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Using SPAD-502 Values to Assess the Nitrogen Status of Apple Trees

Denise Neilsen, Eugene J. Hogue, Gerald H. Neilsen, and Peter Parchomchuk

Agriculture and Agri-Food Canada, Research Centre, Summerland, B.C. V0H 1Z0, Canada

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Abstract. **Four apple (*Malus domestica* Borkh) cultivars ('Fuji', 'Spartan', 'Fiesta', and 'Gala') on Malling 9 (M.9) rootstock were grown in the field with three N rates (5, 20, and 35 g N/tree per year), supplied as $Ca(NO_3)_2$, and fertigated daily for 9 weeks. In the second year, leaf SPAD readings (chlorophyll readings obtained with the Minolta-502 SPAD meter) increased over the growing season for all cultivars, and leaf N decreased. Leaf SPAD and leaf N measurements increased in response to N fertigation rate at all sampling times. 'Gala' consistently had lower SPAD readings than the other cultivars, and, with the exception of the first sampling time, 'Fuji' had higher and 'Fiesta' lower leaf N concentrations than other cultivars. There were strong relationships between leaf N concentration and SPAD readings for all cultivars until mid-July (r^2 = 0.44 to 0.89), but not later in the growing season. Differences in SPAD readings and leaf N concentration due to cultivar and over time were as great as those due to N treatments, indicating that in the future, determination of critical SPAD values for apple leaves must be standardized for cultivar and sampling time. SPAD readings could be used to assess the need for N early in the growing season in fertigated orchards where rapid changes in nutrition programs can be undertaken readily.**

Assessment of the N status of apple trees traditionally has been based on the N concentration of leaves sampled from the midportion of the current season's growth during the period of greatest leaf nutrient concentration stability. This period occurs between 110 and 125 days after full bloom (Faust, 1989). In general, such leaf concentration information has been used to correct the next season's potential nutrient stress by broadcast application of fertilizer in fall, spring, or both. Dwarf trees in modern high-density orchards are potentially more dependent on externally supplied N than the large trees in traditional orchard systems, which may have a considerable amount of stored N. Many high-density orchards use fertigation through drip irrigation systems for supplying water and nutrients in regions with consistent water deficits (Haynes, 1985) and in regions where irrigation is supplementary (Kipp, 1992). In such orchards, rapid remediation of nutritional problems is possible. Thus, a more interactive approach to fruit tree nutrition that would allow monitoring of tree nutrient status throughout the growing season is desirable. This approach is of particular importance for highly capitalized orchard systems, where rapid vegetative growth in the first one or two growing seasons is essential for a good economic return.

The SPAD chlorophyll meter (models 501 and 502; Minolta Corp., Ramsey, N.J.) was used successfully to determine the chlorophyll content of apple leaves (Campbell et al., 1990; Schechter et al., 1992; Singha and Townsend, 1989). It also was used to assess the N status of leaves in a variety of annual crops (Follett et al., 1992; Piekielek and Fox, 1992; Turner and Jund, 1991). Less information is available for perennial crops, although SPAD readings have been related to petiole and leaf blade N contents measured by a variety of techniques in strawberry (*Fragaria* ×*ananassa*) (Himelrick et al., 1993). Although SPAD readings were highly correlated with some strawberry plant N measures, the results were sufficiently inconsistent for the authors to question the usefulness of the technique. They also noted that there was insufficient background information available to relate plant N concentrations to N requirements at various growth and fruiting stages (as is likely for apple trees). However, given the flexibility and speed of SPAD measurements, a possible role was seen for using a SPAD meter to estimate foliar N status in the field.

Our purpose was to determine the effect of N supply, cultivar, and time during the growing season on the N status of 2-year-old apple trees on M.9 rootstock as measured by the SPAD meter readings and leaf N concentration.

Materials and Methods

Within-tree variability. As a preliminary study to assess within-tree variability, every leaf on three 2-year-old 'Spartan' on M.9 trees was measured using the SPAD meter in mid-June. These trees received 30 g of N/tree per year, fertigated daily through a drip irrigation system. Two measurements were made on each leaf on opposite sides of the midrib at the widest point and averaged. Branches were assigned into three categories based on their vertical position (vp) in the tree: bottom, middle, or top third. For each branch, leaves also were assigned to three categories based on their horizontal position (hp) on the branch relative to the trunk: base, middle, or tip. Thus, any leaf could be described by its vp and hp in the tree. To determine the effect of leaf position on SPAD readings, results were analyzed using analysis of variance (ANOVA) with factorial combinations of vp and hp and tree replicates. The vp and hp were treated as fixed effects.

