Fertilizing Nursery and Landscape Tress: Critical Nitrogen Levels Related to Chlorophyll/ Carotenoid and SPAD Readings

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*Leaf N associated with woody plant health ranged between **1.7% and 2.5%** with **values less than 1.7%** being associated with a **low foliar N** content. SPAD value less than 25 are considered the level when N fertilization should start in trees to prevent N-related deficiency.*

The single photon avalanche diode (SPAD), such as the Minolta SPAD-502 (Spectrum Technologies, Inc., Plainfield, IL, U.S.), is a chlorophyll content meter that is commercially available, portable, and used to measure "greenness" based on optical responses when a leaf is exposed to light that in turn is used to estimate foliar Chlorophyll (Chl) concentrations (Kariya et al. 1982). The SPAD-520 makes instantaneous and non-destructive readings on a plant based on the quantification of light intensity (peak wavelength: approximately 650 nm: red light-emitting diode [LED]) absorbed by the tissue sample. A second peak (peak wavelength: approximately 940 nm: infrared LED) is emitted simultaneous with red LED to compensate for thickness of the leaf (Hoel 1998). Percival et al. (2008) found, irrespective of the species that they investigated, *Acer pseudoplatanus, Quercus robur* and *Fagus sylvatica*, high correlations were recorded between SPAD readings, total leaf Chl and carotenoid content, foliar nitrogen (N) content, and leaf photosynthetic efficiency as measured by chlorophyll fluorescence Fv/Fm values; however, a poor correlation between SPAD values and the ratio of total Chl: carotenoid were obtained.

Much of leaf N is incorporated in Chl, so quantifying Chl content gives an indirect measure of nutrient status (Richardson et al., 2002). However, Chl generally accounts for less than 10% of a plant's total N, whereas proteins account for approximately 80% of total plant N (Imsande, 1998). Additionally, the assessment of leaf photosynthetic pigments (Chl and carentoids) is an important indicator of senescence because breakdown of leaf Chl is associated with environmental stress (Brown et al., 1991). Another indicator of stress is the variation in total Chl/carotenoids ratio. A rapid increase in total leaf carotenoid content versus Chl is a widely recognized plant response to stress (Hendry and Price, 1993). Kitao et al. (1998) found that deciduous tree leaves showed their maximum photosynthetic performance in late-June, in their study, which occurred 3-months after first flush at their location.

In higher plants, carotenoids generally consist of 7% to 9% of total leaf photosynthetic pigments, consistent with values indicated by Hall and Rao (1999), Lawlor (2001), and Percival et al. (2008). Chl contains N but carotenes do not. Therefore, when N deficient plants are given N they increase their Chl concentrations but not carotenes and may develop a dark-green hue (Terry 1980; Val et al., 1987). This explains why trees fertilized with only N can be more susceptible to insect and disease infestations as the Chl: carotenoid ratio is imbalanced. Carotenoids are the "quenching" substances in the plant. Initial plant stress response is stomatal closure to conserve transpirational water loss, this in turn results in the production of high-energy reactive oxygen species (ROS) such as superoxide and singlet oxygen (Lawlor, 2001). Buildup of ROS results in oxidization damage to leaf membranes, i.e., chlorophyll bleaching and cellular membrane destruction. To minimize the effects of oxidative stress, plants have evolved an **antioxidant system consisting of carotenoids** that function as protective photooxidative pigments responsible for the **quenching of these ROS** (Kraus and Fletcher, 1994). Because an increase in total leaf carotenoid content is a widely recognized plant stress response (Peñuelas and Filella,1998), quantification of total leaf content can provide indicators of plant responsiveness to stresses frequently encountered in urban and landscape environments (Hendry and Price, 1993; Strauss-Debenedetti and Bazzaz, 1991; Vieira, 1996). In the Percival et al. (2008) study, SPAD readings for *Acer* and *Quercus* ranged from 1 to 49. These were slightly higher on the low end, and slightly lower on the high end, than those reported by Richardson et al. (2002) for *Betula papyrifera*.

