Influence of Potassium Deficiency and Temporary Potassium Deficiency on Nitrogen Metabolism in Leaves and Berries of Wine Grapes

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Executive Summary

The aim of the proposed research was to study several sites with a history of potassium deficiency using a rapid screening procedure for putrescine levels that we developed in previous work on this project. The objective was to validate our screening procedure and provide recommendations regarding sampling time so that putrescine screening can be used routinely for managing potassium deficient vineyards.

During the 1992 season we analyzed leaf samples for putrescine by HPLC and a recently developed TLC screening procedure that increased sample throughput by an order of magnitude. Results show that our screening procedure can certainly be used for Cabernet sauvignon and Chardonnay and can probably be used with other varieties. The screening procedure can be used to detect elevated putrescine in leaves at bloom whether or not leaf symptoms are present. The screening procedure is equally useful at veraison and after harvest. In all cases when samples were scored by TLC as having increased putrescine, the result was confirmed by HPLC. False positives and false negatives were not observed. Putrescine in leaves as estimated by the screening procedure correlated well with a subjective evaluation of the degree of potassium deficiency suffered by the vine.

Results obtained during the 92/93 season served to validate of our screening procedure. We can now provide specific recommendations regarding sampling time so that putrescine screening can be effectively used for managing potassium deficient sites. In addition we have identified several "ideal" locations to apply the procedures we have developed. Interestingly, we found one site in our study that did not fit the typical potassium deficiency syndrome. This site provides an opportunity to characterize other disorders besides potassium deficiency that can lead to increased putrescine levels in the leaves.

Final Report to American Vineyard Foundation

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Validation of the TLC Screening Procedure

Figure 1

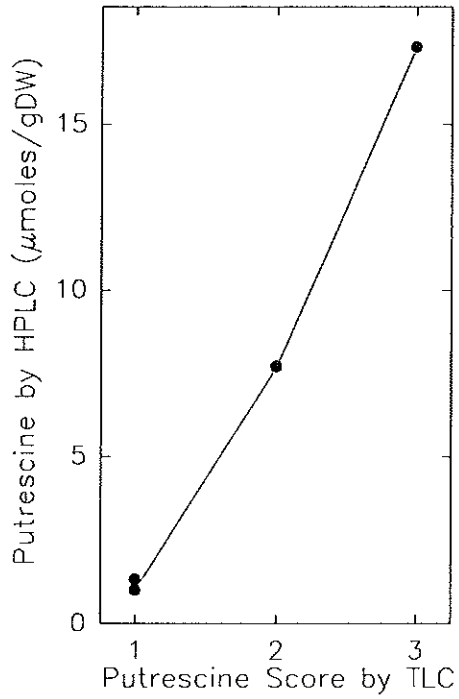
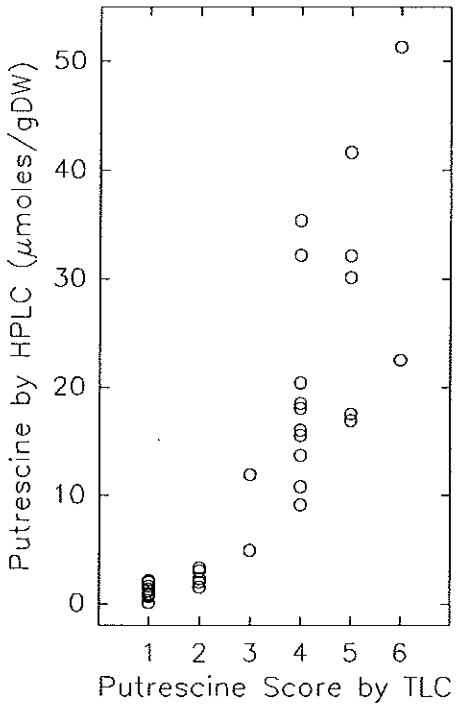
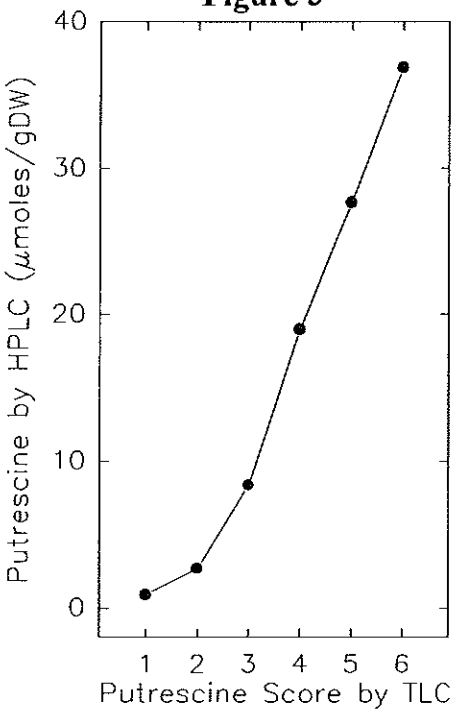