Field experiment. In Spring 1992, 'Fuji', 'Spartan', 'Fiesta', and 'Gala' on M.9 apple trees were planted at 1.5 × 3-m spacing in a Skaha loamy sand soil. Nitrogen treatments of

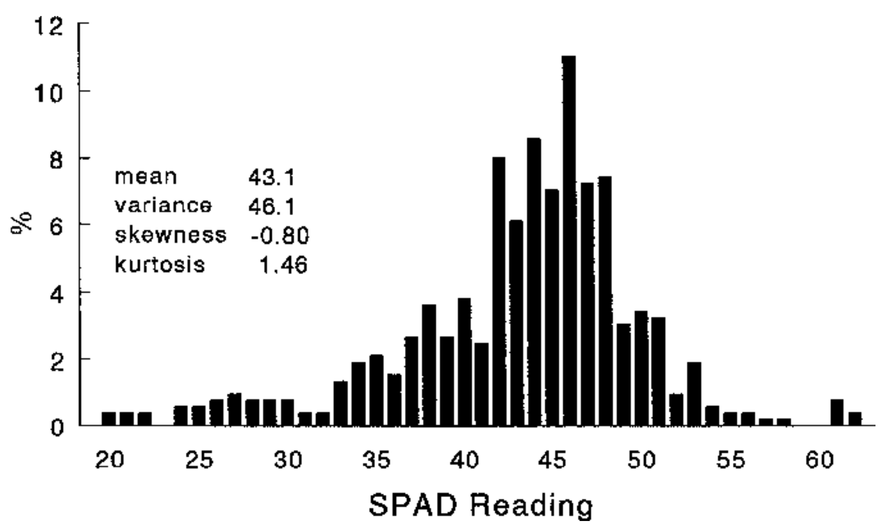

Fig. 1. Percent distribution of leaf SPAD values within three 2-year-old 'Spartan' on M.9 apple trees (n = 525).

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Table 1. Analysis of variance for the effect of leaf position within a tree on leaf SPAD readings of 2-year-old 'Spartan' on M.9 apple trees in mid-June.

Source of variation	df	Mean square	*P* value
Tree (replication)	2	51.19	0.6108
Vertical position (vp)	2	226.5	0.1403
Horizontal position (hp)	2	4567	0.0001
vp × hp	4	6.568	0.9916
Error	16	106.3	

Table 2. Effect of leaf position on leaf SPAD reading of 2-year-old 'Spartan' on M.9 apple trees in mid-June.

Vertical position (on tree)	SPAD	Horizontal position (on branch)	SPAD
Top	41.4	Tip	36.9
Middle	43.6	Middle	45.0
Bottom	44.3	Base	46.8
Significance	NS		***
SE	1.99		1.99

NS, ***Nonsignificant or significant at $P \leq 0.001$, respectively.

5, 20, or 35 g N/tree per year were randomized to the main plot units, cultivars to the subplot units, and sampling date (described later) to the sub-subplot units of a split-plot experiment. The experiment was arranged in a randomized complete-block design. There were three trees per experimental unit and five replications. Nitrogen was supplied as $Ca(NO_3)_2$, fertigated daily for 63 days through a drip irrigation system. In 1993, fertigation started 19 May and ended 20 July. Blossoms were removed after full bloom (5 to 18 May). Soil solution NO_3-N concentrations were monitored twice weekly in the 'Gala' plots using suction lysimeters permanently installed at a 30-cm depth below the drip emitter. Leaf chlorophyll was measured five times during the growing season at 3-week intervals from 27 May to 20 Aug. 1993 using a SPAD meter. To correspond with standard leaf sampling techniques for nutrient analysis, samples were collected from the midportion of the current year's extension growth. Two, nine-leaf samples were collected from each plot, and SPAD readings were made immediately on opposite sides of the midrib of each leaf at the widest part. Thus, for each sample, the SPAD value recorded was an average of 18 readings.

Table 3. Analysis of variance for leaf SPAD readings and leaf N concentration measured five times during the growing season for four apple cultivars grown with three levels of N fertigation (5, 20, and 35 g N/tree per year).

