The Bioindicators of Forest Condition Project: A physiological, remote sensing approach

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Objective measures of forest ecosystem condition are needed to gauge the effects of management activities and natural phenomena on sustainability. The Bioindicators of Forest Condition Project seeks to develop a Forest Condition Rating (FCR) system using a physiological, remote sensing approach. In particular, the goal of the project is to test whether hyperspectral remote sensing may be used to infer stand-level information about pigment concentration, chlorophyll fluorescence, and other physiological features of condition. The project spans a four-year period of intensive sampling in tolerant hardwood forests in Ontario using the Compact Airborne Spectrographic Imager (CASI). For each airborne campaign, concurrent ground-based sampling for leaf physiological features was performed. Controlled laboratory and greenhouse studies were also conducted to derive relationships between leaf-based spectral measurements and physiology in the presence of environmental stresses. The project has identified several promising bioindicators of strain that are discernible from hyperspectral images and related to ground-based physiology. The most promising remote indicator for semi-operational testing is estimation of chlorophyll content, which can be used to classify maple stands on a five-stage scale of health. Chlorophyll fluorescence has also been discerned from spectral signatures, but our studies indicate it may be confounded by chlorophyll content. The intent here is to update the forestry community on progress made, insights gained, and the practical implications of the research.

Keywords: chlorophyll fluorescence, hyperspectral, indices, pigments, reflectance, tolerant hardwoods

Les mesures objectives de l'état de l'écosystème forestier sont nécessaires pour évaluer les impacts des activités d'aménagement et des phénomènes naturels sur la durabilité. Le Projet des bio-indicateurs de l'état des forêts cherche à élaborer un système de classement de la condition des forêts (FCR) au moyen d'une approche de télédétection physiologique. Plus précisément, le 'objectif du projet est de voir si la télédétection hyperspectrale peut être utilisée pour obtenir de l'information au-delà du niveau du peuplement sur la concentration des pigments, la fluorescence de la chlorophylle et d'autres éléments physiologiques de l'état du peuplement. Le projet couvre une période de quatre ans d'intense échantillonnage dans les forêts de feuillus tolérants de l'Ontario, et utilise le Compact Airborne Spectrographic Imager (CASI). Pour chacune des campagnes aéroportées, un échantillonnage parallèle au sol a été effectué sur les éléments physiologiques des feuilles. Des études contrôlées en laboratoire et sous serre ont été entreprises pour établir les relations entre les mesures spectrales des feuilles et leur physiologie en présence de stress environnementaux. Le projet a identifié quelques bio-indicateurs prometteurs de contraintes qui sont perceptibles à partir d'images hyperspectrales et reliées à un état physiologique au sol. L'indicateur le plus prometteur pour des essais partiellement opérationnels se trouve à être l'estimation du contenu en chlorophylle, qui peut être utilisé pour classifier les peuplements d'érable selon cinq classes de santé. La fluorescènce de la chlorophylle a été également discernée parmi les signatures spectrales, mais nos études indiquent qu'elle peut être confondue avec le contenu en chlorophylle. Cet article vise à informer la communauté forestière sur les progrès réalisé, les connaissances acquises et les implications pratiques de cette recherche.

Mots-clés : fluorescence de la chlorophylle, hypersprectrale, indices, pigments, réflexibilité, feuillus tolérants

Introduction

The Bioindicators of Forest Condition Project was initiated in 1996 by the Ontario Forest Research Institute (OFRI) to identify and develop physiological indicators of forest condition (Mohammed *et al.* 1997). The project focus is to develop objective measures of condition at the stand level. The stimulus for initiating the project came from two sources: (1) international and national commitments by policy-makers to develop a standard set of criteria and indicators of forest sustainability (Canadian Council of Forest Ministers 1995); and (2) an ongoing need for operational tools to assess the effects of forest management practices on forest health.

Most current assessments of forest condition are limited to ground-based visual evaluation (e.g., Canadian Forest Service 1999). While the benefits of these conventional field assessments are recognized, they do not reveal changes in physiology that characterize early stress responses. For it has been shown that physiological responses can indicate productivity and adaptability to environmental stress (Chapin 1991, Colombo and Parker 1999). Assessment of forest physiological condition may provide an early indication of decline in stand vigour and productive capacity. Early detection could help to identify stands requiring remedial or salvage action prior to the development of visible damage and, potentially, unrecoverable losses in biomass.

The Bioindicators Project does not intend to diagnose causal agent(s). For the present, we seek to develop a Forest Condition Rating (FCR) System to classify condition on a quantitative scale from healthy to stressed, relative to an acceptable range of values. This is consistent with the accepted definition of forest health where desirable ecosystem functions and process are sustained within a natural range of variability (Canadian For-

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est Service 1999). The range of variability examined here is for key spectral features that correspond to changes in physiological condition (e.g., chlorophyll content). Thus, the efforts here are directed at developing an integrative measure of stand condition rather than identifying the causal agent(s) of stress. In particular, we chose to test the feasibility of hyperspectral remote sensing as a means to link optical above-canopy signals to ground-based bioindicators of physiological status.

