

Using the SPAD 502 Meter to Assess Chlorophyll and Nitrogen Content of Benjamin Fig and Cottonwood Leaves

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SUMMARY. A Minolta SPAD-502 leaf chlorophyll meter was used for nondestructive data collection on the chlorophyll and nitrogen (N) status of benjamin fig (*Ficus benjamina*) and cottonwood (*Populus deltoides*) to quantitatively evaluate foliage quality. The goal was to provide a specific calibration for interpreting SPAD data within a media study for each species. Triplicate SPAD readings were collected from each of six leaves, sampled from forty plants per species, then processed for foliar analysis. Leaf tissue disks were also collected directly over SPAD testing sites for chlorophyll concentration measurement. Significant linear correlations were found between SPAD data and chlorophyll concentrations ($r^2 = 0.90$ in benjamin fig and $r^2 = 0.91$ for cottonwood). A significant, but lower correlation was found between SPAD data and N concentration. The SPAD-N correlations improved from the fifth month to the ninth month harvest ($r^2 = 0.32$ to 0.53 for benjamin fig and 0.26 to 0.42 for cottonwood). The SPAD-502 could be useful for in landscape plant

management, and in time for production situations, but baseline data is needed. Consistent protocol in sample collection and seasonal timing is needed prior to use as a predictor for tissue N levels. Development of species, and perhaps cultivar, specific baseline data and sampling procedures will need development, but could yield a rapid, quantitative, in expensive field diagnostic for foliage quality for making cultural management decisions.

Management of amenity trees, public and private, is increasingly important in a time of elevated environmental consciousness. Plant nutrient status is of interest to landscape managers, as this factor not only affects aesthetic value, but also vigor, susceptibility to pests and diseases and ability to tolerate environmental stress. National standards (American National Standards Institute, 1998) and professional organizations are calling for more technical prescription based fertilization recommendations (Lilly, 2001) for landscape plants as part of a total plant health care approach to landscape management. Additionally, increasing sophistication of production protocols are impacting nutrient and irrigation management in nurseries (Ristvey et al., 2001; Yeager et al., 1997). Field diagnostic tools need to be examined to objectively evaluate nutrient status or foliage quality as a basis for management decisions on cultural practice or for proactive monitoring in a maintenance regime. Tissue sampling and/or other laboratory analyses can become cost prohibitive not only in testing fees, but in the time lost between field sampling and final reports from testing labs, limiting the ability to use best practices over numerous sites and species. Methods to evaluate foliage quality need to streamline the cost and time to interpretable data for nutrient regime or cultural recommendations.

Nitrogen in the root zone influences plant growth and yield in production. The tendency to overfertilize increases maintenance costs and labor, and may result in surface and groundwater pollution (Lea-Cox, 2000; Ristvey et al., 2001). Nitrates (NO_3) in particular are especially mobile in the soil and can readily move through the soil with water. In addition to the economic costs of fertilizer wastage,

contamination with NO_3 in urban and arable areas constitutes a potential human and livestock health hazard (Stevenson, 1986). Other detrimental effects of excessive, or ill-timed fertilization can include cold damage underscoring the importance of developing convenient tools to insure appropriate fertilization programs (Hawkins et al., 1996; Raese, 1997).

Nutrient deficiencies or toxicities are traditionally diagnosed by soil tests and plant tissue analysis. Although these tests provide accurate information for predicting plant nutrient status, they are costly in terms of time and money to the end-user. In addition, these methods require sophisticated laboratory equipment that is often unavailable to the common user. Delay as a result of sample collection and laboratory work may also prevent timely remedial response.

The Minolta SPAD-502 meter (Spectrum Technologies, Plainfield, Ill.) is a hand-held light meter used to measure the relative greenness of leaves in a rapid manner. SPAD is an acronym for soil plant analysis development (Wood et al., 1992). The SPAD meter was designed originally in 1963 for N management in rice (*Oryza sativa*) production in Japan (Wood et al., 1993). The latest model, SPAD-502 determines the relative amount of chlorophyll present by measuring the transmittance of the leaf in two wave bands (600 to 700 and 400 to 500 nm). It gives a reading in arbitrary units, that is proportional to the amount of chlorophyll present. The device is pressed onto the leaf surface and a relative nondestructive greenness reading is taken in a few seconds.