Source of		Leaf SPAD reading		Leaf N concn (%)	
variation	df	Mean square	*P* value	Mean square	*P* value
Nitrogen (N)	2	1439	0.0001	2.698	0.0001
Error A	8	18.20		0.042	
Cultivar (C)	3	199.4	0.0001	2.291	0.0001
N × C	6	10.45	0.3788	0.094	0.0282
Error B	36	9.46		0.035	
Date (D)	4	2745	0.0001	1.654	0.0001
N × D	8	16.81	0.0001	0.190	0.0001
C × D	12	18.01	0.0001	0.230	0.0001
N × C × D	24	3.072	0.7849	0.013	0.5290
Error C	240	3.849		0.014	

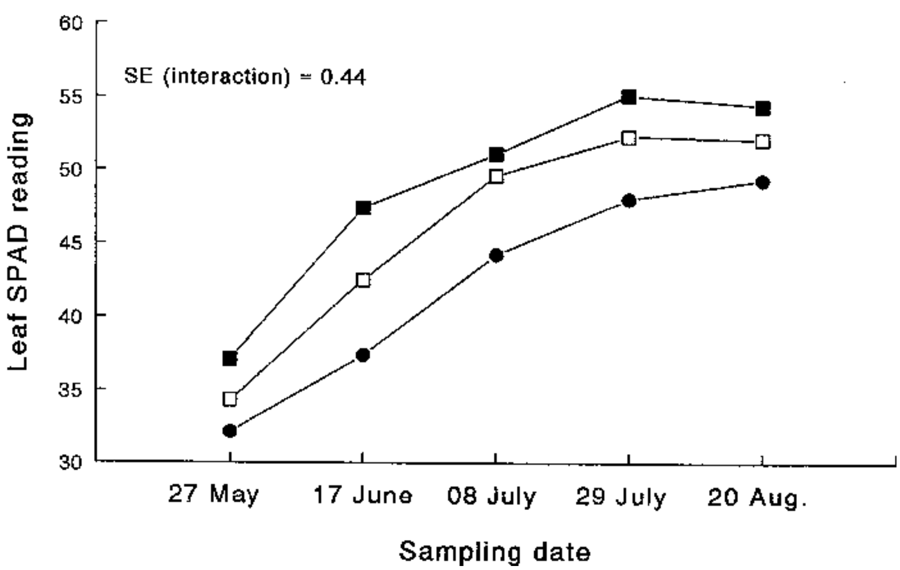

Fig. 2. Effect of N fertigation rate [(●) 5, (❐) 20, or (■) 35 g N/tree per year] and sampling date on leaf SPAD readings for 2-year-old apple trees on M.9 rootstock. Results averaged over all cultivars.

Leaves then were dried at 65C for 24 h, ground in a stainless steel mill, and dry-ashed in a muffle furnace for 3 h. A 250-mg subsample was digested with 40 mg HgO, 1.9 g K_2SO_4, 5 ml of concentrated H_2SO_4, and 3 ml H_2O_2 for 0.5 h on a block digester at 350C. Standard AutoAnalyser procedures were used to determine N (Technicon AutoAnalyser II Industrial Method 334-74 A/A; Technicon).

For all experiments, correlation analysis, regression analysis, and ANOVA were performed using SAS procedures (SAS Institute, Cary, N.C.).

Results and Discussion

Within-tree variability. There was a wide range of SPAD readings within shoot leaves from 2-year-old 'Spartan' on M.9 trees (Fig. 1). The distribution was unimodal with a long tail to the left. The hp of the leaves (proximity to trunk) affected SPAD readings (Table 1). These readings were higher in more shaded portions of the trees closer to the trunk (Table 2.), which indicates higher chlorophyll accumulation in regions of lower light intensity. This result is contrary to findings of Schechter et al. (1992) and Ghosh (1973), who attributed lower chlorophyll content per surface area in spur leaves when compared with shoot leaves to a lack of exposure to light. However, in our study, leaves at the tips of branches were likely not fully expanded and consequently were not at their maximum chlorophyll production level (Kennedy and Johnson, 1981). The variability in leaf chlorophyll underscores the need for systematic and careful sampling if leaf SPAD measurements are to be used early in the growing season as a measure of tree N status.

Field experiment. The N fertigation rate, cultivar, and sampling date affected leaf SPAD readings and N concentration (Table 3). Because there were interactions between the effects of sampling date and fertigation rate and cultivar on leaf measurements, results are presented for each sampling date.

