

Nitrogen Fertilization Has Differential Effects on Red Color Development and Flesh Starch Breakdown of 'Gala' Apple

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Nitrogen management is critical for achieving high yield and good fruit quality in commercial apple production because tree growth, fruit yield and quality are dependent on N supply. Within a certain range, increasing N supply leads to larger fruit and higher soluble solids in 'Gala' apple (Cheng et al., 2007). However, red-fruited

"Our results indicate that increasing N supply delays skin red color development but accelerates flesh starch degradation in 'Gala' apple. These differential effects of nitrogen supply should be taken into account when assessing fruit maturity for optimizing harvest time."

cultivars grown under high N supply often have poor color.

The intensity and extent of coloration is an important consideration in determining harvest time for red-fruited apple cultivars. Early work by Magness et al. (1940) showed a negative relationship between leaf N content and fruit skin color in 'Rome Beauty' apple. Since then, it has been reported in many studies that fruit coloration decreases as the rate of N application increases (e.g. Week et al., 1952; Fallahi et al., 2001). This decrease in coloration with increasing N application is presumably caused by the inhibition of N on anthocyanin synthesis and accumulation. However, delayed chlorophyll degradation might have also contributed. Higher rates of N were associated with greener fruit in 'Golden Delicious' (Nielsen et al., 1984).

Although the effect of N supply on coloration of apples has been well documented, much less attention has been paid to its effect on other aspects of fruit maturity. Because maturity at harvest greatly affects apple fruit quality and storage performance, starch hydrolysis indices (an indicator of starch level), soluble solids concentration (SSC), flesh firmness and the rate of ethylene evolution are often measured, in addition to skin color, to indicate the maturity stage of apple for making decisions on harvest time. N supply does not necessarily affect all the fruit maturity indices in the same manner. High N application was found to enhance pre-harvest fruit drop of 'McIntosh' apples (Hoffman, 1940). High N application was later reported to enhance ethylene evolution and respiration of 'Fuji' apple at harvest (Fallahi et al., 2001) and ethylene evolution of 'Starkspur Golden Delicious' apple after cold storage (Fallahi et al., 1985). Nielsen et al. (2006) observed

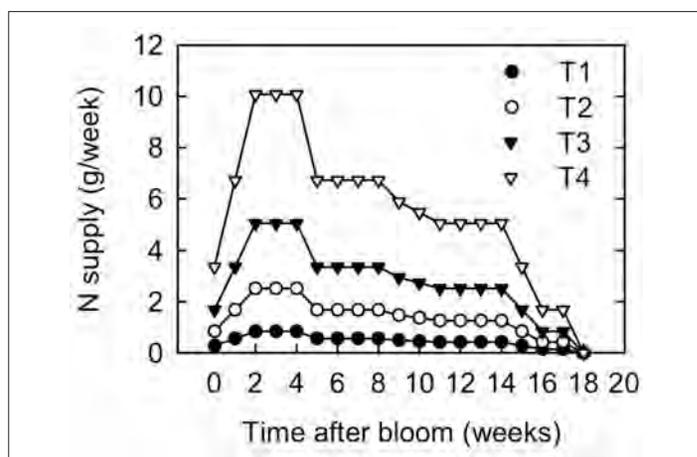


Figure 1. Supply patterns of four levels of nitrogen (T1, T2, T3 and T4) for 7-year-old 'Gala'/M.26 apple trees during the growing season of 2007. The total amount of N provided in T1, T2, T3, and T4 during the growing season is 8.8, 26.4, 52.7 and 105.4 g per tree, which is equivalent to 21.8, 65.5, 131.1.0 and 262.2 lbs of actual N per acre, respectively.

that 'Gala' apple trees receiving low N supply at 0 to 4 weeks after bloom had lower starch index (higher starch) in fruit at harvest than those receiving high N supply during the same period or low N supply at later times. Fallahi et al. (2001) also noticed that 'Fuji' apple trees receiving low N supply had slightly lower starch index (slightly higher starch) than those at higher N supply. All these observations suggest that fruit maturity might be advanced by high N supply. The objective of this study was to determine the effects of N supply on flesh starch degradation relative to the skin pigmentation in 'Gala' apple.