Hyperspectral technologies have progressed markedly in recent years and offer the advantages of rapid assessment and finely resolved spectral and spatial detail. Although hyperspectral satellites are expected to be launched in the near future (Stoney 1997, Ustin and Trabucco 2000), the spatial resolution is coarser than airborne remote sensing platforms. For this reason, the project used a Compact Airborne Spectrographic Imager (CASI), a sensor designed for high spatial and spectral resolution, enabling image data to be clearly associated with test plots. This approach follows the recommendations of a commissioned review (Dendron Resource Surveys 1997) that suggests analysis of hyperspectral information could possibly reveal indicators of physiological health across a wide range of tree species and ages.

Some work has already shown promise using airborne sensors with narrow bands to detect early damage assessment in conifer species undergoing forest decline (Herrmann *et al.* 1988, Rock *et al.* 1988). Another study indicated considerable success detecting non-visual herbicide-induced strain on a mixed conifer stand using a far-field narrow band spectrometer (Carter *et al.* 1996). The CASI has also been used in other forestry applications, such as in land classification, and in evaluation of crown closure, root rot incidence, and stem counts (Davison *et al.* 1999a, 1999b; Leckie *et al.* 1999; Reich and Price 1999).

While applications using hyperspectral sensors appear to be promising, there is still a basic need for more biological validation and interpretation of hyperspectral information before it may be used routinely in remote physiological assessments (Dendron Resource Surveys 1997, Treitz and Howarth 1999). The project team accordingly devised a strategy (Mohammed *et al.* 1997) that incorporates different levels of investigation to ensure that links between bioindicators at the leaf level and the above canopy spectral reflectance could be subjected to scrutiny. A key contribution here is the acquisition of real field data (rather than commonly used simulated data) to develop, validate, and refine predictive models of biological status in forest canopies.

We are not proposing that forest condition assessment will rest solely with remote sensing of spectral reflectance. Instead, this information can form another thematic layer with other current spatial data sources (e.g., ecological characteristics and disturbance). The benefit of incorporating a physiological data layer is that it enhances the traditional approaches of structural-based measures of productivity and condition. By combining these data layers, our physiological and ecological understanding increases. This represents the first step towards a more complete spatial representation of our forests. An early warning system would also complement efforts by others (e.g., Bonan 1993, Band *et al.* 1999) to remotely acquire estimates of net primary productivity, which can be associated with subsequent changes in leaf area at the landscape or regional scales.

Here, we present a project overview, including a discussion of the approaches used and consolidation of our findings (see also Dendron Resource Surveys 1997; Mohammed *et al.* 1997, 2000; Sampson *et al.* 1998; Zarco-Tejada *et al.* 2000a, 2000b, 2000c). We also discuss the challenges and future stages of this work. The overall intent is to update the forestry community on progress made, insights gained, and the practical implications of the research.

Methods

Development of an FCR system, as envisaged here, involves interpreting remotely sensed spectral information with the aid of ground-based assessments of structural and physiological aspects of forest condition. Research activities included controlled laboratory studies, ground-based field assessments, and acquisition of hyperspectral airborne data to identify gradients in key spectral features that might correspond to actual differences in physiology.

Study sites

Study sites were selected from existing plot networks with historical databases. Six sugar maple (*Acer saccharum* M.) sites were selected from Ontario's Hardwood Forest Health plot network (McLaughlin *et al.* 1996). This network was established in 1985 by the Ontario Ministry of the Environment and Energy in response to suspected acid precipitation and low-level ozone damage. The network extends across the range of maple in Ontario and represents over 100 permanent observation plots. Stands are assessed annually using a numerical Decline Index (DI) that combines individual crown component scores of chlorosis and the proportion of dead branches (McLaughlin *et al.* 1996). The DI was used here to select a gradient in stand condition for plots within the Algoma Region, Ontario.

Another six study sites were selected from the provincial Growth and Yield Program. This program includes 4000 permanent sample plots in managed and natural forests across the province (OMNR 1993). Sites were chosen on the basis of several productivity measures (e.g., site class and basal area). The objective was to obtain a gradient in productivity for maple plots in the Algoma Region.

The 12 maple sites in the Algoma Region constitute most of the findings reported here, where airborne and intensive field sampling were done in 1997, 1998, and 1999. To validate these findings, an additional 12 sites throughout southcentral Ontario were selected in the year 2000. In particular, six Hardwood Forest Health Plots (McLaughlin *et al.* 1996), and six sites from areas affected by the ice storm of 1998 in eastern Ontario (Lautenschlager and Nielsen 1999) were selected based on a similar rationale of suspected gradients in vigour.

Airborne, ground and laboratory studies

Airborne remote sensing was conducted with the CASI, using a 72-band configuration and a spatial resolution of approximately 2 m. For each airborne campaign (two per year), leaf photobiology assessments were conducted using tests of chlorophyll fluorescence, pigment concentrations, spectral reflectance and transmittance. Tree- and stand-level parameters were measured by traditional estimates of diameter and stocking and by measurements of leaf area index and stem electrical resistance.