Effect of N fertigation rate and sampling date on leaf SPAD readings. At all sampling times, leaf SPAD readings increased in response to N application rate (Fig. 2). Nitrogen fertigation was initiated 18 May and finished 20 July. Because N was supplied as $Ca(NO_3)_2$, most N uptake likely occurred during the fertigation period between 30 May and 20 July when soil solution NO_3-N concentrations were highest (Fig. 3). Little NO_3-N was present in the soil solution either before or after that time. Thus, fertilizer N likely had the most direct impact on leaf chlorophyll at the second, third, and fourth sample times, whereas at other sample times, recycled or stored N would have been more important. At all N rates, leaf SPAD readings increased over the growing season until the end of July and then leveled off. This increase was not necessarily only in response to N supply because apple leaf thickness also increases over the growing season (Wooge and Barden, 1987), and SPAD values (Campbell et al., 1990) and leaf chlorophyll content increase with leaf thickness (Ghosh, 1972). Thus, the effects of thickness and chlorophyll content on SPAD readings cannot be separated readily. The seasonal effect on leaf thickness was attributed not only to leaf maturation but also to the time in the season of leaf initiation and development (Wooge and Barden, 1987). Thus, increases in leaf thickness, and possibly leaf SPAD readings, over the growing season also may be due to an increasing proportion of younger peripheral leaves in the sample.

Effect of cultivar and sampling date on leaf SPAD readings. For each cultivar, leaf SPAD readings increased until the end of July and then leveled off (Fig. 4). In general, SPAD readings were lower for 'Gala' than for all other cultivars. The magnitude of the differences in SPAD readings among cultivars was similar to that among N rates at the beginning of the growing season but was smaller after the first sampling date. To our knowledge, there is little information published on differences in leaf chlorophyll content among cultivars. Spur-type mutants tend to have thicker leaves with higher chlorophyll contents than their standard counterparts (Looney, 1968; Westwood and Zielinski, 1965). Steffens and Zimmerman (1992) reported that own-rooted 'Gala' or 'Gala' on M.7 had lower leaf chlorophyll concentrations than 'Triple Red Delicious' on either M.7 or its own roots.

Effect of N fertigation rate, cultivar, and sampling date on leaf N concentration. Leaf N concentration increased in response to N fertigation rate, and the response differed among cultivars (Fig. 5). The increase in response to added N was greater for 'Spartan' than for other cultivars. At all N rates, 'Fiesta' had lower concentrations of leaf N, and at the low N rate, 'Fuji' had the highest concentration of leaf N than the other cultivars. The magnitude of the difference in leaf N concentration among cultivars was as great as that among N rates.

At all sampling times, there was an effect of N rate on leaf N concentration (Fig. 6). There was a greater response to N rate during the fertigation period than when there was little NO_3-N available in the soil (Fig. 3). Leaves from trees receiving the low N level (Fig. 6) were close to levels generally accepted as insufficient for good growth in nonbearing young trees (British Columbia Ministry of Agriculture, Fisheries and Food, 1994). Over the growing season, the difference between the highest and lowest treatment effects became much smaller. In general, leaf N concentration decreased over the growing season as previously reported (Rogers et al., 1953), and the effect was slightly more pronounced at higher N fertigation rates.

Differences in leaf N concentration among cultivars varied over the growing season, although, with the exception of the first sampling date, 'Fiesta' had the lowest and 'Fuji' or 'Spartan' the highest concentrations (Fig. 7). Although leaf N concentration decreased, the magnitude of the differences among cultivars remained relatively constant over the growing season, in contrast to the effect of N rate for which the differences lessened (Fig. 6). Thus, the effect of cultivar on leaf N concentration