The utility of SPAD meter use has been extended to other major crops and is now widely accepted in the agronomy industry due to excellent correlation of SPAD-502 readings with chlorophyll content and N status of crops. Leaf chlorophyll content is often well correlated with leaf N status and photosynthetic activity (Evans, 1983; Seeman et al., 1987). Among others, the SPAD-502 meter had been accurate in predicting chlorophyll and N levels in rice (Takebe and Yoneyama, 1989); maize (*Zea mays*) (Wood et al., 1992) and wheat (*Triticum sativum*) (Follett et al., 1992).

Results with horticultural crops have been less satisfactory. Though the relationship between chlorophyll

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content and SPAD-502 readings were still maintained (Yadava, 1986), there was greater variability when attempts were made to correlate N status with SPAD-502 readings. For example, Himelrick et al. (1993) conducted a trial with 'Chandler' strawberry (*Fragaria xananassa*) trial and found poor correlation between SPAD-502 readings and total NO₃ in leaf blades and petioles. Others working with red maples (*Acer rubrum*) (Sibley et al., 1996) and potatoes (*Solanum tuberosum*) (McLaskey, 1997) found either nonsignificant or weak relationships. Li et al. (1998) demonstrated the ability to use SPAD data to predict leaf N status in evaluation of fertilization programs for grapefruit (*Citrus paradisi* on *C. aurantium* rootstock) during the spring flush.

The reason for the reduced ability of chlorophyll meters to predict horticultural crop N needs is not entirely clear. There have been speculations that one reason could be the greater complexity of N source-sink relationships of perennial plants (Wood et al., 1993). Leaf position variation and time of sampling might also be possible factors (Linder, 1980; Neilsen et al., 1995). Leaf thickness was also found to be an additional factor to consider for interpretation leaf N using the SPAD meter (Peng et al., 1992).

The purpose of these experiments was to determine the feasibility of using the SPAD-502 on two tree species to generate a calibration baseline for assessing foliage quality, linking the SPAD data to chlorophyll concentration and leaf N levels. The calibration data was needed for evaluation of the selected species in a pavement material root zone study (Loh, 2000). Benjamin fig was selected to represent an evergreen urban tree species commonly associated with pavement displacement in tropical environments. Cottonwood was used to gather background data for its use as an vigorous test plant for future root growth/pavement displacement studies.

Materials and methods

PLANT MATERIAL. The material in this study was obtained from plants grown in separate greenhouses at Ithaca, N.Y., as part of a substrate evaluation study (Loh, 2000). Benjamin fig was grown under natural photoperiod and irradiance. Greenhouse temperature set points were 30

°C (86 °F) day and 25 °C (77 °F) night temperatures. They were manually watered daily to ensure that water stress was minimized (Loh, 2000). The cultural conditions for cottonwood were similar except that the temperature set points were 25 °C by day and 18 °C (64 °F) by night. Test subjects for both species were clonal. Tissue-cultured benjamin fig plugs [14 to 20 cm (5.5 to 7.9 inches)], and rooted cuttings of a locally growing cottonwood specimen [35 cm (13.8 inches)]. 120 trees per species were set as a split-split plot design over four stone-soil substrates containing sandy loam soil diluted with 3.8 to 5.1 cm (1.5 to 2.0 inches) crushed stone to yield varied soil concentrations over varied total root zone volumes (Loh, 2000). The plants were grown for 5 months and had visually different leaf colors indicating a testable range of chlorophyll concentrations for SPAD-502 calibration due to soil dilution in the treatment levels (Loh, 2000). Plants for sampling were chosen to provide a visual range of color, and maximize the calibration range of the SPAD meter for use in evaluation of treatment effects throughout the study.

CHLOROPHYLL MEASUREMENTS.

