

Fruit Phosphorous and Nitrogen Deficiencies Affect ‘Grand Pearl’ Nectarine Flesh Browning

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Abstract. Fruit flesh browning (FB) is a major component of cold storage disorders that limits fresh and fresh cut fruit consumption. Using fertigation, nutrient deficiencies were imposed on ‘Grand Pearl’ nectarines (*Prunus persica* var. *nectarina*) grown in sand culture for 8 years and postharvest flesh browning was studied over 2 years. Antioxidant activity, polyphenol oxidase activity, total phenolics, and fruit FB potential were evaluated. Nutrient deficiencies did not always result in leaf or fruit tissue deficiency, indicating complex interactions among nutrients during uptake and use in the plant and its fruit. Low phosphorus and nitrogen fruit concentrations were associated with biochemical browning reactions in fruit flesh at harvest and with fruit FB during storage, signs of a shorter market life and lower consumer quality. Currently recommended leaf and fruit nutrient critical values are based only on production and do not address postharvest quality. Further research is needed to determine new recommended leaf and fruit nutrient values suitable for both production and maintaining fruit quality during storage.

Susceptibility of stone fruits to chilling injury (CI) depends on genotype, maturity, storage temperature, length of storage, and preharvest and postharvest manipulation (Lurie and Crisosto, 2005). CI symptoms include lack of juiciness (flesh mealiness or woolliness), FB, black pit cavity, flesh translucency (gel breakdown), red pigment accumulation (bleeding), lack of flavor, and failure to ripen (Lurie and Crisosto, 2005). Nectarine FB is a genetic disorder that can be triggered by a combination of factors such as cultural practices (soil fertilization, irrigation management), postharvest temperature, and storage and shipping processes (Crisosto et al., 1999; Mitchell, 1987). Mineral nutrition is critical for tree fruit growth and production (Johnson and Uriu, 1989), but little is known about the role of nutrition in cold storage disorders, CI expression in fresh cut fruit, and fruit consumption by consumers. Fruit nutrition research has mainly focused on optimizing tree growth and yield with limited attention paid to its effect on stone fruit quality (Crisosto et al., 1997). Studies on apples (Bramlage et al., 1980; Crisosto et al., 1997) and plums (Kotze

et al., 1987) reported an influence of nutrient deficiencies on internal breakdown. Therefore, further research on the influence of preharvest manipulations such as mineral nutrition, irrigation, and cropload on stone fruit postharvest quality and cold storage performance is needed.

Fruit tissue enzymatic browning is a consequence of oxidative degradation of phenolic compounds by polyphenol oxidase (PPO), which leads to production of quinones that polymerize rapidly to form brown-colored products (Kader and Chordas, 1984). The concentration of phenolic compounds, the activity of PPO, and the presence of available oxygen are strongly related to enzymatic browning (Mayer and Harel, 1979). Phenolic composition and concentration are important contributors to fruit antioxidant capacity, which is highly desirable, because it provides several benefits to human health (Robards et al., 1999). In addition, phenolic compounds, in combination with other factors, can improve brown rot (*Monilinia fructicola*) resistance in peaches by acting directly on cutinase and preventing the penetration of this fungal infection within the fruit flesh (Bostock et al., 1999).

Flesh browning can be triggered by fruit bruising; by exposure to oxygen in fresh cut, sliced, and pulped forms; or by thawing fruit after prolonged freezing. In addition, lye-peeling of canned fruit also strongly influences the browning potential of the fruit

(Vamosvgyazo, 1981). Thus, fruit handling and processing are critical factors underlying browning potential, because these activities can damage cell structure. Enzymatic FB is a major component of postharvest CI problems in stone fruit. It is also a commercial problem, because browning can affect fruit quality in fresh or canned forms. Any enzymatic browning is a major industrial concern because the altered sensory and visual characteristics are unattractive to consumers in addition to the quality loss resulting from decreased concentrations of phenolic compounds. It is highly desirable, therefore, to have fruit with high concentrations of phenolic compounds but low postharvest FB during and after cold storage (Ogundiwin et al., 2008). A high concentration of antioxidants such as ascorbic acid is also highly desirable in fruits, because it may prevent some degree of enzymatic browning by inhibiting PPO activity (Teisson, 1972). Furthermore, quinone compounds can be reduced by ascorbic acids, regenerating phenolics and inhibiting browning.