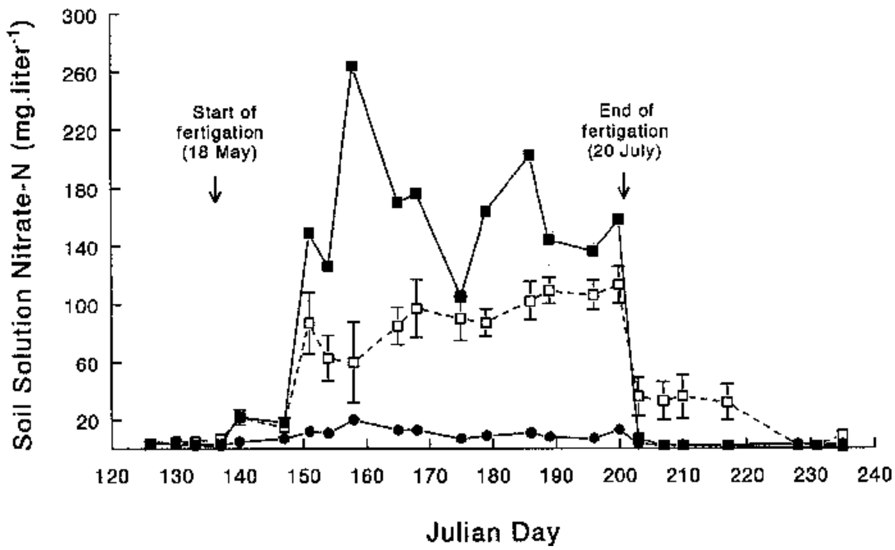

Fig. 3. Concentration of NO_3-N in the soil solution over the 1993 growing season for 2-year-old 'Gala' on M.9 orchard plots fertigated with three levels of $Ca(NO_3)_2$ [(●) 5, (❐) 20, or (■) 35 g N/tree per year]. Vertical bars indicate SE.

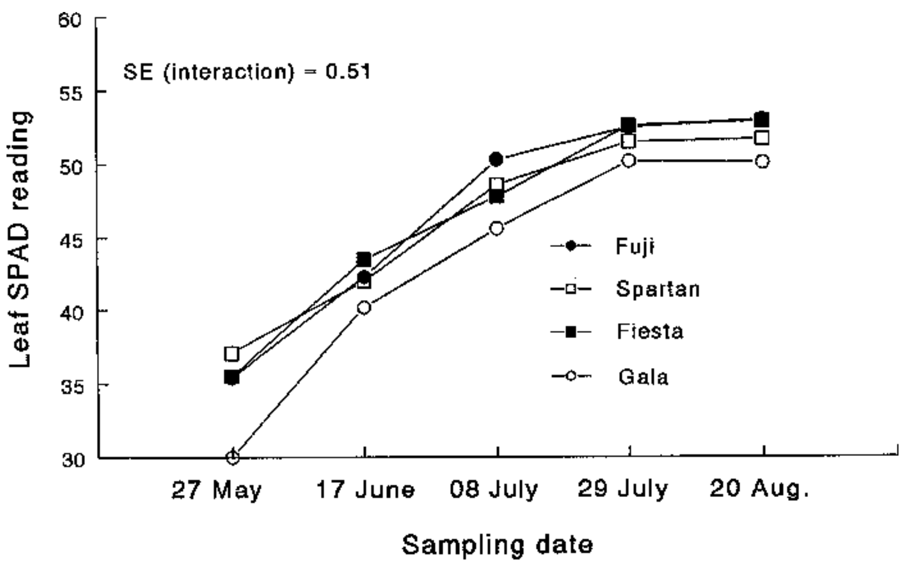

Fig. 4. Effect of cultivar and sampling date on leaf SPAD readings for 2-year-old apple trees on M.9 rootstock. Results averaged over all N fertigation rates.

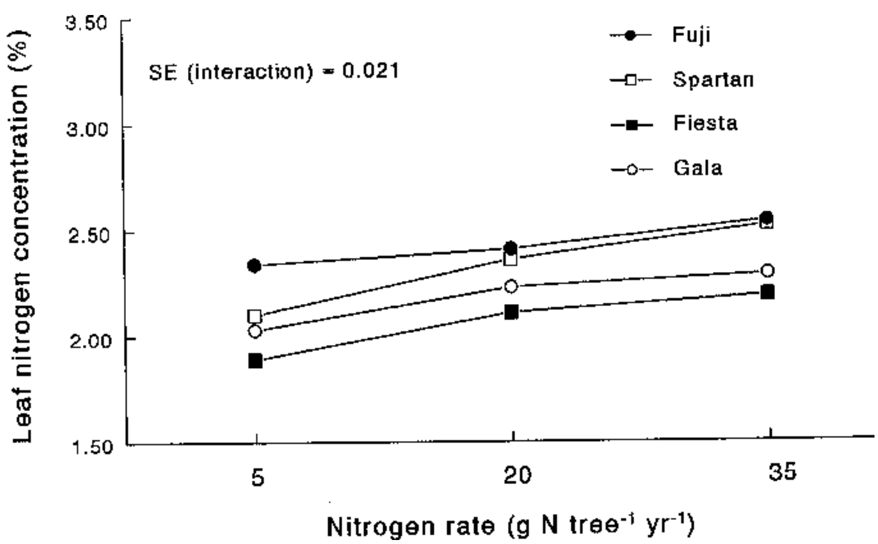

Fig. 5. Effect of N fertigation rate and cultivar on leaf N concentration for 2-year-old apple trees on M.9 rootstock. Results averaged over all sampling times.

was greater than that of N fertigation rate (Fig. 6) at the last two sampling dates.