Cresswell and Weir (1997), working with woody plants in Australia, found the percentage leaf N associated with woody plant health ranged between **1.7% and 2.5%** with **values less than 1.7%** being associated with a **low foliar N** content. Percival et al. (2008) investigated the relationship of % N to SPAD and Chl. Percival et al. (2008), found in their genera of *Acer, Fagus* and *Quercus* sp. that SPAD critical values of between 22 and 25 correlated to Cresswell and Weir's (1997) low foliar N of 1.7%. Consequently, Percival et al. (2008) concluded SPAD value less than 25 are the level when N fertilization should start in these species to prevent N-related deficiency problems. Perry and Hickman (2000), did a survey of 25 woody landscape plants species in California with species from the following genera, *Betula, Fagus, Buxus, Abies, Fraxinus, Ilex, Juniperus, Larix, Pinus*, and *Picea*. Perry and Hickman (2000) only measured leaf % N and found for *Quercus lobata* leaf concentrations of total N ranged from a minimum of 2.14%, to a maximum of 2.85%, and an average of 2.32%. *Acer saccharinum* leaf concentrations of total N ranged from a minimum of 2.03, to a maximum of 3.39%, and an average of 2.52% (Perry and Hickman, 2000) and *Betula pendula* had a minimum of 2.16%, to a maximum of 3.39%, and an average of 2.69%. (Perry and Hickman, 2000). Percival et al. (2008) N levels for *Acer pseudoplatanus* ranged from 0.25% to 3.25% with coinciding SPAD readings between 0.5 – 49. N levels for *Quercus robur* ranged from 1% to 3.4% with coinciding SPAD readings of 1-49 (Percival et al., 2008).

There are several discrepancies in the reported leaf %N levels of Cresswell and Weir (1997) for general woody plant health and those reported by Perry and Hickman (2000), for the two genera that were common to those investigated by Percival et al. (2008). Percival et al. (2008), reported *Acer* minimum N% levels were considerably lower than Cresswell and Weir (1997). Perry and Hickman (2000), although, their maximum values reported did concur with Percival et al., (2008), they did not with Cresswell and Weir (1997). Likewise, Percival et al. (2008), *Quercus* minimum %N levels were considerably lower than Cresswell and Weir (1997) and Perry and Hickman

(2000). Percival et al. (2008) *Quercus* %N, however, was much higher than reported by Perry and Hickman (2000) and Cresswell and Weir (1997).

Standard Practices

Many studies support that top dress applications of N fertilizers in landscape or nursery field trees provided **no benefit** in growth versus **no** fertilizer (Day and Harris, 2007; Harris et al., 2008; Robbins, 2006). In other words, doing nothing was as good as fertilizing with N. Also, N fertilizers did not speed establishment, increase shoot extension or leaf nitrogen (Day and Harris, 2007). However, three factors that affect tree caliper growth beyond N application rate and timing, that possibly confounded these "no benefit" studies were illustrated by other researchers and include, soil compaction, ground cover(s) present, and N form (Fare, 2006; Rao and Rains, 1976; Warren et al., 1993). Nitrate is the preferred form of N application if soils have high pH (above 6.0). Even though Nitrate N is preferred in alkaline soils, Nitrate absorption is more rapid at low pH (Rao and Rains, 1976). Another, issue is type of fertilizer. Controlled release fertilizers (CRF's) which contained minor nutrient packages were found to significantly increase caliper growth and time of development in a study by Mathers et al. (2012) which took place over three years. CRF's containing increased Zn and Mn may be more important to the growth of Red Maple (*Acer rubrum* 'Red Sunset') versus Chanticleer Pear (*Pyrus calleryana* 'Chanticleer') or Red Oak (*Quercus rubra*). N alone provided little response with maple indicating N was not limiting. The fertilizer containing the lowest amount of N and Fe was most beneficial to Red Oak (*Quercus rubra*). Chanticleer Pear (*Pyrus calleryana* 'Chanticleer') was the only species that responded as expected to increased N fertilization, growing larger with more N.