Some stone fruit species are more susceptible to FB than others. Some peach and nectarine cultivars from California breeding programs are highly susceptible to FB, reducing their storage potential (Crisosto et al., 2008, 2009). This study relates nutrient deficiencies to FB and its precursors phenolic concentration, PPO activity, and antioxidant concentration in a high FB, susceptible cultivar. We hypothesize that low concentrations of nutrients and phosphorus (P) will lead to greater FB by modifying the membrane permeability to make browning reaction substrates more readily available.

Materials and Methods

Field setting. In 2000, 60 large tanks, 3.35 m long, 2.44 m wide, and 1.22 m tall (10,000 L), were installed at the Kearney Agricultural Research Center to study the nutrition of stone fruit. Each tank was filled with sand to enable accurate control of the nutrients applied and deficiencies imposed.

A ‘Grand Pearl’ nectarine tree was placed in each tank and trained to a perpendicular V system to ensure a uniform shape. Four different fertilization treatments were imposed for 8 years using a drip irrigation system with two emitters per tank. The treatments were a fully fertilized control, low nitrogen (N), low P, and low potassium (K) with four replications per treatment. The nutrient solutions were based on Hoagland’s solution (Johnson and Crisosto, 2003).

Plant material. In 2008 and 2009, fruit were collected from each tree as they reached commercial maturity based on ground color and firmness. Fruit size, soluble solids concentration (SSC), and titratable acidity (TA) were determined at harvest. In 2009 only, fruit was stored for 11 d at 5 °C for later quality evaluation. After storage, fruit was evaluated for internal breakdown symptoms and other disorders (Crisosto et al., 1994). Internal breakdown (CI) symptoms were classified as FB or bleeding as described (Crisosto et al., 1999). These observations were made on the

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mesocarp around the pit after the fruit was cut transversely along the plane of the suture.

Leaves from each tree were collected in mid-July of both seasons and analyzed for total N using the combustion method and by nitric acid digestion for the rest of the elements according to the UC Davis analytical laboratory protocols (A.O.A.C., I, 2006; Meyer and Keliher, 1992). Approximately 10 fruits from each treatment/tree combination were collected, carefully washed, cut, and stored frozen at -80°C . The fruit material was then ground into a fine-textured powder in liquid N. Fruit nutrients were analyzed only in 2009 according to UC Davis analytical laboratory protocols using total Kjeldahl N for N (Horneck and Miller, 1998; Isaac and Johnson, 1976) and nitric acid digestion for the rest of the elements (Meyer and Keliher, 1992).

Polyphenol oxidase activity measurements. PPO measurements were determined as described (Cheng and Crisosto, 1995). A sample of 0.5 g finely ground fruit material was extracted with 1 mL ice-cold extraction buffer (0.2 M sodium phosphate buffer, pH 6.2, 1 mM EDTA, 5% w/v PVPP, and 1% Triton X100). The mixture was vortexed and incubated on ice for 1 h and then centrifuged at $17,000 \times g$ for 10 min at 4°C . The supernatant (enzyme extract) was collected, kept on ice, and used immediately for the PPO enzyme assay. The enzyme assay contained 0.8 mL 20 mM catechol in 0.1 M phosphate buffer, pH 6.2, and 0.2 mL crude enzyme extract. The increase in absorbance at 420 nm was recorded from 1 to 4 min after the addition of enzyme extract, at 0.5-min intervals, using a Smartspec-Plus Spectrophotometer (BIO-RAD Laboratories, CA). The reactions were linear within the time range. PPO activity was expressed as $\Delta A_{420} \text{ nm}^{-1} \cdot \text{g}^{-1}$ fresh weight.