Relationship between leaf SPAD readings and leaf N concentration. Because cultivar and date significantly affected leaf SPAD and N concentration, correlations between the two variables were performed for each cultivar at each date (Table 4). Stronger relationships existed at the beginning of the growing season than at the end. For all cultivars, there was a rapid decline in r^2 values after the second week in July, which possibly reflects the lack of difference among leaf N concentrations in response to N fertigation rate that occurred at this time (Fig. 6). Regression equations for predicting leaf N concentration from leaf SPAD readings were developed at each date and cultivar (Table 5). At all sampling dates, there were significant differences (data not shown) among either regression line slopes or intercepts, indicating that for prediction purposes, data could not be pooled.

Most studies where leaf SPAD measurements have been used to determine plant N status have been undertaken with annual crops. In most cases, attempts have been made to relate SPAD readings, subsequent N additions, and crop yield (Follett et al., 1992; Piekielek and Fox, 1992; Turner and Jund, 1991). Leaf SPAD readings are sufficiently variable between seasons and locations to make definitive statements about N deficiency difficult, and, although critical values have been assigned for some crops, it has not yet been possible to relate leaf SPAD readings to fertilizer requirements (Fox et al., 1994; Piekielek and Fox, 1992; Turner and Jund, 1991). The N status of apple trees traditionally has been related to leaf N concentration. Although there is some information available that relates N applications to yield and growth, fertilizer recommendations must take into account tree vigor, previous cropping history, fruit quality, and storage potential. Consequently, it is unlikely that leaf SPAD measurements could be used to determine amounts of N required at a given stage in the growing season.

Table 4. Coefficients of determination (r^2) for leaf N concentration and leaf chlorophyll content (SPAD) for four apple cultivars over the 1993 growing season (n = 30).

	Date				
Cultivar	27 May	17 June	08 July	29 July	20 Aug.
Fuji	0.66**	0.60**	0.44**	0.10	0.01
Spartan	0.64**	0.68**	0.70**	0.23**	0.12
Fiesta	0.59**	0.62**	0.89**	0.26**	0.10
Gala	0.52**	0.73**	0.44**	0.10	0.10

**Significant at $P \leq 0.01$.

In our study, the good relationships determined between leaf SPAD readings and leaf N concentration early in the growing season indicates that SPAD measurements may be used to determine need for N fertilizer, but not necessarily the amount required. This ability has several advantages over current N status monitoring techniques used for apple trees. Midseason leaf analysis is the usual method for determining the following growing season's nutrient requirements and is an integral part of nutrient management programs that are based on single fertilizer applications in either fall or spring. Fertigation is a technique that allows application of nutrients at any time during the growing season. Many growers with fertigation systems apply nutrients daily and are capable of responding rapidly to changes in nutrient demand. Thus, SPAD monitoring has a potential role in determining differences in the N status of trees, among cultivars, and over time to aid in decision-making about N fertilizer applications. Within-tree variability predicates a systematic sampling procedure that probably should be similar to standardized procedures for collecting leaf samples for nutrient analysis.