Dr. Elton Smith (1991), performed the only long term (18 years) study ever conducted in woody nursery/ landscape trees and created many of the current recommendations still used today. Dr. Smith's recommended rates for top dress (surface for nursery) or (drilled, sub-surface or surface for landscape) were 220 to 264 lb/ac (nursery) (Smith, 1991), or 6#/ 1000 ft2 or 29.3 g/m^2 (landscape) (Smith, 1986). Smith (1991) was conducted with *Tilia cordata* 'Select,' *Malus* 'Snowdrift' and *Acer* *saccharum* ‘Monumentale.’ Despite soluble agricultural grade (SAG) fertilizers having been found to prove no benefit and CRF’s with minor package being found to be effective, SAG’s are still commonly used in nursery. Soluble or controlled release fertilizers (CRF’s) are used in landscape, however, these do not always contain minor packages. The applications of soluble fertilizers are normally split (spring and fall) to be completed in late spring on or before mid to late June and mid to late autumn before a normal freeze. Thus, the questions of when? – with what? and rate? continue in woody plant studies and practices today.

Impact of pH

Nutrient availability to plants is affected more by pH than by any other factor. In high pH soils, ions of micronutrients such as iron (Fe) and Manganese (Mn) precipitate and the availability of these elements decreases. Plants, thus, may express deficiencies of iron (Fe) and manganese (Mn) in these conditions. Phosphorus may also become deficient in alkaline conditions as it complexes with calcium (Ca) to form insoluble calcium phosphates. Deficiencies of most of the micronutrients can be corrected by adjusting soil pH. However, it is very difficult to adjust pH downwards. It is much easier to raise pH. In some cases, the soil pH maybe so high that the sulfate reducing bacteria necessary to convert elemental sulfur (S) to the only form in which plant can take it up (SO_4^-) are in such low numbers that no conversion will occur (Reisenauer et al., 1973). Many region of the Midwest have high pH or alkaline soils and have the scenario listed above, where sulfate reducing bacteria are limited.

Transplant mortality is a significant concern and expensive in terms of replant, warranties, customer satisfaction and time for landscape managers. Also, in the landscape correcting nutrient deficiencies is more important early in the life of the tree versus as the tree matures. Smith (1991) showed that trees over 6 years of age showed a marked decline in response to fertilizer or requirement. Smith (1991) showed that *Tilia* declined in nutrient requirements by 16 years, *Malus* at 10-12 years and Struve (2002) indicated *Acer* at 14 years.

Three common caliper trees sold for the urban landscape market in the Midwest are *Quercus ellipsoidalis* (Northern Pin Oak), (5 – 6.5) *Acer rubrum* 'Frank Red' (Red

Pointe® Maple), and *Betula nigra* 'Cully' (Heritage Birch). All of these species prefer acidic soils and as pH increases above 7.0, iron (Fe) chlorosis develops with pin oak which prefers pH 5-6.5, and 'Cully' birch which prefers pH 3 to 6.5 and manganese (Mn) chlorosis with Red Pointe maple which prefers 5.1 to 7. The preferred pH ranges cited by species above, agree with the greatest availability of Fe or Mn for these species. Not only do genera and species differ in pH preferences, but differences exist in ability to absorb and translocate nutrients and ability to accumulate. The difference in Fe requirements, for instance, are much higher in *Quercus palustris* (nursery average 139 ppm) versus *Betula nigra* (survey average 107 ppm) (Jones et al., 1991) although both develop Fe chlorosis. Whereas, with Mn, *Acer rubrum* has a lower demand (survey average 20 ppm) versus *Betula nigra* (survey average 29 ppm) and a high demand for Fe (683 ppm) (Jones et al., 1991) even though *Acer rubrum* will develop Mn chlorosis and *Betula nigra* Fe chlorosis.

Fig. 1*.* *Quercus ellipsoidalis* (Northern pin oak) June 2017. The accepted level of Fe for Pin oak (*Quercus palustris*) (closest related species) taken at the same as used in this experiment is 139 ppm (Jones et al., 1991) and %N is 1.97% which correlates reasonably with Cresswell and Weir (1997) and Percival et al. (2008) critical level of foliar N. The tree shown above was growing in soil pH of 7.8 in SW Ohio and were critical low in Fe at 33.2 ppm and border line for %N at 2.2.