Total phenolics and antioxidant extraction. A sample of 0.4 g finely ground fruit powder was extracted with 1 mL 80% methanol. The mixture was incubated at 4°C overnight and then centrifuged for 10 min at $17,000 \times g$ at 4°C to collect the supernatant. The supernatant extracts were stored at -80°C until analysis for total phenolics and antioxidant activity.

Total phenolics determination. Total phenolics were determined by the Folin-Ciocalteu method as described (Singleton and Rossi, 1965) and expressed as gallic acid equivalents ($\mu\text{g GAE/g}$ fresh weight). In addition, absorbance at 280 nm was recorded using a Smartspec-Plus Spectrophotometer (BIO-RAD Laboratories).

Total antioxidants determination. Antioxidant concentration was determined by the DPPH method (Brand-Williams et al., 1995). Fifty microliters sample extract was added to 950 μL 0.1 mM DPPH and kept overnight in the dark. Absorbance at 515 nm was determined using a Smartspec Plus Spectrophotometer (BIO-RAD Laboratories) and compared with a Trolox standard curve. Results were expressed in μg Trolox equivalent antioxidant activity/g fresh weight.

Browning potential determination. A sample of 0.4 g finely ground powder was extracted with 1 mL cold 0.1 M sodium phosphate buffer

(pH 4.0). The homogenate was centrifuged at $17,000 \times g$ at 4°C for 10 min. The absorbance of the supernatant at 420 nm was recorded using a Smartspec Plus Spectrophotometer (BIO-RAD Laboratories) immediately (A0), after 1 h (A1), and after 4 h (A4) incubation at 30°C . Browning potential was calculated as a difference in absorbance (420 nm) for each of two periods: A0 to A1 and A1 to A4 (Cheng and Crisosto, 1995).

Statistical analysis. Data were analyzed as a complete randomized design using the Statistical Analysis System (SAS Institute Inc., Cary, NC) personal computer program for analysis of variance with least significant difference means separation and stepwise regression analyses. Stepwise analysis included those parameters involved in the browning reaction such as total antioxidants, total phenolics, and polyphenol oxidase activity to determine the most important variable influencing browning potential.

Results

Leaf nutrient concentration. During the two seasons, leaf macro- and micronutrient concentrations of nectarine trees growing in the sand tanks were very similar and within the UC Davis recommended range (Johnson and Uriu, 1989). In 2009, K concentrations were similar among treatments, ranging from 0.98% to 1.17% (Table 1). Leaf micronutrient concentrations of nectarine trees growing in the sand

tank were unaffected by any treatment (data not shown). Leaf N concentration was significantly lower in trees from the low N and K treatments, whereas leaf P concentrations were very similar except for the low P treatment. Leaf calcium (Ca) concentration was greatest in the low N treatment and lowest in the low K and P treatments. The control and low N treatments had the highest leaf magnesium concentrations.

Fruit nutrient concentration. The N, K, and Ca concentrations measured in fruit tissues were within the ranges previously published (USDA, 2009). Only fruit from the low P treatment had concentrations below those obtained from a survey of fruit populations (Table 2). Fruit from control trees had the most N followed by fruit from the low P and K treatments, whereas fruit from the low N treatment had the least. Phosphorous concentrations in fruit were affected significantly by the nutrient deficit treatments with the low P treatment having the least P by a significant margin. Potassium and Ca concentrations in fruit were unaffected by any treatment. The low K treatment did not reduce fruit K, P, or Ca concentrations but reduced N within the normal fruit range. The low N treatment significantly reduced fruit N to $\approx 50\%$ of control fruit without affecting fruit K, P, or Ca. The low P treatment reduced fruit P ($\approx 80\%$) and N ($\approx 20\%$) concentrations without reducing those of K or Ca.