Experimental Procedures

Trees and N treatments. Seven-year-old 'Gala'/M.26 trees were grown in 55-L black plastic pots in acid-washed sand (pH 6.2) at a spacing of 3.5 × 11 feet (equivalent to 1129 trees/acre) in east-west rows at Cornell Orchards in Ithaca, NY. They were trained as tall spindles and had produced regular crops in the previous four years. Uniform trees were selected before budbreak in 2007, and each tree was fertigated with 4 L of Hoagland's No. 2 solution at one of four N concentrations twice per week from bloom (11 May) to 2 weeks before harvest, except during active shoot growth when the trees were fertigated three times per week.

The N concentration used during active shoot growth was 5.0, 15.0, 30.0, or 60 mM (from NH_4NO_3), but when shoot growth slowed down, the N concentrations were lowered. The weekly supply of N for the trees in the four N treatments is shown in Figure 1. Each tree received a total of 8.8, 26.4, 52.7, or 105.4 g actual N from bloom to harvest, which is equivalent to 21.8, 65.5, 131.1 or 262.2 lbs actual N per acre. Each N treatment was replicated five times with single tree per replicate in a completely randomized design. Irrigation was provided with two spray sticks per tree and the trees were well-watered throughout the growing season. The crop load of these trees was adjusted to 8 fruit per cm^2 trunk cross-sectional area (TCA) by hand thinning when the king fruit was 10 mm, and this crop load was maintained to fruit harvest. All the trees received standard disease and insect control throughout the growing season.

Twenty leaves per tree were taken from the middle of the extension growths at 90 days after bloom for leaf N analysis.

Measurements of fruit yield and quality Fruit number, fruit yield per tree, and average fruit weight were measured at fruit harvest on 18 Sept. 2007, 130 days after full bloom (DAFB). Forty well-exposed fruits from each tree were picked randomly at harvest, divided into five groups with 8 fruit each, weighed, and placed in perforated polyethylene bags. One group was evaluated for various quality attributes at harvest. The other four groups of fruit were stored at 2°C, and evaluated for quality at 5-week intervals for a total of 20 weeks. Fruit firmness was measured from the two peeled sides of each fruit by an EPT-1-R Penetrometer (Lake City Technical Products Inc., Kelowna, BC, Canada). Soluble solids concentration (%) was measured from the expressed juice of the fruit used for firmness test with an Atago ATA-60 PAL-1 portable digital refractometer (Atago USA, Inc., Bellevue, WA). The remaining fruit tissues were peeled and cut equatorially, frozen in liquid nitrogen and stored at -80°C for fruit N and starch measurements.

Measurements of anthocyanins, chlorophylls and starch Peel discs (1 cm^2 in size) on the sun-exposed side of fully exposed fruit were selected for uniformity in color and taken at 83 days after full bloom (DAFB) (when pigmentation just began), 111 DAFB, and at harvest (130 DAFB), respectively, frozen in liquid N_2 and stored at -80°C until analysis. Total anthocyanins were extracted from two peel discs with 3 ml of methanol:water:HCl (85:12:3, v/v), kept in the dark at room temperature overnight, and the absorbance at 520 nm was measured on samples diluted with pH 1.0 and 4.5 buffers. Peel chlorophyll content was extracted with 80% acetone and measured photometrically. The starch concentration in the fruit flesh was measured enzymatically.

Measurements of leaf and fruit N Leaf samples were dried in a forced-air oven, and ground. Frozen fruit tissues were pulverized and freeze-dried. Tissue N was measured using an FP-428 C/N analyzer (LECO Corp., St. Joseph, MI).

Results

Leaf and fruit N status Leaf N content increased in a curvilinear fashion as N supply increased, with the value being doubled from the lowest N supply (T1, 8.8 g N per tree) to the highest N supply (T4, 105.4 g N per tree) (Figure 2A). In contrast, fruit N content increased proportionally to increasing N supply (Figure 2B).

Yield and fruit quality at harvest Trees receiving the lowest N supply (T1, 8.8 g N per tree) had significantly lower yield