Figure 2



In order to validate our screening procedure we needed to compare TLC spot ratings with actual putrescine values as determined by HPLC. We made such a comparison at several times during the season. Figure 1 shows the relationship between putrescine rating (1 to 3) as determined by TLC and the actual values obtained by HPLC analysis for 4 leaf samples taken at bloom from a potassium deficient Cabernet sauvignon site. In this case (as in all cases) there was a good correlation between the rating given by TLC analysis and the actual putrescine value in the leaf as determined by HPLC analysis. At a second site sampled more extensively postharvest we observed a similar relationship (Figure 2). In this example many more data points were used to establish the relationship and spots given the same rating by TLC exhibited a range of values by HPLC. In this analysis the putrescine levels observed on the TLC plates were given a rating of 1 to 6 based on the intensity of the spot rather than the 1 to 3 scale used in Figure 1. The number of classes into which the TLC spots are separated is arbitrary, but if too many classes are used the method offers little discrimination. This is not a limitation on the method since there were no false positives nor false negatives in this data set. This is an important feature of any screening procedure, *i.e.* that false positives are not generated and that samples with elevated levels are not overlooked. Despite the overlap between classes seen in Figure 2, in all instances where putrescine was normal by HPLC the spot was rated 1 or 2 by TLC. Furthermore, when putrescine was elevated the spots were rated 3,4,5 or 6 by TLC. In this example the highest putrescine level was 50 times normal and it is not critical for a vineyard manager

Figure 3



to know if the putrescine in the leaves is 50 times normal or only 20 times the usual value. In either case there is potassium deficiency that warrants treatment. This example clearly shows the utility of the TLC screening procedure. Figure 2 shows all of the data points obtained at this particular site and Figure 3 shows the average putrescine for the range of values obtained by HPLC.

Timing of Sampling for Putrescine Analysis

Table 1

SAMPLE	PUTRESCINE (µMOLES/gDW)	FOLD ELEVATED
Normal 1991	3.8	-
Moderate 1991	37.3	9.3
Severe 1991	24.5	6.3
Normal 1992	2.3	-
"Symptoms" 1992	5.1	2.2

A vineyard that had been studied during the 1991 season was resampled during bloom in 1992. During the 1991 season the vineyard exhibited putrescine values from 6 to 9 fold elevated. Potassium fertilization was initiated after the 1991 sampling and the vineyard was resampled at 80% bloom during the 1992 season. In the region of the vineyard that was normal during the 1991 season the putrescine value was 2.3 µmoles per gram of dried leaf. Samples taken from the region of the vineyard where moderate or severe symptoms had been observed in 1991 showed putrescine levels 2.2 times higher, despite the observation that leaves in the "symptoms" area **did not** yet exhibit potassium deficiency. Vines in that region of the vineyard subsequently developed symptoms. This result shows that resampling problem sites at bloom can uncover elevated putrescine levels prior to the onset of symptoms.

Elevated Putrescine in Different Varieties at Bloom

Table 2

SAMPLE	PUTRESCINE (μMOLES/gDW)	FOLD ELEVATED
Normal Chardonnay 85%Bloom	1.33	
Symptom Chardonnay 85% Bloom	7.69	5.7
Normal Cabernet 25% Bloom	1.01	
Symptom Cabernet 25% Bloom	17.3	17.1

Our previous study of putrescine and potassium deficiency at bloom was confined to Cabernet sauvignon. During the 1992 season we compared a potassium deficient Cabernet sauvignon site and a nearby Chardonnay vineyard showing leaf symptoms of potassium deficiency. The results are shown in Table 2. In both cases symptoms began to appear at bloom and the results show that putrescine was already elevated in both varieties. The combined results from Table 1 and Table 2 show that bloom time sampling can reveal elevated putrescine in leaves from potassium deficient areas whether or not symptoms are present. This is an important result for application of our research to vineyard management where potassium deficiency is chronic or suspected. Our results are applicable to Cabernet sauvignon and Chardonnay; and we believe they could be shown to be useful in other varieties as well.

Correlation of Vine Symptom Rating and Putrescine Screening Value in Vines with Potassium Deficiency Sampled Postharvest

Table 3

Vine Score	Putrescine Score
0	1
2	2
2	4
2	3
2	3
0	1
2	2
2	3
1	1
1	2
0	1
3	3
3	3
2	2
2	3
0	1
2	4
2	4
2	4
2	4