Fruit yield and quality. In 2008, only low K and P reduced fruit yield, whereas in 2009, low N also reduced fruit yield (Tables 3 and 4).

Table 1. 'Grand Pearl' nectarine midseason leaf nutrient concentrations for four fertilization treatments during the growing season.

Treatment	N (%)	P (%)	K (%)	Ca (%)	Mg (%)
2008					
Control	3.17 a	0.17 b	0.60 ab	2.49 b	0.96 ab
Low K	2.84 bc	0.23 a	0.46 b	2.32 bc	0.70 c
Low N	2.71 c	0.16 b	0.70 a	3.06 a	1.06 a
Low P	3.11 ab	0.11 c	0.49 b	1.97 c	0.81 bc
Significance Pr > F	0.009	<0.0001	0.01	0.001	0.007
2009					
Control	3.15 a	0.19 b	1.17	2.12 a	0.77 ab
Low K	2.75 b	0.25 a	0.98	2.11 a	0.61 b
Low N	2.46 b	0.16 b	1.28	2.39 a	0.84 a
Low P	3.08 a	0.08 c	1.06	1.46 b	0.61 b
Significance Pr > F	0.001	<0.0001	0.42	0.02	0.03

Mean separation done by least significant difference test at $P = 0.05$. Similar letters within a column indicate no significant difference.

N = nitrogen; P = phosphorus; K = potassium; Ca = calcium; Mg = magnesium.

Table 2. 'Grand Pearl' nectarine fruit nutrient concentrations and flesh browning evaluation at 11 d after storage at 5°C for four soil fertilization treatments during the 2009 growing season.

Treatment	N (%)	P (%)	K (%)	Ca (%)	Flesh browning score (1–6)	Flesh browning (%)
Control	0.33 a	0.050 b	0.36	0.007 ab	1.20 b	19.82 b
Low K	0.27 b	0.060 a	0.32	0.009 a	2.00 a	55.06 a
Low N	0.16 c	0.040 c	0.35	0.006 b	1.90 a	51.75 a
Low P	0.27 b	0.010 d	0.34	0.009 ab	2.07 a	67.08 a
Significance Pr > F	0.001	<0.0001	0.55	0.1	0.001	0.0008

Mean separation done by least significant difference test at $P = 0.05$. Similar letters within a column indicate no significant difference. Browning scores: 1 = none; 2 = very slight browning in the pit cavity; 3 = slight browning in the pit cavity and surrounding tissue; 4 = moderate browning on less than 50% of the flesh; 5 = severe browning on 50% to 75% of the flesh; 6 = extreme browning covering most of the flesh. N = nitrogen; P = phosphorus; K = potassium; Ca = calcium.

Fruit weight was also reduced in fruit from the low K and P treatments but not from the low N treatment. Fruit from the low K and P treatments had a higher SSC than fruit from the control and low N treatments, whereas TA ($\approx 0.3\%$) was unaffected by any treatment. Soluble solids content was higher in 2009 (25.8%, low crop load) than in 2008 (16.1%, high crop load). Fruit cheek firmness was only affected in 2009 by the low N and P treatments.

Cold storage disorders. In 2009, fruit from the low N, P, and K treatments had higher flesh browning incidence expressed as score and percentage. Fruit from deficient treatments had ≈ 3.0 times more fruit with flesh browning ($\approx 50\%$ to 60%) than fruit

from the control treatment ($\approx 20\%$) after storage for 11 d at 5°C (Table 2).

Biochemical measures. Total phenolics and antioxidant activity was not significantly affected by low K or low N (Table 5). However, fruit from the low P treatment had higher total phenolics and antioxidant activity than fruit from the other treatments. During the two seasons, PPO activity was significantly higher in fruit from the low P and K treatments than in the other treatments. In 2009, PPO activity was also greater in fruit from the low N treatment than in the non-nutrient-deprived control. In both seasons, browning potential was higher in fruit from the low P treatment than in fruit from the other treatments.

