Pyruvate Kinase Activity and Protein Concentration in 'd'Anjou' Pear Fruit with Cork Spot

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Abstract. An assay for pyruvate kinase (PK) was tested as a diagnostic tool for cork spot, a major physiological disorder in pear *Pyrus communis* L. cv. d'Anjou fruit. PK activity and Ca and protein concentrations were measured in peel of normal and affected fruit during selected months in 2 years. Protein concentration was more closely associated with cork spot than PK activity or Ca concentration. These preliminary results suggested the PK assay was a poor diagnostic tool for cork spot.

Cork spot is one of the major disorders in 'd'Anjou' pear fruit in the United States. It is assumed to be similar to bitter pit in apple and blossom-end rot in tomato and watermelon (Shear, 1975). Cork spot is generally linked with low Ca concentration in the fruit (Mason and Welsh, 1970; Raese, 1986, 1988; Richardson and Al-Ani, 1982; Richardson and Lombard, 1979; Woodbridge, 1971). Although Ca is the mineral element most often related to this fruit disorder, the results of fruit Ca analysis can sometimes vary widely and, therefore, cannot be used reliably to diagnose the problem (Bevacqua, 1989; Kupferman, 1988; Raese, 1986). The lack of a reliable analytical technique led to a search for an alternative method of identifying cork spot. The present study evaluated the feasibility of assaying pyruvate kinase (EC 2.7.1.40; PK) activity as a diagnostic tool. PK activity, Ca concentration, and protein concentration were compared to see which measurement was most closely associated with cork spot and, thus, might possess the greater value as a diagnostic tool for the disorder.

The use of a PK assay as an indicator for Ca-related disorders in fruit crops was proposed by Bar-Akiva et al. (1976). Calcium is an inhibitor of PK activity (Lavon et al., 1988a, 1988b; Tomlinson and Turner, 1973) and Bar-Akiva et al. (1976) showed that Ca

deficiency in citrus caused an increase in PK activity.

Fruit samples were collected from six mature trees at the Mid-Columbia Agricultural Research and Extension Center (Table 1). Three of the trees were selected because of their histories of producing normal or marketable fruit. The other three trees were chosen because they had histories of producing fruit with a high incidence of the disorder. In 1988 and 1989, a tree prone to cork spot, designated K6, produced relatively normal harvests and was reclassified as a normal tree for those years (Table 1).

Samples designated as normal (N) were always prepared from fruit completely free of cork spot symptoms from trees with a low incidence of the disorder. Samples designated as cork spotted (CS) were prepared from fruit with obvious symptoms of cork spot that were from trees with a high incidence of the problem. An exception to this was the CS samples from May, June, July, and August of the 1988 crop year. Since cork spot symptoms emerged 21 days before harvest (7 Sept.) in 1988, the samples collected before that time possessed no outward symptoms and were simply fruit from trees that

historically produced a high incidence of cork spot.

We selected six mature fruit or an equivalent mass of fruitlets from the same tree to form one sample. Fruit was collected from different heights and from all four sides of the tree. Fruit was stored at $-0.5 (\pm 0.5)$ between harvest and analysis.

The fruit were rinsed with distilled water and wiped dry. The peel of the calyx half of the fruit was removed with a paring knife, chopped into 4.0-mm² pieces, and immediately covered with a cloth wetted with 0.5% (w/v) Na₂S₂O₅ solution to minimize oxidation. The tissue first was frozen at -20°C for 12 hr and then freeze-dried for 6 days. Fresh and dry weights were recorded for each sample. The sample was rendered into powder and stored in plastic bags at -20°C . Sample collection was replicated three times.

Lyophilized tissue (0.5 g) was homogenized in a mortar and pestle with 0.5 g insoluble polyvinyl polypyrrolidone, 1.5 g white quartz sand, and 10.0 ml of an extraction buffer that consisted of 50 mM imidazole-HCl (pH 7.0), 3 mM dithiothreitol, 5 mM ascorbic acid, 3 mM Na₂S₂O₅, 14 mM 2-mercaptoethanol, 1 mM EDTA, and 20% (v/v) glycerol. The macerated tissue was squeezed through two layers of Miracloth and centrifuged at $37,000 \times g$ for 20 min at 20°C . The supernatant was retained as the crude extract for the enzyme assay. The extraction procedure was performed at 20°C .

PK activity was assayed by the colorimetric measurement of the pyruvate produced from phosphoenolpyruvate in the presence of adenosine - 5' - diphosphate (Bevacqua, 1989; Boyer, 1973; Lavon et al., 1988a, 1988b). The results were analyzed as specific activity (nmol pyruvate/min per milligram protein) or total activity (nmol pyruvate/min per gram dry weight or fresh weight).

The samples were analyzed for Ca concentration by an inductively coupled argon plasma apparatus. The 1988 postharvest samples were analyzed by atomic absorption spectrophotometer. Results were expressed as parts per million dry weight.

Soluble protein levels in the crude extract were assayed by Peterson's modification of the micro-Lowry method with precipitation

Table 1. Yield and incidence of cork spot at harvest for sample trees for 1987 through 1989².