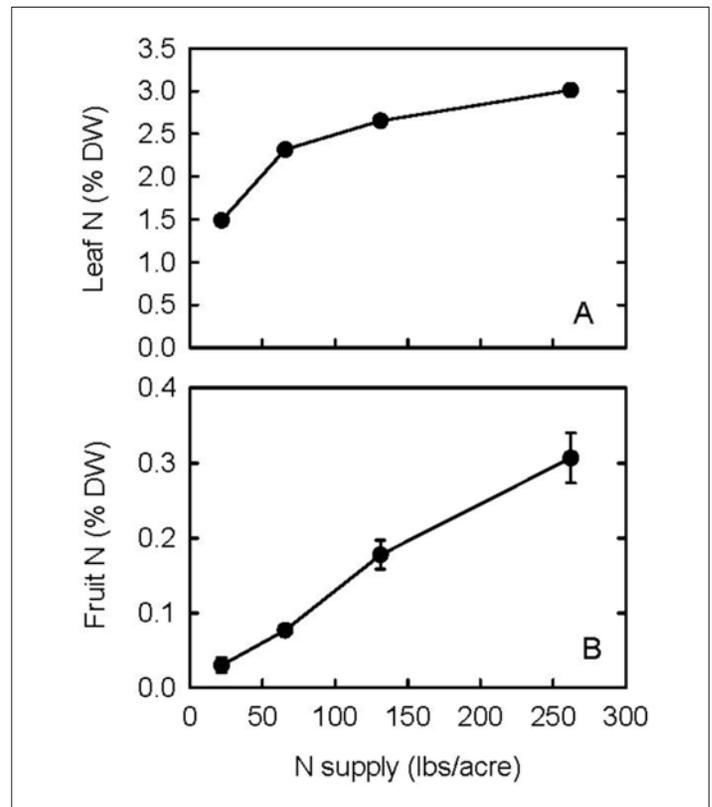


Figure 2. Leaf and fruit nitrogen status of 'Gala'/M.26 trees in response to N supply levels. Leaf samples were taken at 90 days after bloom and fruit samples were taken at harvest.

efficiency, smaller fruit, lower soluble solids concentration, but firmer fruit than those in T2 (26.4 g N per tree), T3 (52.7 g N per tree) and T4 (105.4 g N per tree) treatments (Table 1). Trees in T3 treatment produced the highest fruit yield and the largest fruit (197.6g/fruit). Fruit soluble solids concentration was significantly lower in T1 than in the other N treatments, whereas no significant difference was detected between T2, T3 and T4 treatments. Fruit firmness tended to decrease with increasing N supply.

Fruit color, anthocyanins and chlorophylls in fruit skin

Red color development was significantly delayed by high N treatments (Figure 3). This was reflected in the concentration of anthocyanins in the fruit skin (see below). Since Gale Gala is a red strain of Gala, the sun-exposed side of the fruit in all N treatments had developed enough color by harvest, but the red color on the shaded sided decreased with increasing N supply.

Table 1. Yield, fruit size, firmness and soluble solids concentration (SSC) of 'Gala' apple fruit at harvest as affected by different levels of N fertilization.

N Treatments (g N/tree)	Yield (kg/tree)	Yield efficiency (kg/ cm^2 TCA)	Fruit weight (g/fruit)	SSC ($^{\circ}$ Brix)	Firmness (lbs)
8.8	17.8b	1.33b	155.1c	13.6 b	18.0 a
26.4	18.6b	1.58a	181.2b	14.6 a	16.7 b
52.7	20.9a	1.64a	197.6a	15.2 a	16.1 bc
105.4	19.9ab	1.53a	191.3ab	15.0 a	15.6 c

Differences among treatments followed by different letters are significant at 0.05 level (LSD)

TCA: trunk cross-sectional area

The fruit skin on the exposed side had low concentrations of anthocyanins at 83 DAFB when pigmentation just began, increased steadily till 111 DAFB, and then increased at a faster rate towards maturity (Figure 4A). At harvest (130 DAFB), skin anthocyanin concentrations in T2 and T3 were comparable, but were higher than those in T4. In the skin of the fruit from T1, however, a much higher rate of anthocyanin accumulation occurred from 83 to 111 DAFB, which resulted in a much higher concentration of anthocyanins than those in the other three N treatments.

Chlorophyll concentrations in fruit skin decreased in all four N treatments from 83 to 130 DAFB (Figure 4B). Fruit from trees in T1 had significantly lower chlorophylls than those from the other three N treatments at 83 DAFB. The decrease in chlorophyll concentrations from 83 to 130 DAFB was significantly delayed by increasing N supply, though the difference in chlorophyll concentrations tended to diminish as fruit developed towards maturity.

Fruit starch concentration before and after harvest Starch concentration in the flesh was higher at higher N supply at 38 days before harvest (Figure 5). Starch concentration underwent a sharp decrease from 38 days before harvest to harvest, and the slope of the decrease was steeper at higher N supply, leading to decreased starch concentration with increasing N supply at harvest. During cold storage, starch degradation continued in all four N treatments, but starch degradation was completed earlier at higher N supply.