In addition, basic and interpretive studies were conducted that involved laboratory and greenhouse investigations of the relationships between leaf spectra, chlorophyll fluorescence,

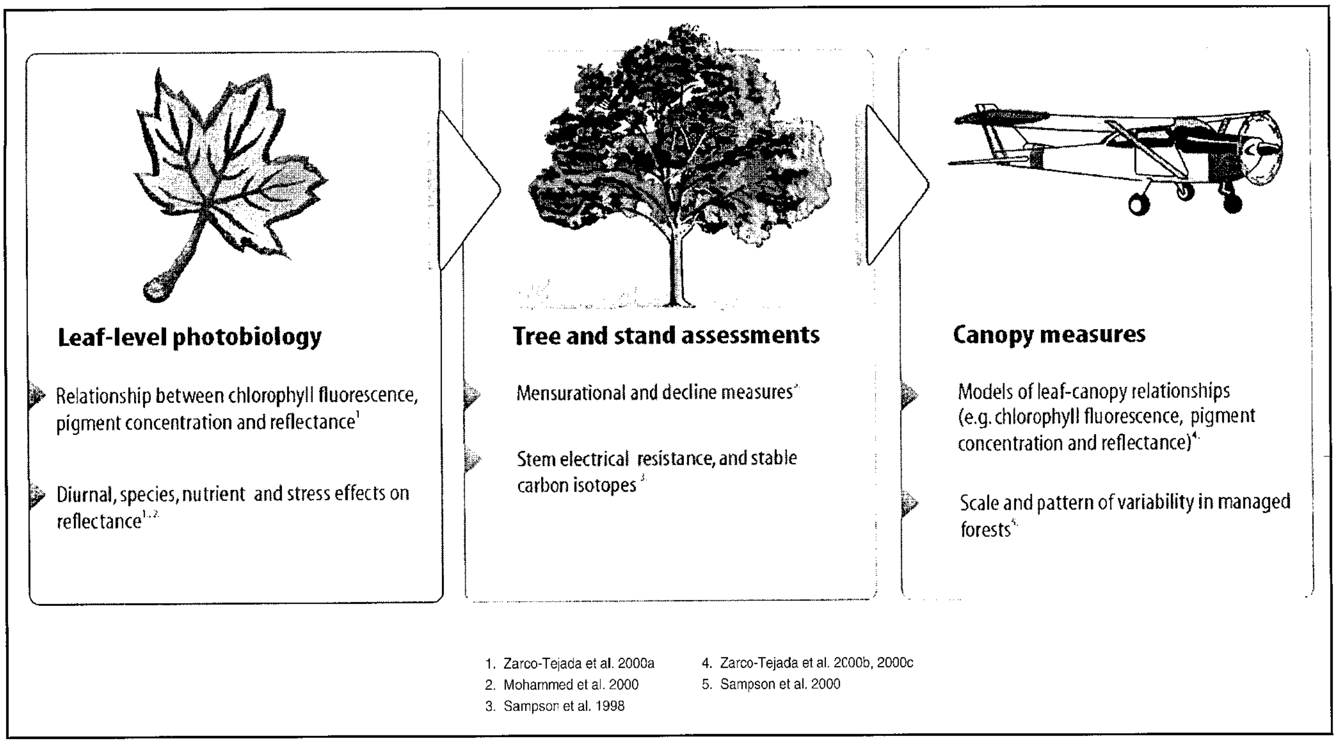

Fig. 1. Overview of approach – from leaf level investigations through to individual tree and stand assessments – and the corresponding studies used to develop bioindicators of forest condition.

and pigment concentration; diurnal effects on leaf reflectance; species effects on leaf reflectance; and other natural and stress-induced effects on leaf reflectance. At the canopy level in the field, diurnal effects on canopy reflectance, and the influence of stand structure on reflectance were studied. Bridging the leaf and field canopy-level studies were canopy CASI experiments completed in the laboratory using maple seedlings and examining diurnal effects as well as the influence of chlorophyll pigments and fluorescence on reflectance.

Detailed methods for these studies have been presented in other reports (e.g., Mohammed *et al.* 1997, 2000; Sampson *et al.* 1998, 2000; Zarco-Tejada *et al.* 2000a, 2000b, 2000c) with a general schematic of the project provided in Fig. 1. Together, these studies sought to investigate spectral relationships to physiology at the leaf level, and also to explore the potential to infer leaf-level information from canopy- or stand-level remote sensing of hyperspectral reflectance.

Results and Discussion

Leaf level studies are considered to be the foundation for the development of remote sensing sampling strategies and interpretation of data. The basic questions investigated at this level are whether spectral indices can be used to identify physiological strain prior to the appearance of visual symptoms in plants, and if so, what underlying properties or measures can be used to interpret the significance of the response. Addressing these questions provides some insight in scaling-up to the canopy level. That is, can spectral changes be measured at the stand level and, if so, what factors influence its behaviour? The reported results and discussion follow this systematic approach from leaf-level to canopy.

Leaf Level Detection of Vegetation Stress

As part of the Bioindicators Project, Mohammed *et al.* (2000) undertook a study to explore biological factors influencing leaf spectral reflectance. The first question of interest is whether early detection of strain in plants is possible at the leaf level. For this application, several spectral indices were investigated in controlled laboratory and field studies.