In a cooperative experiment with Mary Bianchi, Farm Advisor San Luis Obispo County, we compared vine symptom ratings and values assigned to analysis of leaf samples by our putrescine TLC screening procedure. The purpose of this work was to examine the relationship between a vine rating system used to assess severity of potassium deficiency, and the amount of putrescine found in leaf samples as evaluated by TLC screening. Mary collected 20 leaf samples from individual Cabernet sauvignon vines, and at the same time rated the vine for the severity of potassium deficiency. The vine rating is subjective and based on a scale of 0 to 3 depending on the degree of leaf symptoms and vine stunting, zero being normal. The putrescine rating was based on a scale of 1 to 4 depending upon the intensity of the putrescine spot. The

experiment was done "blind" *i.e.* the person doing the putrescine rating was unaware of the value Mary had assigned to the vine. After the putrescine values were obtained the data was collated into Table 3. In all cases where vines were rated with no potassium deficiency (*i.e.* a Vine Rating of 0), leaves exhibited normal putrescine levels (*i.e.* a Putrescine Score of 1). With one exception (sample 9) vines given a rating reflecting some degree of potassium deficiency showed elevated putrescine in the leaf sample. Vine 9 was given a rating of 1 by Mary but evaluated as having normal putrescine by the TLC method. All vines given a rating of 2 or 3 showed elevated putrescine in the leaves by TLC. This result clearly shows that vines suspected of suffering potassium deficiency can be rapidly and reliably confirmed as having elevated putrescine in the leaves. In the absence of elevated putrescine, potassium deficiency can be ruled out of the diagnosis.

A Case of Elevated Leaf Putrescine Not Typical of Potassium Deficiency

Figure 4

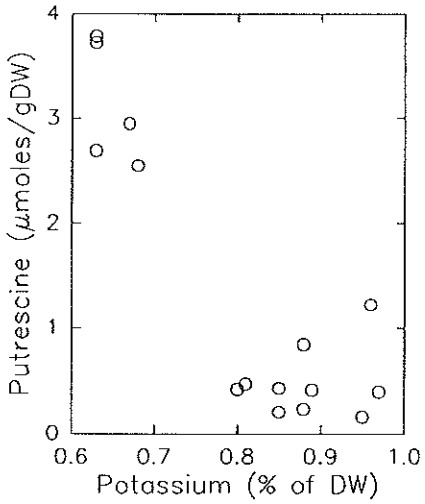
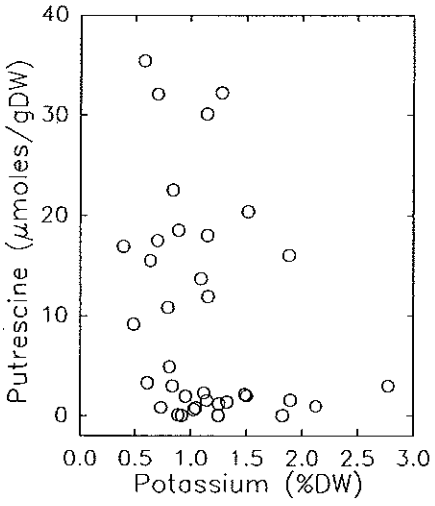


Figure 5



We have studied several potassium deficiency sites in both Cabernet sauvignon and Chardonnay. Whenever we have compared leaf putrescine with the potassium level found in the leaf, we have seen the typical relationship shown in Figure 4. Above some threshold value of potassium in the leaves the putrescine is normal, and below the threshold the putrescine is elevated. During the 1992 season we studied a Chardonnay site brought to our attention by Rhonda Smith, Farm Advisor Sonoma County. This site had leaf symptoms typical of potassium deficiency in one segment of the vineyard, and by screening we confirmed that putrescine in the leaves was indeed elevated. However, when we measured the potassium in the leaves and constructed a correlation between leaf potassium and putrescine we found the results shown in Figure 5. This is a very unusual relationship between leaf potassium and leaf putrescine. In some cases the putrescine is elevated but the potassium level is considered normal. In other cases the potassium seems low but the putrescine is not elevated. We must

emphasize that this example stands alone in all of the sites we have studied where this kind of pattern has emerged. We currently believe that this is not a simple case of potassium deficiency, and that one or more other conditions may exist in the vineyard that contributes to elevated putrescine in the leaves and the associated foliar symptoms. As part of this project we plan to re-sample this site at bloom in order to further characterize this situation.

This unusual site is perhaps the exception that illustrates the usefulness of the screening procedure we have developed in the course of this project. A truly potassium deficient site shows a characteristic relationship between putrescine in the leaves and leaf potassium (Figure 4). As suggested by the work during the 1992/93 project, this relationship can be useful for diagnosing potassium deficiency and monitoring the effectiveness of treatments to alleviate the condition.

Our screening procedure can certainly be used for Cabernet sauvignon and Chardonnay and can probably be used with other varieties. The screening procedure can be used to detect elevated putrescine in leaves at bloom whether or not leaf symptoms are present. The screening procedure is equally useful at veraison and after harvest. In all cases when samples were scored by TLC as having increased putrescine the result was confirmed by HPLC as being elevated. False positives and false negatives were not observed. Putrescine in leaves as estimated by the screening procedure correlated well with a subjective evaluation of the degree of potassium deficiency suffered by the vine.

Outside Presentations of Research

1. This work was presented at a seminar entitled *Recent Advances in Viticulture and Enology* conducted by the Department of Viticulture and Enology at U.C. Davis on April 1, 1993.

2. An abstract based on this work was submitted and accepted for an oral presentation at the annual meeting of the American Society for Enology and Viticulture to be held in Sacramento, CA on June 22-25, 1993.