Soluble solids concentration and firmness at harvest and during storage Soluble solids concentration (SSC) and firmness showed noticeable differences in the fruit with different levels of N supply at harvest and during storage (Figure 6). At harvest, fruit SSC was significantly higher in T2, T3 and T4 treatments than in T1 treatment. Fruit SSC in T1 increased during cold storage, reached a maximum at 105 days after harvest (DAH), and then decreased slightly (Figure 6A). For fruits in the other three N treatments, however, SSC continued to decrease from harvest to 140 days after harvest. No significant difference was detected in SSC between any N treatments after 105 DAH.

At harvest, fruit firmness was lower at higher N supply (Figure 6B). Fruit firmness decreased linearly from harvest to 140 DAH in all four N supply levels. Fruit firmness was negatively related to the level of N supply, with the highest fruit firmness measured in the lowest N treatment.

Discussion

Larger fruit size and higher yield efficiency with increasing N supply found in this study (Table 1) was expected since our previous work clearly showed that increasing N supply improves leaf N status, leaf and whole tree photosynthetic capacity, and leaf area to fruit ratio, leading to more cells per fruit, larger fruit and higher yield (Cheng et al., 2007). Compared with our previous study, a wider range of N supply was used and a wider leaf N

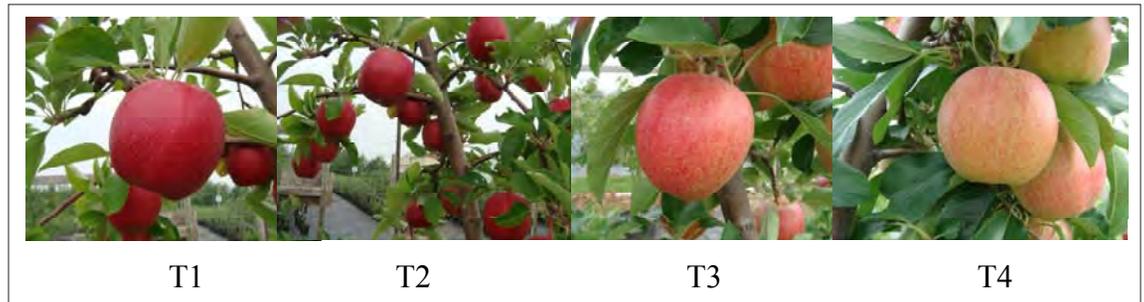


Figure 3. Effects of nitrogen supply on fruit skin color of 'Gala' apples. This photo was taken at 20 days before harvest. The total amount of N provided in T1, T2, T3, and T4 during the growing season is 8.8, 26.4, 52.7 and 105.4 g per tree, respectively.

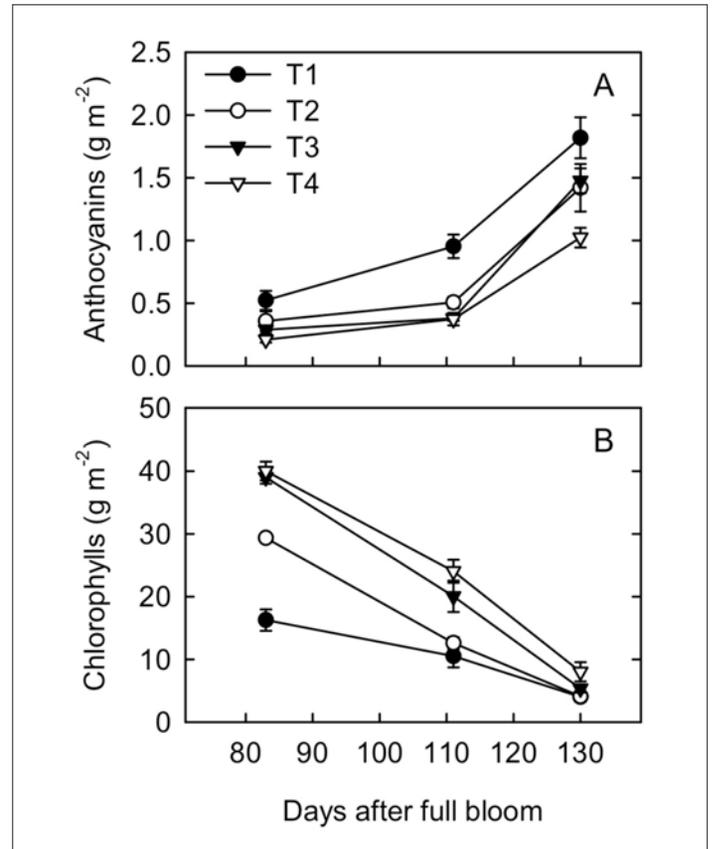


Figure 4. Effects of nitrogen supply on anthocyanin concentration (A) and chlorophyll content (B) in 'Gala' skin during fruit development and maturation. The total amount of N provided in T1, T2, T3, and T4 during the growing season is 8.8, 26.4, 52.7 and 105.4 g per tree, respectively. Each point is mean \pm SE of five replicates.

status was achieved in this experiment (Figure 2A), which may explain why fruit size and yield efficiency did not show further responses when N supply was increased beyond a certain level.

