Fig. 1 A and B). Murphy and Riley (18) recommended using the A_{882}, but many standard spectrophotometers and disposable (polystyrene) cuvettes are unreliable above 800 nm. The relationship between phosphate concentration in standard solutions and absorbance was linear at 882 and 710 nm throughout the range of phosphate concentrations encountered in plant tissues, although the response (slope) was less at 710 than 882 nm (Fig. 2). Hence, the combined results of Figs. 1 and 2 indicate that the A_{710} or A_{882} can be used with this method to obtain a sensitive and linear indicator of EP levels in tissue extracts. A routine lab spectrophotometer has the resolution and stability to determine P accurately with this assay (at 710 nm), and the acetic acid extract can be analyzed for other minerals including K, NO_3-N, and NH_4-N.

Sample size and extraction time have significant effects on the analysis of P (24) and S (20) in soil, and of minerals in plant tissue (15). The importance of sample size to the EP determined by the acetic acid procedure was tested by repeating the assay on aliquots of various sizes from the same sample. The samples were obtained from vines of different varieties and with different P status. Increasing sample size from 50 to 500 mg had virtually no effect on EP regardless of P status or of variety (Fig. 3). Subsequent experiments have shown that similar results can be obtained with 10-mg samples and 10-ml extraction volumes, although variability did increase (Fig. 3). The effect of extraction time upon EP was of particular interest, since the acetic acid extraction for 15 min yielded 60% or less of TP. However, unlike soil P extraction methods, there was no effect on the final P concentration when extraction time of aliquots from the same samples was decreased to 8 min or increased to 120 min (Fig. 4). Therefore, sample size and extraction time did not significantly affect the EP recovered from tissue samples of different varieties having a wide range in EP concentration.

Although the results indicated that EP can vary significantly in leaf tissue, the relationship of EP to vine P status is yet undefined. One means of evaluating whether EP reflects P status is to relate EP to TP of the same tissue, since TP is the conventional measure of P status in fruit crops. The relationship between EP and TP was determined for bloomtime petioles of four premium wine grape varieties with different P status. EP was linearly related to TP (Fig. 5, $r^2 = 0.96$) over the range of TP (1.0 g P/kg dry weight to 6.0 g P/kg dry weight) commonly observed in plant tissue (17). Thus, EP appears to reflect (with equal sensitivity to TP) the wide range in P status observed in several varieties. EP may be a measure of the inorganic P reserves within grapevines. The regression of EP on TP intercepts the TP axis at 0.35 g P/kg dry weight (Fig. 5), indicating the presence of a significant P fraction at zero EP. Bieleski (3) obtained similar results with *Spirodela*. Inorganic P was used to support growth under P-deficient conditions and growth ceased when the inorganic P pool disappeared (3). The remaining P fraction was attributed to organic phosphate, which is associated with phospholipids, ester phosphates, and RNA and is stable during extraction (3).

Both lamina (1, 2, 5, 9) and petioles (2, 4, 7–9, 23) have been used with other P assays to determine P status of grapevines. The ratio of TP petioles/TP lamina also has been suggested as an indicator of P status (2, 9, 25). In order to determine which tissue better indicates whole plant P status, the acetic acid extraction procedure was applied to lamina and petiole samples that varied in P content. If EP of petioles and lamina responded similarly to varying P content, the relationship between EP lamina/EP petiole and EP lamina would not have a slope (i.e., for each increase in lamina EP there would be a corresponding increase in petiole EP and the EP lamina/EP petiole ratio would remain unchanged). Although a linear relationship ($r^2 = 0.88$) between the ratio of EP lamina/EP petioles and EP in lamina was observed, the line has a positive slope (Fig. 6). Therefore, EP accumulates more in lamina than in petioles with increasing P content. As a result of this larger signal, vine P status is more readily quantitated by lamina EP than petiole EP. Lamina remain more sensitive than petioles to changes in vine P status during the season, since the slope of the regression line was the same for samples collected at bloomtime or veraison (onset of fruit ripening) (Fig. 6).

The time of sampling may affect analyses and interpretation of P status, since the lamina EP declined between bloom and veraison (Fig. 6). In order to measure accurately and reproducibly the nutrient status of a perennial such as grape, sampling date takes on added importance, since a planting date or otherwise easily identifiable calendar reference is not available.