The use of spectral indices is a common method of measuring sensitivity of vegetation to stress (Dendron Resource Surveys 1997). For example, Gitelson and Merzlyak (1996) found a high correlation between R700/R750 and R550/R750 and the chlorophyll content of maple and chestnut (*Castanea* spp.) trees. The advantage of using ratios combining sensitive and insensitive bands is that the latter function has baselines that factor out variability due to causes other than variations in leaf chlorophyll content.

Several investigators have related changes in chlorophyll concentration to the shift in the spectral red edge – the region of rapid transition (increase) between red and near infrared reflectance (e.g., Horler *et al.* 1983, Vogelmann *et al.* 1993, and Gitelson *et al.* 1996). This shift has been associated with plant stress, forest decline and leaf development (e.g., Rock *et al.* 1988, Boochs *et al.* 1990, Miller *et al.* 1991, Hoque *et al.* 1992). These studies exploit the fact that chlorophyll and other pigment molecules (e.g., carotenoids and anthocyanins) are the principal factors affecting reflectance and absorption of radiation in the visible part of the electromagnetic spectrum (Walter-Shea and Norman 1991). A thorough review of photosynthetic pigments is provided by Stockburger and Mitchell (1999).

For the leaf-level studies in the Bioindicators Project (Mohammed *et al.* 2000), the red edge inflection point was found

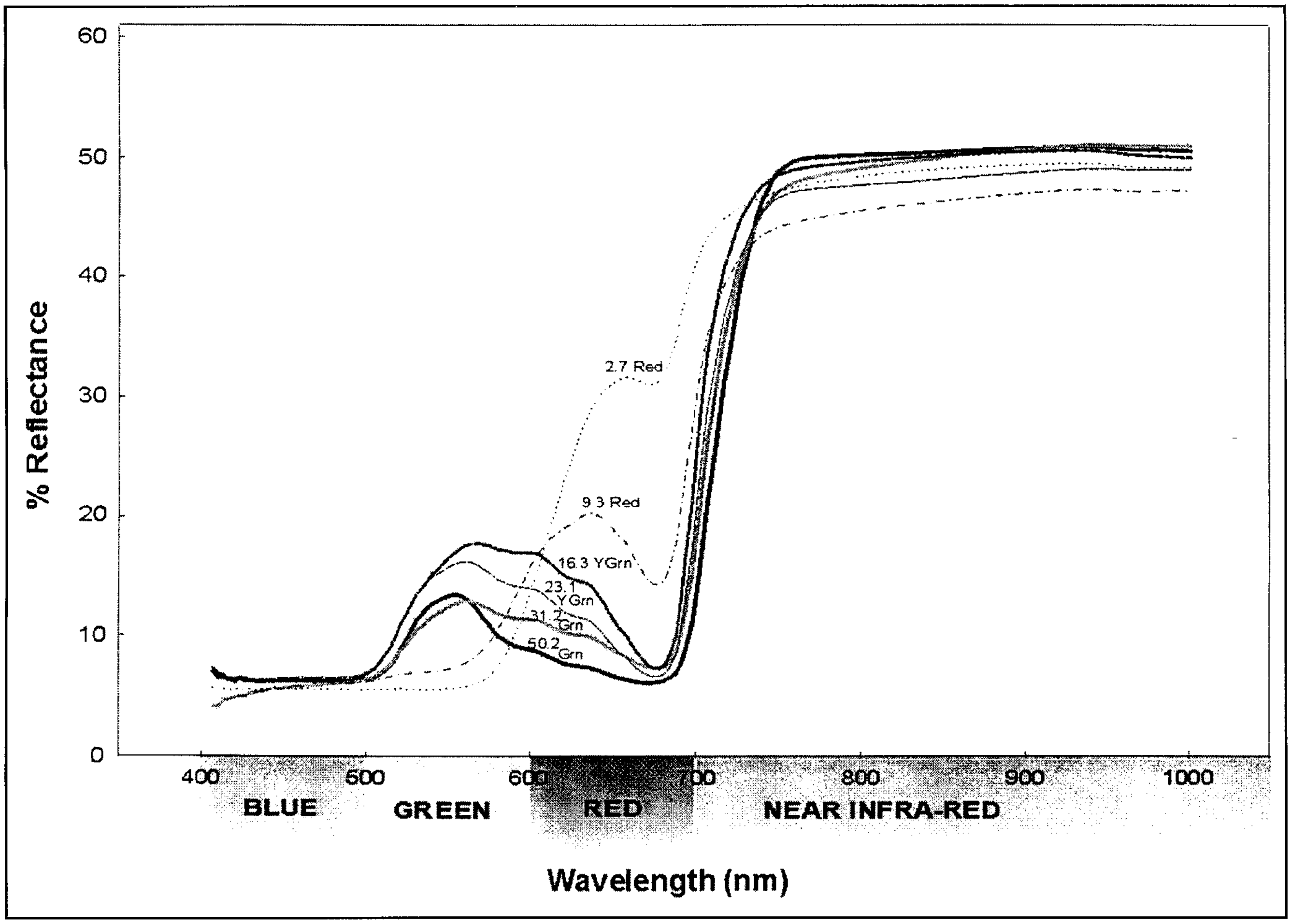

Fig. 2. Senescence effects of reflectance of maple (*Acer saccharum* M.). Chlorophyll *a* concentration in µg·cm^{-2} and leaf colour are shown (Mohammed *et al.* 2000).