Apple fruit coloration depends on the synthesis of anthocyanins, which is genetically determined but is also influenced by various environmental factors. In this study, fruit red color development was significantly delayed by high N treatments (Figs 3, 4A). At harvest, although the exposed side of the fruit in all treatments had developed enough color, the red color on the shaded side decreased with increasing N supply. These results clearly indicate that low N supply enhances, but high N supply attenuates anthocyanin synthesis and accumulation in apple skin. This is consistent with what was found in many previous

experiments, i.e. fruit skin color decreases with increasing N application. In addition to a direct effect, high N supply could also indirectly affect anthocyanin synthesis and accumulation by stimulating shoot growth, which reduces light exposure of the fruit via shading. However, because only fully-exposed fruit on the south side of the tree canopy were used in the current study, this indirect effect would be small for the fruit measured, if any.

Schulz (1986) stressed that intense anthocyanin formation is possible only with the onset or completion of chlorophyll degradation. High N nutrition increases chlorophyll concentration but inhibits anthocyanin formation (Saure, 1990). Reger (1944) found an inverse relationship between the presence of chlorophylls and anthocyanins in the epidermal and hypodermal cells of apple. Several observations on green parts of other species confirm that chlorophylls may absorb much of the red light, thus reducing its efficiency in regulating the phytochrome system, and as a result much higher irradiation is required in green tissues than in etiolated tissues to achieve the same phytochrome control. In this study, significantly higher chlorophyll content was found in the skin under higher N supply (Figure 4B). This suggests that the poor fruit color under high N supply might partly result from high concentrations of chlorophylls present in the fruit skin, which not only mask the display of anthocyanins as surface color but also delay the synthesis of anthocyanins.

While skin pigmentation was delayed by high N supply, flesh starch degradation was accelerated by high N supply (Figure 5). This is consistent with lower starch indices observed by Neilsen et al. (2006) on 'Gala' and by Fallahi et al. (2001) on 'Fuji' apples under low N supply. The accelerated starch breakdown under high N supply might be related to the higher rate of ethylene evolution measured in fruit grown under high N supply (Fallahi et al., 2001). These differential effects of N supply on skin pigmentation and starch degradation have important implications for maturity management, especially if non-destructive techniques such as hyperspectral near infrared reflectance imaging is used to assess the maturity stage of fruit, which is solely based on two non-destructive spectral indices, i.e. the chlorophyll decrease and the anthocyanin increase. If skin coloration is solely used for determining fruit maturity stage, it may overestimate the maturity stage of fruit grown under low N supply whereas underestimate the maturity stage of fruit grown under high N supply. In commercial production, if growers have to wait for better color development on high N fruit, the fruit may be too advanced in maturity to be stored for long-term. Therefore, it is essential to combine assessment of skin color with starch degradation patterns to determine fruit maturity stage before making decisions on optimal harvest time. Of course, a better approach is to optimize nitrogen management in terms of timing and amount based on tree nitrogen demand and tree N status to prevent the production of high N fruit in the first place (Cheng and Raba, 2009; Cheng, 2010).

The lower SSC measured at harvest in the low N fruit in this experiment and in our previous study (Cheng et al., 2007) is partially caused by delayed starch degradation because SSC actually increased during cold storage in the low N fruit due to continued starch degradation (Figure 5, 6A). The decrease in fruit SSC in the other three N treatments during cold storage indicates that the amount of glucose released from starch degradation did not make up for the loss of sugars to respiration. Apples grown under high N supply have higher respiration at harvest and dur-

ing storage than those grown under low N supply (Smock and Boynton, 1944; Fallahi et al., 1985, 2001).