Iron (Fe)

Pronounced chlorosis of either Fe or Mn deficiency results in reduced chlorophyll (Chl) synthesis (Abadía et al., 2011). Fe deficiencies actually reduce all the photosynthetic piments in leaves, i.e. (Chl) *a*, Chl *b*, carotene, and xanthophyll (Terry, 1980). Carotene and xanthophyll are the two groups that make up carotenoids in plants. Chl is essential not only as a photosynthetic pigment, but also as a structural component in living organisms. The reduced level of Chl molecules decreases the photosynthetic efficiency (Wang et al., 2018). Fe is also a cofactor in at least 139 enzymes that catalyze unique biochemical reactions. Fe thus plays many essential roles in plant growth and development including thylakoid synthesis and chloroplast development but also as indicated above photosynthetic pigments synthesis).

Fig. 2. *Betula nigra* 'Cully' (Heritage Birch) June 2017. The accepted level of Fe for Heritage Birch, taken at the same as used in this experiment is **107 ppm** (Jones et al., 1991) and %N is **1.5%** which correlates with Cresswell and Weir (1997) and Percival et al. (2008) critical level of foliar N. Starting in the top left corner of the picture and moving left to right are leaves from an experiment conducted in 2017-2018 in SW Ohio and numbered treatment 1, 2, 3. Staring in the bottom left corner and moving left to right are treatment 4, 5 and 6. %N was never at a critical level for these birch trees/ leaves; however, Fe was critical in treatment (1) 84.5 Fe ppm, (2) 67.8 Fe ppm, (3) 69.1 Fe ppm, (5) 73 Fe ppm and (6) 98.3 Fe ppm. Treatment four was borderline for Fe deficiency at 102 Fe ppm.

Manganese (Mn)

Mn plays an important role in oxidation and reduction processes in plants, such as the electron transport in photosynthesis (PS), especially PSII (Jones et al., 1991). Mn also plays a role in Chl production and activates IAA oxidases (Jones et al., 1991). Mn acts as an activating factor which causes the activation of more than 35 different enzymes (Mousavi, et al, 2011). Due to the metabolic role of Mn in the nitrate-reducing enzyme activity and activation of enzymes which play roles on carbohydrate metabolism, use of fertilizers containing Mn increases efficiency of photosynthesis and carbohydrates synthesis such as starch (Mousavi et al, 2011). Mn deficiencies decrease PS efficiency and therefore crop yield and quality are reduced in turn. Mn is taken up and transferred in the form of Mn^{2+} in plants (Mousavi, et al, 2011). Transfer in the meristematic tissues is gradual, thus the young organs of plants are rich of Mn and deficiencies show first in young tissues as does Fe deficiencies. Nitrogen (N) deficiencies by contrast appear first in older tissue. This is due to nutrient mobility in the plant, with Fe and Mn are phloem non-mobile, and N is phloem mobile. Calcareous soils, soils with high pH and especially in soils with poor ventilation (compacted) are prone to Mn deficiency.