to be well correlated with chlorophyll content. Also, the Photochemical Reflectance Index (PRI) reported by Peñuelas *et al.* (1995b) was responsive to senescence, herbicide, and other stresses, and effects were evident prior to the appearance of visual symptoms such as chlorosis, browning, and reduced growth. The PRI may have application across a range of species, based on our findings and earlier results of Gamon *et al.* (1997). The advantage of such indicators would be their ability to bypass the confounding effects of species. Normalized difference vegetation indices had limited correlation to pigments and other indicators of stress status. The implication of the work by Mohammed *et al.* (2000) is that certain indices such as PRI may be able to serve at the leaf level as a non-specific indicator of early strain in plants, but further study is required.

Within individual species of four coniferous species (jack pine, red pine, white spruce (*Picea glauca* (Moench) Voss), and black spruce (*Picea mariana* (Mill.) B.S.P.)) studied here, additional indices were promising previsual indicators of subsequent growth and necrosis (Mohammed *et al.* 2000). For example, an index based on the ratio of blue to red (R440/R690) wavebands produced strong correlations ($r>0.80$) with subsequent growth and necrosis in stressed plants. In contrast, neither chlorophyll content nor carotenoid concentration measured during the summer were indicative of later effects on growth and browning.

Species Effects

Forty-four species were examined here for species effects. Some general patterns were revealed, such as: lower reflectance in the near-infrared in conifer foliage compared to broadleaved species; higher blue reflectance in species with blue hues in their foliage compared to those without; and more rapid decay of the green spectral peak in deciduous tree and shrub species compared to evergreens and many herbaceous and ground-cover species. However, the influence of species was superseded by other factors within a species, such as leaf age, leaf side, and stress status.

As shown in Fig. 2, senescence in maple produces substantial changes in reflectance characteristics. This and other natural variation (e.g., sun versus shade leaves) must be taken into account when collecting samples. To minimize these effects, canopy and individual leaf sampling in the Bioindicators Project were collected under full leaf expansion prior to onset of autumn senescence. As well, the samples collected were from the upper canopy, i.e., sun leaves.

Although leaf reflectance could not provide accurate identification of species, one cannot necessarily conclude that canopy reflectance, which incorporates species-specific tree architectural effects, will be equally unsuccessful. In addition, for the purpose of assessing forest condition an important question remains as to whether it is necessary to distinguish species within a stand before applying physiological bioindicators, since some evidence suggests that optical surrogates can be used to judge stand condition.

Leaf Properties Affecting Spectral Response

While early-detection of strain was possible using spectral indices, the underlying properties of pigments must also be understood to ensure reliable interpretation. For instance, carotenoids (pigments that protect the photosynthetic reaction centres) do not absorb strongly in the red, but are found to absorb concurrently with chlorophyll in the 300–500 nm spectral region (Peñuelas *et al.* 1995a), making unambiguous interpretation of spectral changes in this region difficult. However, perhaps chlorophyll fluorescence could provide a suitable alternative, since changes in chlorophyll function can precede changes in chlorophyll content.

Chlorophyll Fluorescence

Chlorophyll fluorescence is red and far-red light produced in plant photosynthetic tissues upon excitation with natural or artificial light (Kautsky and Hirsch 1931, Papageorgiou 1975). It has been shown that chlorophyll fluorescence can be quite sensitive to changes in plant physiological properties, resulting in practical applications in assessing pre-visual signs of damage (Mohammed *et al.* 1995). Moreover, the technique possesses the advantage of being rapid, non-destructive, and non-invasive (Mohammed *et al.* 1995). Most applications of chlorophyll fluorescence in near-field studies have been with the use of high intensity lasers (usually with ultraviolet excitation) and relatively small sample to sensor distances.

In the last 15 to 20 years, fluorescence has been used increasingly with forest tree species in studies of dormancy induction, cold hardiness, light acclimation, heat damage, water stress, disease effects, nutrient deficiencies, forest decline, and other applications (Mohammed *et al.* 1995). Chlorophyll fluorescence is also used operationally to assess the viability of seedling planting stock in Ontario, prior to planting (Sampson *et al.* 1997). A notable advantage is that chlorophyll fluorescence has been shown to be a useful tool in identifying previsual strain, often before other tests reveal stress effects.

The chlorophyll fluorescence parameter Fv/Fmax (ratio of variable to maximal fluorescence) was correlated with ground-based assessments of tree height X diameter (Spearman $r = 0.57$) for the 12 maple stands, indicating that Fv/Fmax could be used to rank the sites. In addition, in controlled field studies with stressed jack pine, black spruce, and red pine, Fv/Fmax measured in July was indicative of growth (Pearson $r = 0.89$ to 1.00) and percent browning (Pearson $r = -0.90$ to -0.99) measured in October of the same year.