Discussion

Although most nutrient analyses and recommendations are based on leaf sampling, we focused primarily on fruit tissue concentrations, because these are likelier to provide a sensitive measure that correlates with fruit postharvest disorders such as FB. Reducing specific nutrients provided to the tree using an otherwise complete nutrient mixture applied to the sand tanks did not always reduce the specific targeted nutrient in fruit, indicating complex interactions among nutrient translocation and use within the tree. Subjecting 'Grand Pearl' nectarines to low concentrations of N, P, and K affected fruit biochemistry and visual FB after prolonged cold storage. Our work demonstrated that low P and N affect the intensity and incidence of fruit FB during cold storage, independently of which of these two nutrients was deficient (Table 4). The low P treatment reduced both P and N concentrations in the fruit, whereas the low N treatment decreased fruit P without affecting N. The low K treatment did not affect fruit K and Ca concentrations but did reduce N and P. This highlights the complex nutrient interactions through which limited availability of one nutrient produced imbalances that affected the total fruit nutrient status. From the biochemical analysis, we conclude that low N, K, and P treatments had a significant effect on browning potential precursors in 2009. However, only low P concentration in the fruit had a consistent effect on browning potential and its precursors during both seasons. Unfortunately, fruit nutrient concentrations were not measured in 2008.

Fruit with low P concentrations had increased phenolics concentrations, which act as substrates for the enzyme PPO, which produces quinones that turn tissues brown. In addition, P concentration may have a role in cell membrane permeability and loss of integrity with subsequent destruction of fruit cellular compartmentation (Mayer and Harel, 1979), allowing the phenolic substrates to be more accessible to PPO. Because the substrate (phenolic compounds) and the enzyme (PPO) required for the browning reaction are normally confined in separate cellular compartments (the vacuole and cytoplasm, respectively) (Amiot et al., 1996), the browning reaction does not normally take place. However, when the membrane integrity or permeability of each compartment is disrupted, phenolic compounds become accessible to PPOs and the oxidative reaction takes place, leading to tissue browning. Phosphorous limitation, in combination with other factors such as cold temperature storage, may trigger this situation. Plants can replace membrane phospholipids with galactolipids (non-phosphorous lipids) in response to P-limited conditions (Tjellstrom et al., 2008). However, it is not clear how this replacement affects the integrity and permeability of the membrane and consequent internal disorders. Contrary to what we expected, low P treatments had the most antioxidants, which were supposed to

Table 3. 'Grand Pearl' nectarine yield, fruit fresh weight, red color, soluble solid concentration, and titratable acidity measured at harvest for four soil fertilization treatments during the 2008 growing season.

Treatment	Yield (kg/tree)	Fresh fruit wt (g)	SSC (%)	TA (%)	Cheek firmness (N)
Control	39.81 a	133.80 ab	15.44 b	0.31	59.61
Low K	21.73 bc	116.37 b	16.79 ab	0.30	63.62
Low N	33.16 ab	140.72 a	14.13 b	0.28	55.69
Low P	15.58 c	88.45 c	18.46 a	0.34	62.75
Significance Pr > F	0.0006	0.0009	0.03	0.28	0.47

Mean separation done by least significant difference test at $P = 0.05$. Similar letters within a column indicate no significant difference.

SSC = soluble solids concentration; TA = titratable acidity; K = potassium; N = nitrogen; P = phosphorus.

Table 4. 'Grand Pearl' nectarine yield, fruit fresh weight, red color, soluble solids concentration, and titratable acidity measured at harvest on four soil fertilization treatments during the 2009 growing season.