Single fully expanded leaf samples from 25 plants of each species were obtained at the fifth month in culture, on 15 June 1999. Triplicate readings using a SPAD-502 were taken around the midpoint near the midrib of each leaf sample and averaged (Peng et al., 1992). A 10-mm-diameter (0.4-inch) leaf disc was then obtained from this area for its corresponding chlorophyll determination. Chlorophyll was extracted by N,N-dimethylformamide (DMF) and analyzed spectrophotometrically using the methods described by Moran and Porath (1980). The leaf discs were weighed before further analysis to ensure that the efficiency of extraction was not unduly influenced by differing leaf thickness. Leaf disks were kept in vials of DMF in the dark at 4 °C (39 °F) for 30 h. This duration was determined to be the optimal extraction time in a prior experiment for the plant species and laboratory conditions (Loh, 2000). The extracts were assayed using a spectrophotometer (model 552; Perkin Elmer; Shelton, Conn.) at 647 nm and 664 nm. Total chlorophyll concentrations (converted to µg·g⁻¹ dry tissue) were calculated based on Moran's formula [chloro-

phyll total µg·mL⁻¹ = 7.04 (absorption at 664 nm) + 20.27 (absorption at 647 nm)] (Moran, 1982).

NITROGEN CONTENT DETERMINATION.

Multiple leaf samples from 40 plants of each species were obtained at the fifth and ninth month destructive harvests in culture as part of the substrate study (Loh, 2000), representing whole subplots of the split-split block design. The two data sets were compared for differences with respect to the sampling time as part of the formal treatment analysis of the related substrate study. To provide sufficient plant material for tissue analysis, six leaves were pooled from a single plant to form one sample. Whole healthy leaves were sampled randomly from the plant, avoiding the first and last three leaves from any one shoot or apex. The mean of three SPAD-502 measurements taken around the midpoint near the midrib of each leaf was used in calculating the six leaf sample mean as the SPAD datum linked to a given tissue sample. Plant tissue analyses were conducted at the Fruit and Vegetable Science Analytical Laboratory of Cornell University (Ithaca, N.Y.). Sample N content was determined using the Kjeldahl method (Wolf, 1982). A dry ashing procedure (Greweling, 1976) and analysis for other tissue nutrient levels was conducted due to check N levels for data consistency with the Kjeldahl method. The additional data on other tissue nutrient levels were analyzed for interference with the SPAD-502 readings by other nutrient deficiencies or toxicity (Turner and Jund, 1991). While multiple mineral component variables added to a regression model with a best subsets regression procedure yielded statistically significant improvements to any specific data set on a given date, the data was not conclusive and minimally effective in the calibration of the tool for diagnostic purposes (Loh, 2000) and is not reported. Nitrogen content was verified at the end of the experiment by running three parallel leaf samples through both the Kjeldahl method and a N/protein combustion analyzer (model NA 2100; Carlo Erba Instruments, Raleigh, N.C.).

DATA ANALYSIS. Data from each sampling set were analyzed using regression procedures of MINITAB Release 12 (MINITAB Inc., 1998). Further analysis to detect differences between various regression lines were

performed using GLM procedures of SAS Version 6 (SAS Institute, 1990).