Correlation between chlorophyll fluorescence and spectral reflectance has been suggested previously (Peñuelas *et al.* 1995b, 1997, 1998; Gamon *et al.* 1997), but efforts to quantify this linkage have been somewhat speculative. Here, the work of Zarco-Tejada *et al.* (2000a) permitted the isolation and modelling of chlorophyll fluorescence emissions on apparent spectral reflectance at the leaf level. The effects of natural chlorophyll fluorescence were observable in the red edge spectral region, with peaks at about 750 nm and 690 nm. Laboratory measurements were made with a Li-Cor Model 1800 integrating sphere apparatus coupled to an Ocean Optics Model ST1000 fibre spectrometer in which the same leaves were illuminated alternately with and without fluorescence-exciting radiation in order to separate the fluorescence emission component from the reflectance spectrum. The expected effects of chlorophyll fluorescence emission on the apparent spectral reflectance from a single leaf were also simulated theoretically with the development of a Fluorescence-Reflectance-Transmittance (FRT) model using the doubling radiative transfer method (Rosema *et al.* 1991)

These studies are of practical importance since they imply that spectral indices of reflectance may be useful indicators of fluorescence rather than having to rely on a laser or other active source to induce fluorescence. Moreover, the leaf-based model described in Zarco-Tejada *et al.* (2000a) demonstrates how changes in chlorophyll and pigment concentrations result in corresponding changes to spectral measures of fluorescence. However, our results indicate that the spectral reflectance indices of fluorescence may be confounded by relatively greater changes in leaf chlorophyll content. It appears, therefore, that if natural solar-induced fluorescence signals are to be exploited with remote sensing, measurement strategies must be developed that take this linkage into account.

Nutrient Concentration

Another stress-related factor that likely contributes to spectral response in plants is nutrient status. While there are many indications that mineral nutrition is a factor, a comprehensive understanding of the role of nutrient deficiencies in stress resistance is lacking (Bernier *et al.* 1989, Ellsworth *et al.* 1994, Liu *et al.* 1997). As summarized by Liu *et al.* (1997), primary environmental stresses in declining sugar maple stands have been identified as summer drought, and low soil pH and base cation pools. Current theory suggests that plant responses to nutrient deficiencies or imbalances may be important in mediating physiological responses to other environmental stresses (Ellsworth *et al.* 1994). An effort was made here to evaluate the possible relationship between foliar nutrient content and chlorophyll concentration.

For the 12 maples sites in the Bioindicators Project, total chlorophyll concentration was not significantly correlated with any leaf nutrients sampled in 1999, but in 1998 there was a significant, positive correlation with leaf Ca, Mg and Na (correlation coefficient ranged from 0.22 to 0.62). There was also a negative correlation ($r= -0.32$) to K content. These results are consistent with the findings of others (e.g., Bernier and Brazeau 1988a, 1988b, Foster *et al.* 1989, Watmough *et al.* 1999) that mention Ca, Mg and K deficiencies may play a significant role in the decline of maple forests.

While our project does not emphasize diagnosis of specific field stress agents, it is recognized that techniques involving dendrochemistry would complement the approaches used here as a means to identify the cause and timing of decline. As described by Watmough *et al.* (1999), dendrochemical analysis has indicated that declining trees often exhibit low radial growth decades before crown symptoms of decline are visible as chlorosis, necrosis, or dieback. This analysis allows historical changes in tree chemistry to be documented, which provides greater insight into the possible cause(s) and timing of decline symptoms. For instance, Watmough *et al.* (1999) have used dendrochemical analysis in maple to suggest a link between Ca and Mg deficiencies to acid deposition.

Other Leaf Factors

Other leaf factors affecting nonvisual leaf reflectance were studied here (Mohammed *et al.* 2000) and included leaf age, structure, and developmental factors. For example, we found that young leaves in sugar maple or aging leaves in which chlorophyll breakdown had begun (e.g., one-year-old foliage of white pine (*Pinus strobus* L.) produced higher reflectance in certain regions of the visible spectrum. The region most affected depended on the species, e.g., young maple foliage was red in colour, hence, showed an increased red reflectance. In white pine and eastern hemlock (*Tsuga canadensis* L. Carr.), aging or young foliage was lighter green than current-year mature or near mature foliage, and consequently, reflectance was higher in the green bands. The implications of these findings for scaling up spectral reflectance to the remote scale is that spectral results can be affected by the relative proportions of young and old foliage in a canopy, and these effects will vary depending on the species. These effects were minimized in this project by obtaining CASI data under full leaf expansion prior to senescence in a predominately single-species stand.

Leaf structural and developmental features are key drivers of leaf-based spectral characteristics. For example, Middleton *et al.* (1998) found significant differences for adaxial (upper) versus abaxial (lower) leaf surfaces of white pine, black spruce (*Picea mariana* (Mill.) B.S.P.), and trembling aspen (*Populus tremuloides* Michx). Adaxial surfaces of foliage were suggested to be likely the most important because these dominate the view from above. They found that species differences among tree overstories were distinctly expressed in the adaxial near-infrared spectra, but no species effect on the near-infrared region was evident in the understories across sites.