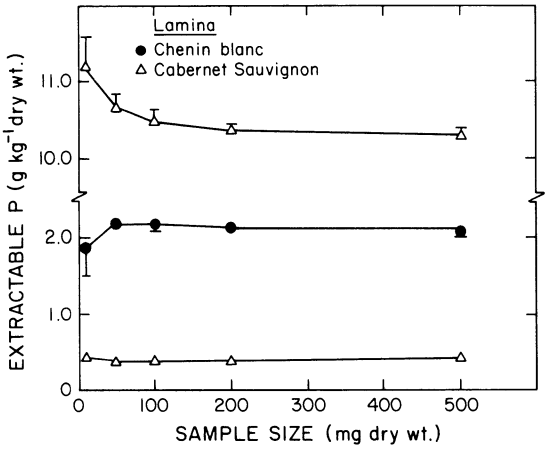

Fig. 3. Extractable P of various sample sizes of 'Chenin blanc' and 'Cabernet Sauvignon' lamina. Data are mean of five replicate samples ± SE. SE is smaller than symbol when error bar is not shown.

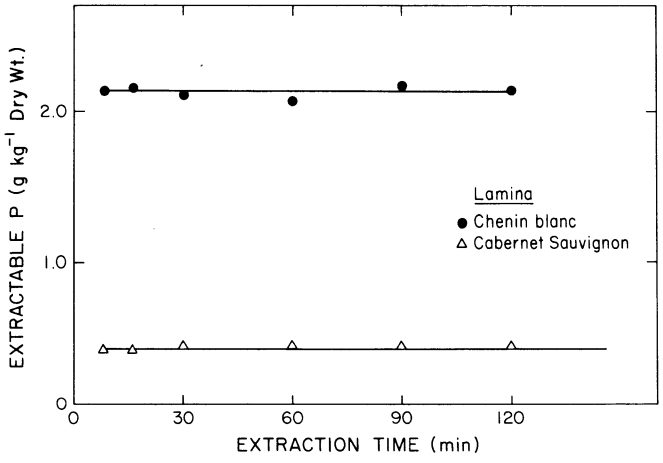

Fig. 4. Extractable P of 'Chenin blanc' and 'Cabernet Sauvignon' lamina for aliquots subjected to various extraction times. Data are mean of five replicate samples ± SE. SE is smaller than symbol when error bar is not shown.

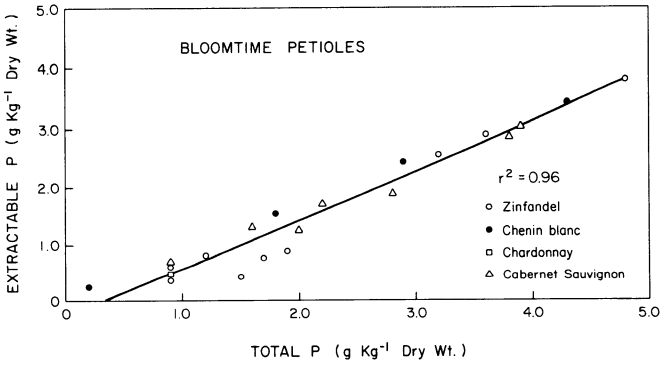

Fig. 5. Extractable P and total P in bloomtime petioles of four premium wine grape varieties in which P status had been varied with P fertilizer applications.

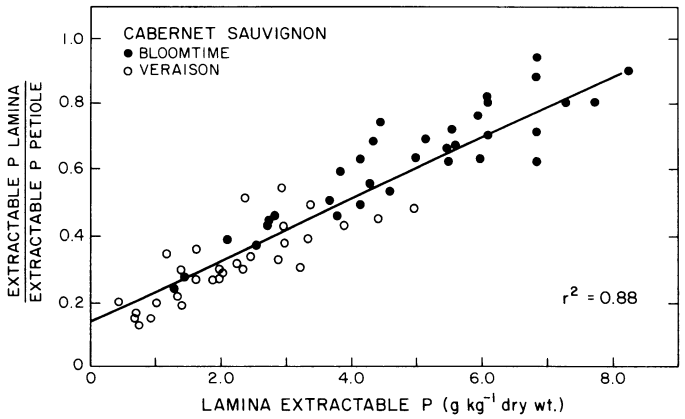

Fig. 6. The relationship between lamina EP/petiole EP and lamina EP of 'Cabernet Sauvignon' leaves. Leaves were sampled from vines of varying P status at bloomtime and veraison.