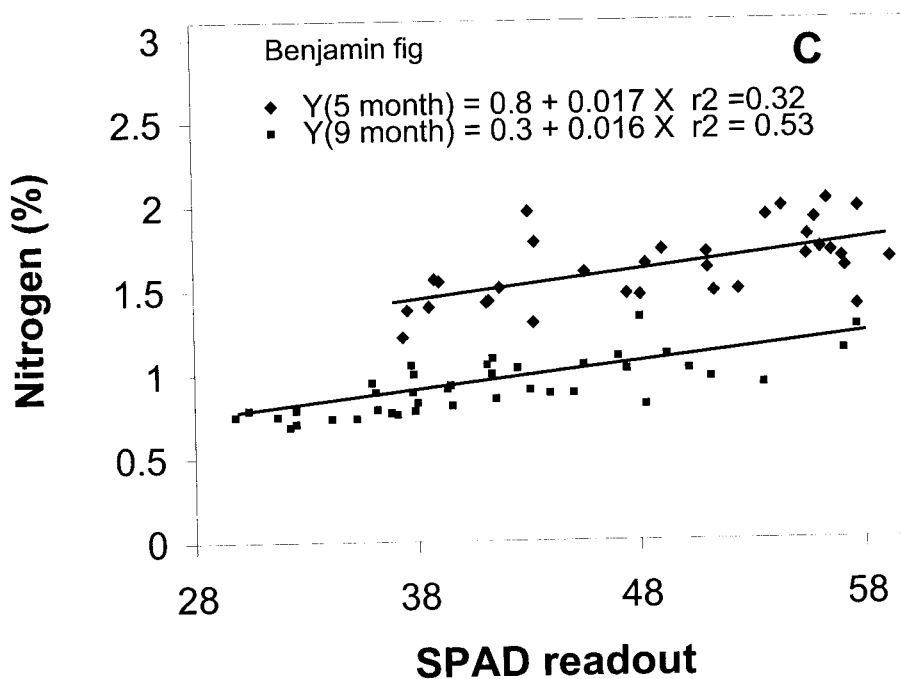
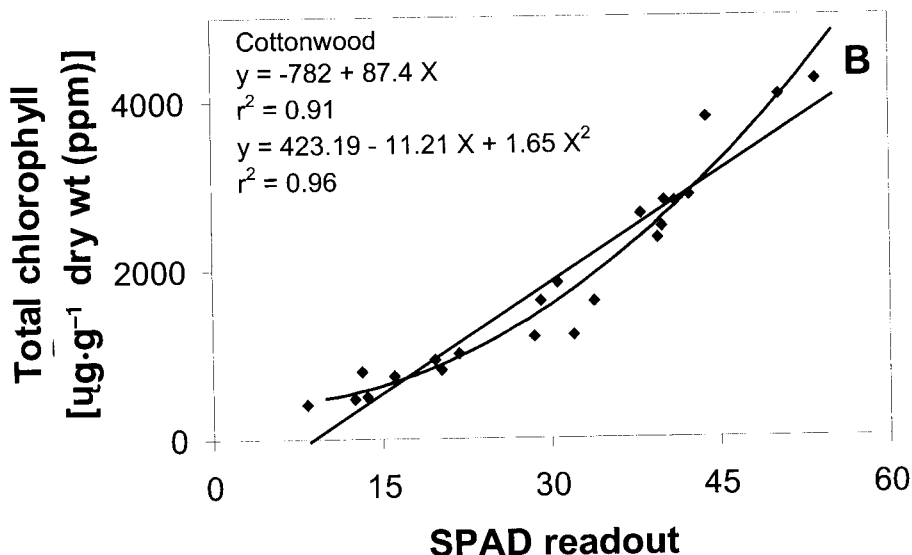
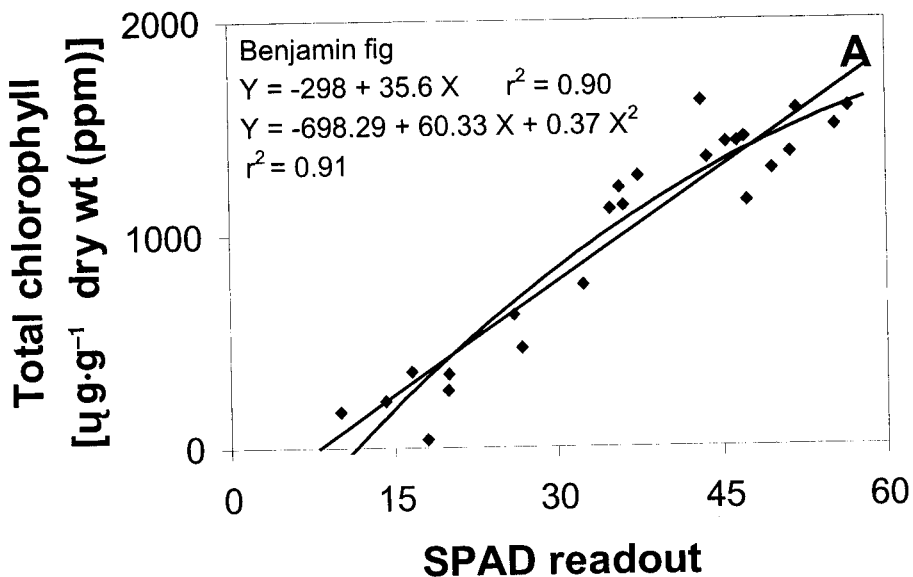
Results and discussion

RELATIONSHIP BETWEEN LEAF CHLOROPHYLL CONCENTRATION AND SPAD-502 READINGS. Measured SPAD-502 readings were significantly correlated with their chlorophyll content ($P < 0.001$ for both benjamin fig and cottonwood). Linear models were adequate in explaining the relationships (Fig. 1A and B) with r^2 of 0.90 and 0.91 for benjamin fig and cottonwood, respectively. Consistent with findings by other workers (Marquard and Tipton, 1987; Schaper and Chacko, 1991) who reported that SPAD-502 meters give differing prediction responses for different plant species, the calibration lines from this investigation were species specific. Thus, any attempt to draw calibration models necessitate individual regression models be developed for each particular species and cultivar. The quadratic model proposed by Castelli et al. (1996) produced an insignificantly different fit with the benjamin fig data ($r^2 = 0.91$), and an improved fit the cottonwood data ($r^2 = 0.96$) (Fig. 1B).