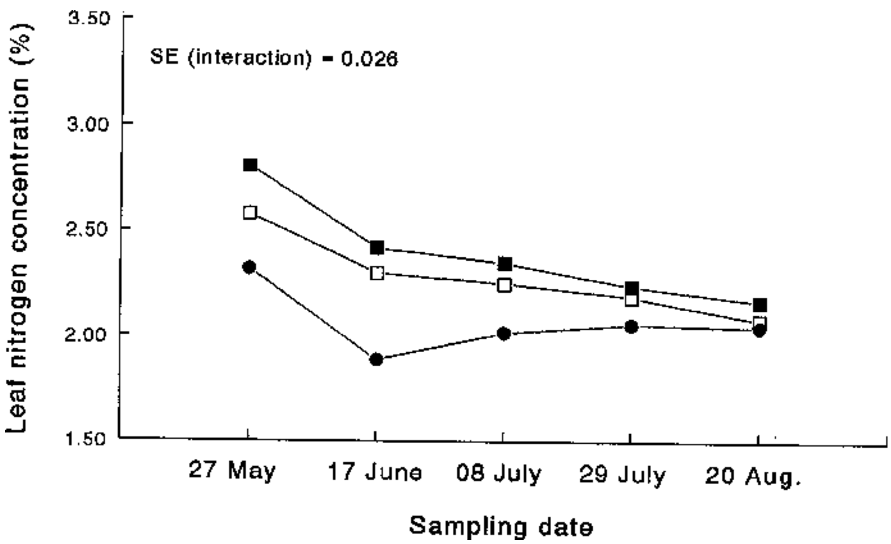

Fig. 6. Effect of N fertigation rate [(●) 5, (❐) 20, and (■) 35 g N/tree per year] and sampling date on leaf N concentration for 2-year-old apple trees on M.9 rootstock. Results averaged over all cultivars.

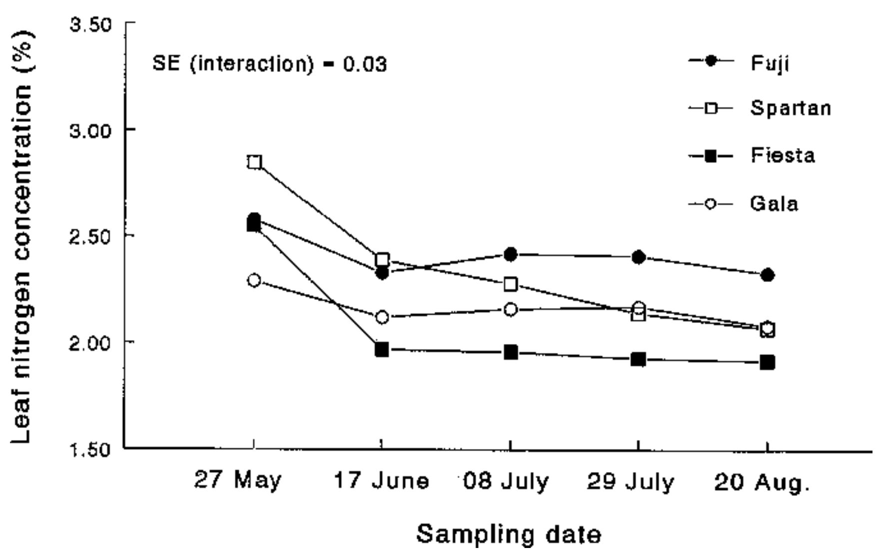

Fig. 7. Effect of cultivar and sampling date on leaf N concentration for 2-year-old apple trees on M.9 rootstock. Results averaged over all rates of N fertigation.

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Table 5. Regression equations[z] for predicting leaf N concentration (Y) from leaf SPAD reading (X) for four apple cultivars at various times over the growing season ($s_{Y \cdot X}$ = standard error of the estimate).

	Date			
Cultivar	27 May	17 June	08 July	29 July
Fuji	Y = 0.31 + 0.06X	Y = 0.05 + 0.05X	Y = 0.7 + 0.03X	
	$s_{Y \cdot X}$ = 0.16	$s_{Y \cdot X}$ = 0.15	$s_{Y \cdot X}$ = 0.14	
Spartan	Y = –0.03 + 0.08X	Y = –0.74 + 0.07X	Y = –0.50 + 0.06X	Y = 0.33 + 0.04X
	$s_{Y \cdot X}$ = 0.19	$s_{Y \cdot X}$ = 0.19	$s_{Y \cdot X}$ = 0.14	$s_{Y \cdot X}$ = 0.26
Fiesta	Y = –0.24 + 0.08X	Y = 0.10 + 0.04X	Y = 0.12 + 0.04X	Y = 0.80 + 0.02X
	$s_{Y \cdot X}$ = 0.17	$s_{Y \cdot X}$ = 0.17	$s_{Y \cdot X}$ = 0.07	$s_{Y \cdot X}$ = 0.15
Gala	Y = 0.18 + 0.07X	Y = –0.18 + 0.06X	Y = 0.96 + 0.03X	
	$s_{Y \cdot X}$ = 0.18	$s_{Y \cdot X}$ = 0.16	$s_{Y \cdot X}$ = 0.12	

[z]Presented for cultivar and date combinations where there was a significant correlation.

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