Fruit firmness was found to be negatively related to the level of N supply both at harvest (Table 1) and during cold storage (Figure 6B). This is consistent with previous findings that greater firmness was associated with lower N levels in apple fruit (Smock and Boynton, 1944; Regnaold et al., 2001). Since fruit firmness is one of the most important criteria for consumers of apples, the

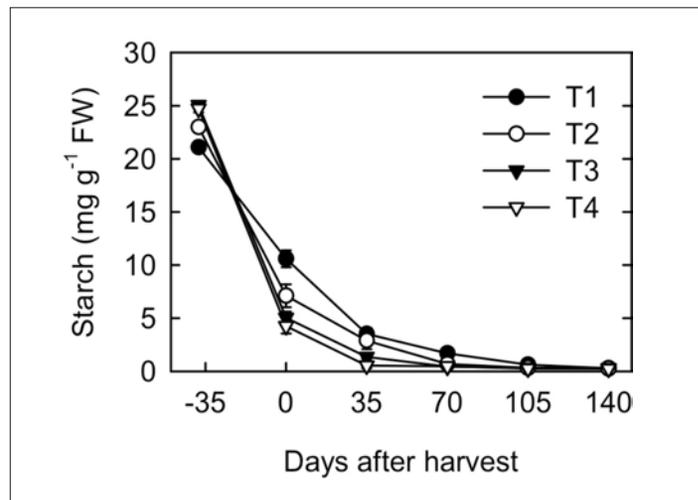


Figure 5. Effects of nitrogen supply on flesh starch concentration of 'Gala' apples before harvest and during storage. The total amount of N provided in T1, T2, T3, and T4 during the growing season is 8.8, 26.4, 52.7 and 105.4 g per tree, respectively. Each point is mean \pm SE of five replicates.

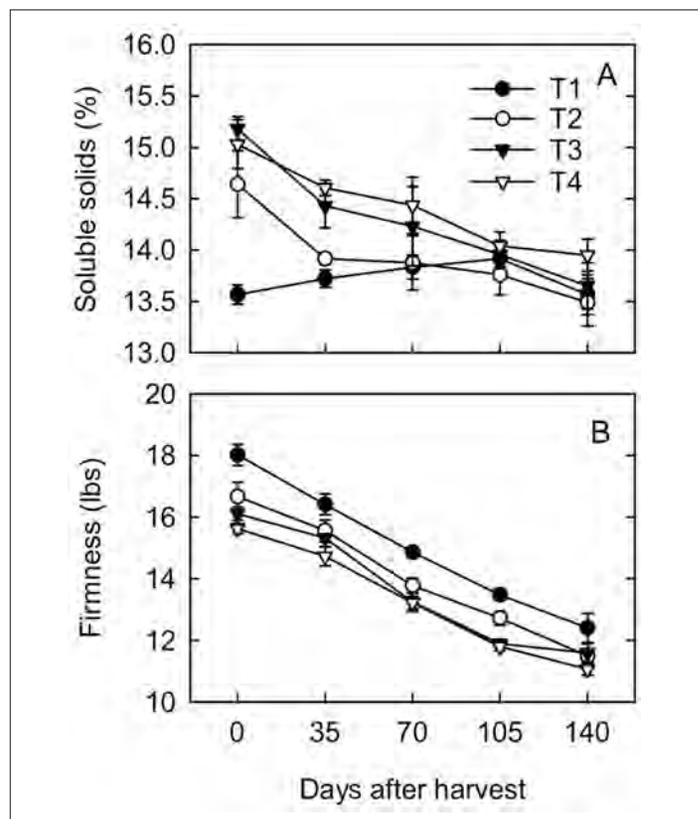


Figure 6. Effects of nitrogen supply on fruit soluble solids concentration (A) and firmness (B) of 'Gala' apples at harvest and during storage. The total amount of N provided in T1, T2, T3, and T4 during the growing season is 8.8, 26.4, 52.7 and 105.4 g per tree, respectively. Each point is mean \pm SE of five replicates.

greater firmness in the low N fruit would be advantageous in the marketplace if the smaller fruit size and lower soluble solids under low N supply are not weighed more heavily by consumers. Apples under low N supply also had higher firmness after storage, indicating their better long-term storability.

In conclusion, increasing N supply delays skin red color development but accelerates flesh starch degradation in 'Gala' apples. These differential effects of nitrogen supply should be taken into account when assessing fruit maturity for optimizing harvest time.

Acknowledgments

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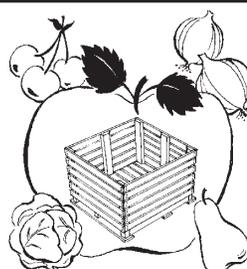
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