Using stacked layers of leaves, Miller *et al.* (1991) studied the influence of seasonal patterns in red-edge leaf reflectance characteristics in 10 species, including sugar maple. They found that while red-edge features changed with seasonal stage of development, there was a sustained period in which values in deciduous trees tended generally to longer wavelengths, between approximately June 5 and August 10 in southern Ontario. This nine-week period could possibly be used as a sampling window for red-edge features that would be minimally influenced by season.

In addition to investigating seasonal patterns, diurnally based changes in the ratio of blue to red reflectance was observed here in sugar maple. The value of this index increased during the day, a result of both an increase in blue and a decrease in red reflectance, which was not accompanied by changes in chlorophyll concentration. Apparent reflectance in the blue region may be influenced by concentrations of both carotenoid pigments and blue-fluorescence-producing biochemicals such as ferulic acid (Lichtenthaler and Schweiger 1998) and NADPH (Chappelle *et al.* 1991). But as shown here, changes in red reflectance could proceed, at least in part, from quenching of chlorophyll fluorescence at midday (Zarco-Tejada 2000). There is also evidence in the literature that variations in other leaf biochemical contents, such as cellulose, lignins, proteins, and nitrogen can be detected through leaf reflectance spectra, typically in the short wave infrared region (e.g., 1200–2400 nm) (Yoder and Pettigrew-Crosby 1995).

Leaf water content is generally considered to be the primary factor affecting leaf reflectance in the infrared wavelengths from roughly 1300–2500 nm (Treitz and Howarth 1999). Carter (1991) found that water content influences the spectra across the 400 to 2500 nm region, with the greatest effects in the water absorption band near 1450, 1940, and 2500 nm; sensitivity maxima were also located between 400 and 720 nm. As an indicator of plant strain, however, leaf water content is less sensitive than leaf chlorophyll content, appearing only at advanced stages of leaf dehydration (Carter 1993). Efforts in this study have, therefore, focused on chlorophyll content.

While leaf thickness may be an important factor of leaf-level spectral characteristics, this feature is not expected to be of critical importance in canopy assessments. Some authors have undertaken to remove the confounding influence of leaf thickness in laboratory studies by using optically thick stacked layers of leaves either by measurement technique (Boyer *et al.* 1988, Miller *et al.* 1991) or in this project by simulation with infinite reflectance formulae (Zarco-Tejada *et al.* 2000b).

Canopy Level Detection of Stress

An intermediate step in linking leaf level response to canopy reflectance was laboratory studies using near-range CASI measurements. Laboratory CASI measurements verified a quantitative link between canopy reflectance of potted maple seedlings and chlorophyll fluorescence (Zarco-Tejada *et al.* 2000b). Optical indices in the 680–690 nm region exhibited the highest levels of correlation with chlorophyll fluorescence, and indices from the red-edge region showed the best relationships with chlorophyll content. These findings were further demonstrated by airborne CASI data for the study sites as shown in Fig. 3. Each level of investigation – from leaf to optically thick (or leaf stack) and canopy models – progressively improved the ability to estimate physiological indicators, such as chlorophyll content and chlorophyll fluorescence.

Similarly, deriving leaf-feature estimates from canopy data (i.e., numerically inverting canopy reflectance models by an iterative process) was performed. By inverting a coupled leaf and canopy reflectance model, total chlorophyll estimation was possible, with r^2=0.56, RMSE=5.7 μg/cm^2 (1998) and r^2=0.48 RMSE=6.1 μg/cm^2 (1999); and r^2=0.42 RMSE=3.0 μg/cm^2 (2000) (Zarco-Tejada *et al.* 2000c). An example of the resulting imagery is shown in Fig. 4, where five classes of chlorophyll concentration were applied.

Various stressors have been associated with a decrease in the chlorophyll content of foliage in sugar maple and conifers . For example, nutrient stress caused by low levels of Ca and Mg and air pollution stress caused by ozone exposure (Volin *et al.* 1993, Tjoelker *et al.* 1995) were reported to decrease chlorophyll levels in sugar maple leaves. This suggests that chlorophyll content reductions can be indicative of the stress condition of maple stands.

Chlorophyll estimation could provide a valuable tool in assessing forest condition when used in conjunction with other data sources (e.g., forest inventory data, insect and disease surveys). These results provide evidence that hyperspectral sensors offer a means to track changes in leaf chlorophyll pigment content and perhaps fluorescence in vegetation canopies. Investigations are underway to determine the suitability of satellite sensors (e.g., MERIS that is to be launched by the European Space Agency in the year 2001) and the robustness of chlorophyll estimations in other species.