Therefore, plant phenological stages are used. In grapevines, bloom and veraison are two such stages.

As lamina EP decreased between bloom and veraison, the lamina EP/petiole EP ratio also decreased (Fig. 6). This decrease suggests that P was remobilized from basal lamina to other active sinks (shoot tips or developing buds and berries) following anthesis. Alternatively, TP may have remained constant, and the decrease in lamina EP could be due to a growth dilution effect (21) or to an increase in the nonextractable P fraction. Therefore, the decrease in lamina EP was investigated by sampling leaves opposite basal clusters at various times after bloom. Lamina EP decreased significantly between bloom and week four in both P-treated and control vines but did not change significantly thereafter (Fig. 7A). EP on a per-leaf basis (Fig. 7B) and TP (Fig. 7C) also decreased between bloom and week four, similar to EP on a concentration basis. As a result, the EP/TP ratio was unchanged during this period. Therefore, remobilization of P appears to be responsible for the decrease in basal lamina EP during the postbloom period. Although the remobilization of inorganic P from high P tissue to low P tissue is a known response to P deficiency, there was a similar decline of lamina EP in vines of high and low P status (69% and 62%, respectively).

Similar decreases in petiole EP of 'Chenin blanc' (5) and 'Thompson Seedless' petiole TP (4) have been reported between bloom and veraison, but this may not be the case for all grape cultivars (5). In contrast, Conradie (6) reported that, in vines grown under nonlimited P conditions, the total P content of leaves increased between bloom and veraison, but he did not separate basal (mature) leaves from other (growing) leaves. Hence, the postbloom increase in P content (6) may have been the net result of continued P uptake by roots as well as redistribution of P to growing leaves from mature leaves and from other vine parts. The P content of leaves decreased between veraison and harvest, whereas the P content of clusters increased (6), which suggests that P moved from leaves to fruit.

Although the coefficient of variability was reduced in both the control (19%) and P-treated (10%) vines between bloom and week eight, it appears that due to remobilization of EP from basal lamina and due to the smaller differences between P treatments that occur late in the season (Fig. 7A), the best time to determine the P status of grapevines is at bloom. In addition to nutrient status and sample variability, sampling date should also take into consideration insect and disease damage, and the effects of water stress and senesence on leaf condition. Bloomtime leaf samples are usually in better condition than samples taken later in the season. Critical levels for other elements have also been established for bloomtime samples, thereby reducing the total number of samples required to assess the overall nutrient status of a vineyard. Samples taken late in the season may, however, be used to detect differences in P-treated and control vines.