Criterion	Tree designation					
	E5	F20	K13	K6	P6	P7
1987						
Yield (kg)	227	293	259	20	56	58
Total fruit (no.)	970	1320	1110	73	264	253
Cork spot (%)	1	1	2	36	23	24
1988						
Yield (kg)	270	117	161	171	294	150
Total fruit (no.)	1317	456	627	687	1542	749
Cork spot (%)	1	5	4	7	15	23
1989						
Yield (kg)	215	157	176	160	167	99
Total fruit (no.)	1157	781	925	814	1085	579
Cork spot (%)	4	6	2	6	21	41

¹In 1987, trees E5, F20, and K13 were classified as normal (N) and trees K6, P6, and P7 were classified as prone to cork spot (CS). In 1988 and 1989, tree K6 produced relatively normal harvests and was reclassified as a normal tree for those years.

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Table 2. Total activity of pyruvate kinase (PK), and Ca and protein concentrations in 'd'Anjou' pear peel from normal (N) and cork spotted (CS) samples for selected months in 1987 and 1988.

Crop yr	Time of analysis (month)	Total PK activity (nmol pyruvate/min per gram dry wt × 1000)		Ca concn (ppm dry wt)		Protein concn (mg·g ⁻¹ dry wt)	
		N	CS	N	CS	N	CS
1987	August	0.18	0.55	416	347	4.69	5.92
1987	January	0.45	0.42	648	491*	2.71	3.69
1987	February	0.76	0.66	648	580	3.08	2.71
1987	May	0.41	0.58	na	na	4.44	5.43
1988	May	3.28	3.33	5280	5410	13.14	13.77
1988	June	1.38	2.07*	3510	4780	4.43	6.42**
1988	July	1.00	1.39	1350	1470	3.33	5.68**
1988	August	1.26	1.47	909	1010	4.46	5.97**
1988	September	1.23	1.04	565	850*	4.88	5.92**
1988	November [†]	2.34	2.62*	1130	1530	5.54	8.82**
1988	December [†]	2.34	2.11	1430	1380	4.56	7.04**
1988	January [†]	2.06	1.91	1300	1690	7.35	6.78

[†]Results for November, December, and January of 1988 crop year are based on a comparison of fruit from two sample trees E5 and P7, whereas the earlier results are based on a comparison of fruit from six sample trees (Table 1).

(Peterson, 1977). The results were analyzed as milligrams per milliliter, milligrams per gram dry weight, and milligrams per gram fresh weight.

Results were statistically analyzed separately for each month. Monthly means for N and CS samples were calculated from three to 12 measurements. A *t* test (Sokal and Rohlf, 1981) was used to test for significant differences between the means for N and CS samples in a given month.

Total PK activity was measurably different between N and CS fruit in June and November of the 1988 cropping season, but not in any of the other 10 months in which the assays were performed (Table 2). These results were the same whether total activity was expressed on a dry- or fresh-weight basis. Specific activity was eliminated as a diagnostic measure for cork spot because the relationship between N and CS fruit reversed from the 1988 harvest (August) to the 1989 harvest (September). Thus, PK activity, regardless of the units of measurement, appears a poor candidate for diagnosing cork spot.

Calcium concentration was lower in CS fruit than in N fruit in January of the 1987 crop year. However, Ca concentration was higher in CS fruit than in N fruit in September of the 1988 crop year (Table 2). These variable results and the inability to detect measurable differences in the other months of the 1987 and 1988 crop years clearly illustrate the weakness of using Ca analysis to diagnose cork spot.

Protein concentration (mg·g⁻¹ dry weight) provided the greatest measurable difference between N and CS fruit for the most months in the 1987 and 1988 crop years. There were strong positive correlations between the re-

sults based on the above unit and those based on milligrams per milliliter ($r = 0.91$) milligrams per gram fresh weight ($r = 0.94$), indicating that regardless of the units of measurement, the assay for protein concentration yielded similar results. Protein concentration was higher in disordered fruit than in N fruit in June through September and in November and December of the 1988 crop year (Table 2). Protein concentration expressed as milligrams per milliliter was also higher in affected fruit than in N fruit in Aug. 1987.

The specific activity of an enzyme is expressed on a protein basis. It was for this reason that protein concentration was assayed in each sample. This led to the unexpected but important finding that CS fruit generally contained higher protein concentrations than N fruit. It was a pattern generally free of the wide variability, reversals, or lack of measurable differences often associated with fruit Ca analysis or the PK assays. High protein content has also been reported for apple fruit with bitter pit and other Ca-related disorders, such as internal breakdown (Faust and Shear, 1968, 1969). The symptoms of cork spot first became apparent in our sample trees 21 days before harvest in the 1988 crop year. Yet, the protein assay detected differences between fruit from normal trees and fruit from trees prone to cork spot as early as June, i.e., 3 months before harvest (Table 2). Faust and Shear (1968) reported that apple tissue with corking disorders had higher protein content than normal tissue and that the increase had occurred by the time the first visible signs of the disorder developed. The present results, although based on few trees, and the earlier ones indicate that the assay for protein concentration merits further study for its poten-

tial to diagnose or predict cork spot early in the growing season.

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