Treatment	Yield (kg/tree)	Fruit fresh wt (g)	SSC (%)	TA (%)	Cheek firmness (N)
Control	9.77 a	127.08 a	24.52 b	0.35	55.25 a
Low K	3.51 b	86.77 b	27.41 a	0.39	59.22 a
Low N	4.96 b	105.51 a	25.54 ab	0.32	30.28 b
Low P	4.41 b	72.03 b	26.22 a	0.35	38.72 b
Significance Pr > F	0.02	<0.0001	0.02	0.38	0.0005

Mean separation done by LSD test at $P = 0.05$. Similar letters within a column indicate no significant difference.

SSC = soluble solids concentration; TA = titratable acidity; K = potassium; N = nitrogen; P = phosphorus.

Table 5. 'Grand Pearl' nectarine antioxidant activity, total phenolics, PPO activity, and browning potential for four soil fertilization treatments during 2008 and 2009 growing seasons.

Treatment	Antioxidant activity TEAA ($\mu\text{g TE/g FW}$)	Total phenolics ($\mu\text{g GAE/g FW}$)	PPO ($\Delta\text{A420/min/g FW}$)	Browning potential (ΔA420) 1–4 h
2008				
Control	621.02 b	388.33 b	0.53 c	0.13 b
Low K	740.97 b	448.86 b	0.94 ab	0.19 b
Low N	915.99 b	499.65 ab	0.65 bc	0.16 b
Low P	1491.73 a	620.09 a	1.30 a	0.39 a
Significance Pr > F	0.009	0.03	0.006	0.0005
2009				
Control	1691.37 b	752.75 b	1.96 b	0.19 c
Low K	2788.73 ab	1066.78 ab	3.05 a	0.81 ab
Low N	2307.09 b	940.02 b	2.89 ab	0.66 bc
Low P	4871.80 a	1574.22 a	2.41 ab	1.21 a
Significance Pr > F	0.01	0.02	0.09	0.001

Mean separation done by least significant difference test at $P = 0.05$. Similar letters within a column indicate no significant difference.

PPO = polyphenol oxidase; TEAA = Trolox equivalent antioxidant activity; FW = fresh weight; GAE = gallic acid equivalents.

counteract oxidation and retard browning. Because many of the phenolic compounds present in fruit can be antioxidants, oxidation substrates, or both (Amiot et al., 1996), we suspect that most of the antioxidants measured in the fruit flesh were good substrates for the oxidation reaction in addition to being antioxidants. Results from stepwise regression analysis (data not shown) demonstrated that antioxidant capacity and PPO were the most important factors in the FB reaction, suggesting that most of the antioxidants measured were phenolic compounds acting as PPO substrates.

Although the fruit and leaf nutrient concentrations among soil fertilization treatments were similar to the recommended and expected concentrations, differences were observed. Midsummer leaf P values below 0.1% are considered deficient in peach and nectarine trees, but currently recommended leaf concentrations are mostly based on production measures (yield and size) (Johnson and Uriu, 1989). Leaf critical values obtained in all treatments were lower than the values recommended for plums in South Africa, where midsummer leaf values of at least 0.22% are required to minimize cold storage disorders (Kotze et al., 1987). Furthermore, recommended fruit nutrient concentrations were obtained based on surveys of small fruit populations and do not necessarily ensure fruit quality or postharvest life. Therefore, it is necessary to adjust current critical values for fruit quality purposes. Although P deficiency problems are rarely present in California, some deficiencies have been reported in other production sites where soil P concentrations are low such as North Carolina, Australia, New Zealand, and some parts of Europe, where trees have responded to P applications (Childers and Sherman, 1988). This preliminary study also suggests that low N affected the P concentration in fruit tissue. This should be studied in more detail to determine whether low N can induce low P in fruit under commercial conditions or if this result was just an artifact of our experimental design.

This preliminary work demonstrates the important role of preharvest management practices such as fertilization on postharvest life and consumer quality and encourages further research in this area.

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