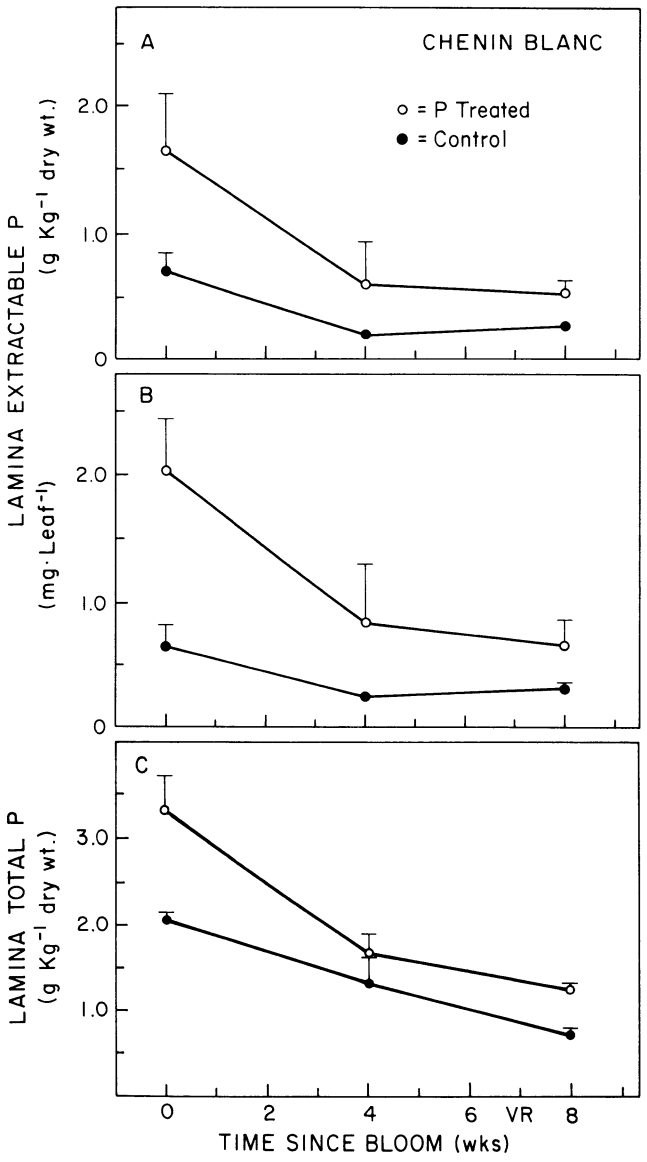

Fig. 7. Extractable phosphorus concentration (**A**), content (**B**), and total P concentration (**C**) for 'Chenin blanc' leaves sampled at various times from bloom through veraison (VR). Data are mean of three replicates ± SE. SE is smaller than symbol when error bar is not shown.

In addition to accurate determination of tissue P levels and to proper sampling techniques, it is essential that any assay of P status of perennial fruit crops is correlated with plant growth and yield parameters. Therefore, vine growth and yield components of P-treated and control 'Chenin blanc' vines were determined. Under P-deficient conditions, the inorganic P pool was depleted and vegetative growth reduced. This reduction is indicated by the low EP/TP ratio in lamina and low pruning weights (Fig. 8). As a result of P fertilizer applications, the EP/

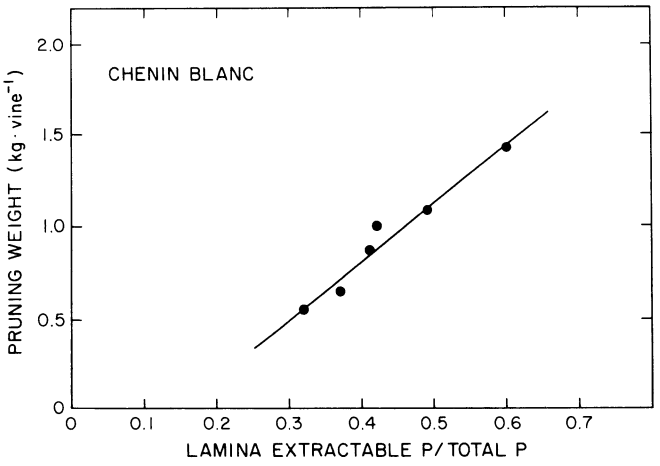

Fig. 8. The relationship between bloomtime lamina EP/TP and pruning weight of vines in which P status had been varied with P fertilizer applications.

Table 1. 'Chenin blanc' lamina EP, berry weight, and yield for control and P-treated vines.

P treatment (kg P/ vine)	Lamina EP (g P/kg dry wt)	Berry wt (g)	Yield/vine (kg)
0	0.71 ± 0.078[z]	1.32 ± 0.11	2.3 ± 0.66
0.05	0.89 ± 0.333	1.29 ± 0.08	5.0 ± 0.05
0.14	1.64 ± 0.267	1.51 ± 0.03	5.1 ± 1.80

[z] Mean ± SE of three samples.

TP ratio increased from 0.3 to 0.6 and pruning weight increased from 0.5 to 1.5 kg/vine (Fig. 8). The increased P status also was associated with increased yields. As lamina EP increased from 0.71 to 1.64 g P/kg dry weight, berry weight increased from 1.32 to 1.51 g/berry, and yield increased from 2.3 to 5.1 kg/vine (Table 1).

In summary, the results of this study have shown that the acetic acid extraction of P from grape leaves is an analytically sound and sensitive method for the determination of P status of grapevines. The absorbance spectra for solutions of the HPM complex developed from standards and from leaf extracts indicated absorbance peaks at 710 and 882 nm. Both A_{710} and A_{882} provide sensitive measures of leaf EP with this method. Lamina EP appears to be a more sensitive indicator of whole vine P status than petiole EP due to a larger signal; however, both lamina and petiole EP responded to P treatments. Lamina EP levels at anthesis are more likely to separate P-sufficient from P-deficient vines than EP levels established later in the season, although EP levels late in the season may be used to determine vine response to P application. Finally, lamina EP levels at anthesis were increased by P application and were associated with increased yield and pruning weight of vines.