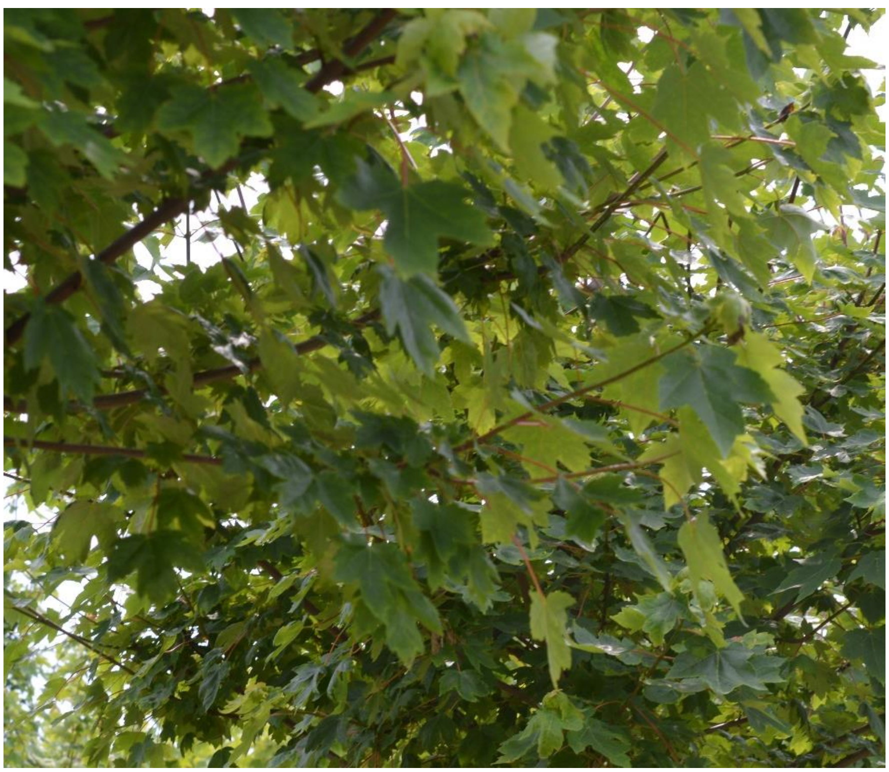

Fig. 3. *Acer rubrum* 'Frank Red' (Red Pointe Maple) June 2017. The accepted level of Mn for red maple (*Acer rubrum*) (not specific to cultivar) taken at the same time as used in this experiment is 20 ppm (Jones et al., 1991) and %N is 2.61% (Jones et al., 1991) which does not match Cresswell and Weir (1997) or Percival et al. (2008) critical level of foliar N of 1.7%. In the tree/leaves above %N is deficient at 2.5 and Mn ppm is border line deficient at 29.7.

Nitrogen (N)

Nitrogen is one of the most widely distributed elements in nature. It is present in the atmosphere, the lithosphere and the hydrosphere. The atmosphere is the main reservoir of N (Delwiche, 1981). The soil accounts for only a minute fraction of lithospheric N, and of this soil N, only a small proportion is directly available to plants. Both nitrate ($N0_3^-$) and ammonium (NH_4^+) are forms of N that can be taken up and metabolized by plants. Nitrate is often a preferential source for plants, but much depends on the plant species and other environmental factors. A number of reports indicate that the uptake of both N-forms is temperature dependent, rates of uptake being depressed by lower temperatures (Clarkson and Warner, 1979). The most important difference between $N0_3^-$ and NH_4^+ uptake is in their sensitivity to pH. NH_4^+ uptake takes place best in a neutral medium and it is depressed as the pH falls. The converse is true for $N0_3^-$ absorption. Uptake of $N0_3^-$ is more rapid at low pH values (Rao and Rains, 1976). N is the highest required nutrient in plants. It is also the most common macro-nutrient deficient in plants. Not only is N required in large amounts, it is constantly leached and volatilized away. N deficient plants are often stunted, slow growing and weak growth, small leaves, esp. older leaves. General chlorosis progresses from light green to yellow and can be accompanied by excessive bud dormancy. Chlorosis can progress to necrosis of leaves and eventually abscission in advanced stages of more severe N deficiencies (Jones et al., 1991).

Conclusions

Despite the discrepancies reported above and the lack of specific information for the three species we are discussing, we assume the common genera values of the **1.7% N level** reported by Cresswell and Weir (1997) and supported by Percival et al. (2008), are the critical N level for *Quercus* and *Acer* as found by Percival et al. (2008) with **coinciding SPAD readings of less than 25**. In a study, we recently concluded with the species discussed in this article (Mathers et al., 2019) and based on the other research reported above, we found that the SPAD-502 meter was a useful tool in determining when to fertilize with the critical %N, Fe and Mn levels lined up with SPAD readings below 25. Considering that it is difficult to get a response from N fertilizer applied to trees, as it is difficult to know when and with what fertilizer to use, it seems the SPAD-502 meter may provide help with your fertilizer budget decisions. The cost of natural gas and thus NH_4 has remained quite stable since 2016, but as we know, under-fertilizing, or over-fertilizing with only N, always cuts into the bottom-line and reduces transplant survival including winter hardiness, all the while increasing pest and disease controls, and establishment times. Try the SPAD – research shows it helps and it seems we can use the help!

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