RELATIONSHIP BETWEEN LEAF N CONTENT AND SPAD-502 READINGS.

Linear correlation of leaf N concentrations with their corresponding SPAD-502 meter readings were significant, but of limited predictive value as a calibration line (Fig. 1C and D). The correlation for both species improved on the second harvest (Fig. 1C and D). Significant differences between the early spring and late summer harvests were based on regression line slopes for benjamin fig ($P_{\text{slope}} = 0.04$, $P_{\text{intercept}} = 0.06$) and intercepts for cottonwood ($P_{\text{slope}} = 0.18$, $P_{\text{intercept}} = 0.001$). Thus, for prediction purposes, sampling time is an important consideration and the data could not be pooled.

There are several possible reasons for variation in the N-SPAD relationship. An actively photosynthesizing leaf in spring may harbor pools of nonchlorophyll related N (for example, free NO_3 in vacuoles waiting to be transported out to other sinks) (Marschner, 1995). The pools of N will not be captured by the SPAD-502 meter, thus registering a lower reading. The process of NO_3 reduction and assimilation in leaves involves the enzyme nitrate reductase. Santoro and



Magalhaes (1983) found that the activity of this enzyme decreases rapidly with leaf age. Thus, it is highly possible that the older summer leaves have a higher proportion of their N in the NO_3^- form; and this is not reflected in the Kjeldahl procedure.

While commonly used, the Kjeldahl method has the drawback of assaying only a portion of the nonreduced N (NO_3^-) (Mills and Jones, 1996). If the leaf type contains a substantial proportion of N in this form, the precision of the method may be questionable. The discrepancy between the Kjeldahl pro-

cedure and the combustion method when parallel samples were examined was not large enough (Fig. 1E) to fully account for the N content differences observed earlier between spring and summer leaves. The Kjeldahl procedure was able to capture more than 80 percent of the true N content for the points tested at the end of the study.

Finally, one of the sources of error is sampling difficulty. Unlike the earlier protocol for chlorophyll determination, the portions of leaf surface used for SPAD-502 measurements did not correspond exactly to the leaf discs from

which N was measured. The requirement of a sizable volume of plant material for assay demanded that six different leaves were pooled. Varying leaf thickness affects the precision of SPAD-502 meter readings (Thompson et al., 1996), thus increasing variability. Difference in leaf thickness is therefore another possible explanation for the low coefficient of determination obtained with the relationships between leaf N and SPAD-502 readings.

The SPAD-502 meter provides a quick, nondestructive measure of the relative greenness of leaves at a specific moment of time. Chlorophyll concentrations in leaves are thus well correlated with meter readings for benjamin fig and cottonwood. Currently, there are difficulties in extending this technology to detect nutrient levels, particularly N content in perennial plants. Similarly, while the tool provides a reportable datum useful in controlled settings for research application, there is a great deal of work needed if it is to be used as a field diagnostic tool as a base for cultural management decisions. Issues of sampling protocol such as leaf physiological age, position, sampling time, interactions with other mineral content, and complex source-sink relationships associated with perennial plants are likely sources of variability.

Studies have shown that most woody plants have the ability to adjust growth rates to varying levels of nutrients (Ericsson, 1981; Ericsson and Ingstad, 1988; Johnson, 1993). Thus the foliage of most landscape trees usually appears an acceptable green even though nutrient deficiencies may already be limiting growth. This will further confound the use of SPAD-502 meters in detecting nutrient deficiency situations as a single landscape field diagnostic tool.