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Influence of Drought Stress and Mycorrhizae on Growth of Two Native Forbs

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Abstract. **Seedlings of *Baptisia australis* (L.) R. Br. and *Liatris aspera* Michx., grown in prairie soil with no additional P, benefited significantly from inoculation with *Glomus etunicatum* Becker and Gerd., regardless of whether they were adequately watered or moderately or severely drought-stressed. In the presence of additional P, growth of severely droughted inoculated seedlings for both plant species was not significantly greater than noninoculated plants. When the influence of four *Glomus* species on growth of the two forbs was compared under drought-stress conditions with no supplemental P, growth of both plant species was signficantly improved by all fungal species compared to noninoculated controls. Preinoculated seedlings of both plant species were transplanted into disturbed-site soils with indigenous vesicular–arbuscular mycorrhizal (VAM) fungi present and subjected to severe moisture stress. After 12 weeks, inoculated seedlings were significantly larger than noninoculated seedlings for all soil types, with or without additional fertilizer (0.15 kg P/m^3 + 0.075 kg N/m^3). Under conditions of drought stress and low fertility, preinoculated seedlings of both *B. australis* and *L. aspera* grew significantly larger than noninoculated seedlings.**

Native forbs (wildflowers) are desirable for use in low-maintenance landscapes because they are adapted to climatic stress, resist weed competition and injury from insects and diseases, and reduce labor and material costs. Evolving under conditions of strong competition with grasses, initial growth of forbs is slow, with investment of more tissue in roots than aboveground foliage (6). Due to this deep root system, forbs are difficult to transplant from a native to a landscape setting, a factor, along with slow seedling growth, that makes introduction of these species to the landscape industry difficult.

The use of container-grown transplants is desirable for landscape establishment, especially for these deep-rooted forb species. Preinoculation with VAM fungi has been shown to increase transplant survival and regrowth of several container-grown horticulture plants (19), at least in part due to absorption and translocation of immobile nutrients by the fungi, thereby improving the nutrient status of the host plant (13). This growth enhancement may be even more pronounced in plants having a taproot system common to many native forbs (2), because feeder roots of these species are found below the zone of decomposition in the zone of reduced available nutrients. Forb roots have been found to be mycorrhizal to depths of 210 cm (27), possibly suggesting a dependency on VAM fungi for nutrient absorption.

VAM fungi also improve the water relations of host plants (19). It has been suggested these mycorrhizal effects on host plant–water relations depend on host plant nutrition and the fertility level of the soil (1, 21, 23). At low soil P levels, mycorrhizal fungi may improve plant water relations (21, 23), while at high P levels this effect was small or eliminated (18, 21, 23). In low-maintenance landscapes, where low fertility and water stress are limiting factors, VAM fungi may play a key role in landscape plant establishment and survival.

Transplant survival, regrowth, winter survival, and inflorescence count of several wildflower species can be improved with preinoculation with VAM fungi under adequately watered, low-fertility conditions (28). This study examines whether a) VAM fungi improve the drought tolerance of two native perennial forbs, b) this influence is affected by different fungal species, and c) any growth benefits from pretransplant inoculation with VAM fungi will be evident after transplanting into disturbed soils containing indigenous VAM fungi.

Materials and Methods

Acid-scarified seeds (soaked in 32.4 N sulfuric acid for 1 hr) of *B. australis* (blue wild indigo, BWI) and stratified seeds (stored in cold, moist storage for 8 weeks) of *L. aspera* (rough gayfeather, RG) were germinated in vermiculite for use in the following studies.

The first two experiments described were harvested after 12 weeks, while the final study was maintained for 16 weeks to maximize growth differences among treatments. All experiments were conducted in a 25° day, 15°C night greenhouse and arranged in a completely randomized design. At harvest, top, root, and total dry weights were determined. The dried roots were stained in trypan blue (22) and examined microscopically to assess percentage of root colonization and colonization intensity (16). An analysis of variance ($P = 0.05$) was performed

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