In spite of these limitations, the SPAD-502 meter remains potentially useful as a quick nondestructive diagnostic tool for the management of woody perennials. As part of a field diagnostic process with soil tests, the tool may still be quite useful for rapid assessment of plant status in the field. A cataloging of chlorophyll values for individual species is a work with significant potential. To reduce prediction variability, a more systematic sampling procedure involving standardizing leaf position and sampling time will have to be worked out. This is similar for example to the corn industry where standards in a well-

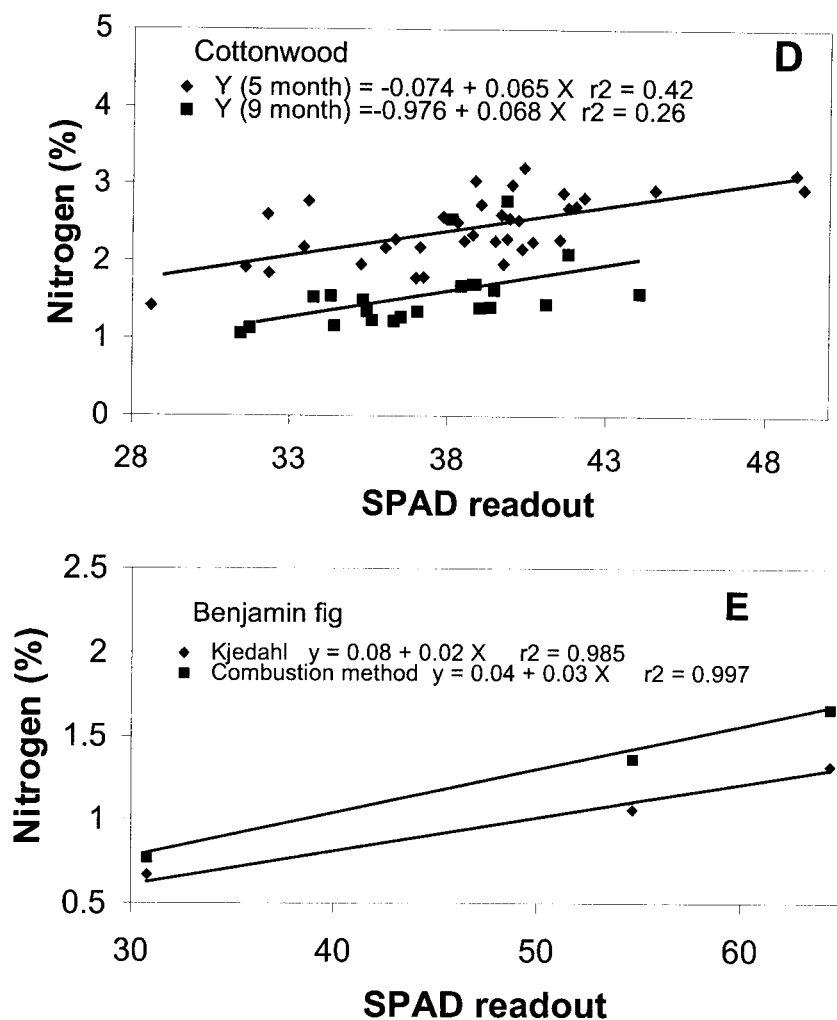


Fig. 1. Calibration curves for SPAD 502 meter relating SPAD readout to leaf chlorophyll concentration in benjamin fig (A) and cottonwood (B). SPAD data was collected in the fifth month of active growth on fully expanded highlight leaves, then processed for chlorophyll content. Calibration curves relating SPAD readout to leaf nitrogen content in benjamin fig (C) and cottonwood (D). SPAD data collected in the fifth and ninth months and processed for nitrogen content via the Kjeldahl method. Comparison of Kjeldahl and combustion methods (E) for determination of leaf nitrogen levels in benjamin fig as related to SPAD data for use in evaluating error in calibration curves to relate SPAD readout to tissue nitrogen levels. SPAD readout values represent the mean of three readings for the chlorophyll calibration sample (A, B), and 18 readings (three per leaf over six leaves) for tissue nitrogen calibration (C, D, E).

fertilized area are grown side by side with the actual crops where specific leaf sampling protocol is established. In this way, internally normalized data can be obtained from these standards and the data are calibrated for each particular field, hybrid, stage of growth, and set of cultural practices, to lend themselves a common basis for interpretation (Schepers et al., 1992). This may have significant implications for production and maintenance programs of specific landscape plants, although the feasibility of producing such a comprehensive program on a diverse species inventory in either scenario is currently highly questionable.

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