Analysis of the influence of chlorophyll fluorescence on canopy reflectance is expected to provide insight into promising

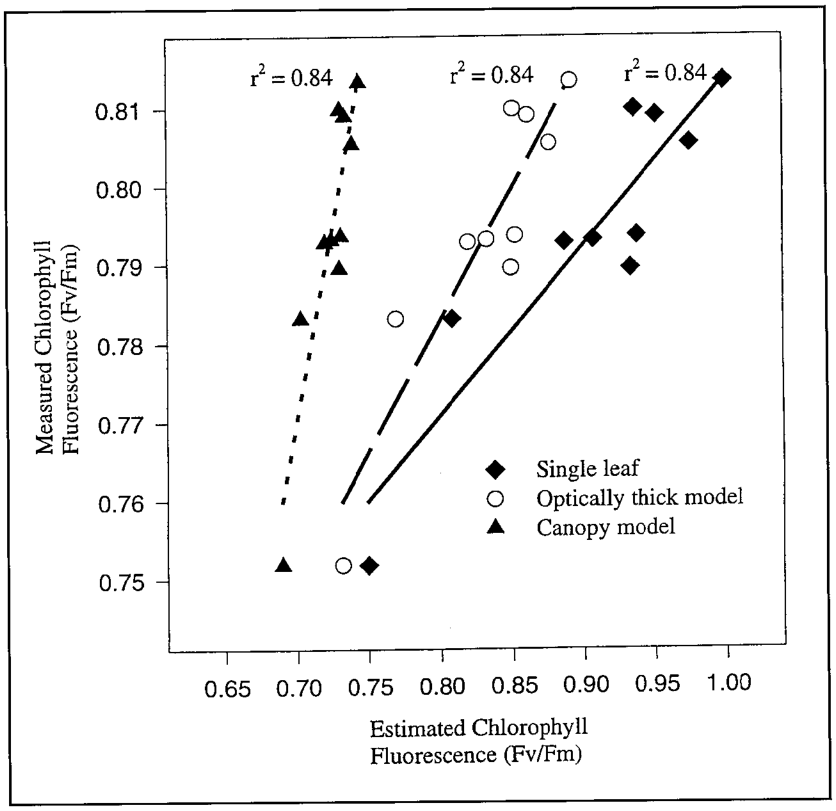

Fig. 3. Estimation of chlorophyll fluorescence (Fv/Fm) from CASI data using a red-edge, spectral derivative index developed at the leaf level through to optically thick (Lillestaeter 1982) and canopy reflectance simulation models (Kuusk 1996). The data corresponds to 10 maple study sites in the Algoma Region, ON collected in July 1998. It can be seen that estimations improve (linear regression slope progressively approaches unity) when the optical indices are calculated using the optically thick and then the canopy reflectance model. (Figure adapted from Zarco-Tejada *et al.* 2000b).

remote sensing approaches. Existing studies of fluorescence at the individual plant level (see Mohammed *et al.* 1995) exemplify the robust nature of the measure and the range of possible applications. The potential ability to assess canopy fluorescence without high-powered laser instruments is of practical importance. While current findings appear promising, a firm conclusion on this point is premature. Research is needed to determine whether inferences of natural chlorophyll fluorescence can be done with useful accuracy and to the extent where confounding factors (such as pigment content variability, acquisition conditions and canopy architecture) can be adequately minimized (Zarco-Tejada *et al.* 2000b).

Canopy Properties Affecting Spectral Response

As described by Duggin and Robinove (1990), the analysis of absorption phenomena is challenging for many reasons, ranging from physical characteristics of the canopy to atmospheric and instrumentation influences. While these latter influences have been addressed to a large extent (refer to Gray *et al.* 1997), it was considered necessary here to examine differences in forest structure (i.e., the amount and orientation of plant material) and individual tree condition.

Forest Structure

Forest structure has been considered the most important variable in determining canopy reflectance (Treitz and Howarth 1996). The influence of structural changes on spectral response was studied by first determining the level of spatial variation in reflectance data for different silvicultural practices (clearcut, selection and shelterwood) within maple stands. The study site was the Turkey Lakes Harvesting Impacts Project (Morrison *et al.* 1999), which is in close proximity to the other maple sites investigated and is characterized as a mature, maple forest.

Spatial variability was determined using a geostatistical approach termed semivariogram analysis (Curran and Atkinson 1998). The analysis revealed a suitable resolution (or scale) of approximately 9 m and 5 m for examining structural and physiological features, respectively (Sampson *et al.* 2000). While multiple scales of variation exist in nature (Marceau *et al.* 1994), the results here provide some guidance in considering the suitability of existing data sets and in developing sampling strategies in tolerant hardwoods. It is also consistent with the hypothesis that scale dependency exists in nature where underlying processes are best described (Hay *et al.* 1997). In effect, it provides a means to assess which airborne or satellite sensors meet the spatial resolution requirements to assess forest condition. It is significant that the resolutions used in the Bioindicators Project (3–5 m) satisfy the requirements for applicability of this spatial variability study.

Once patterns of spatial variability were described, possible factors contributing to these patterns were explored. A multivariate analysis of both structural (e.g., biomass and canopy opening) and functional (e.g., pigment concentrations) variables was performed for the range of silvicultural treatments. The analysis revealed that the degree of crown opening influenced the level of spectral variability; the highest levels of variability were recorded for shelterwood and selection systems in contrast to the clearcut and control. The degree of variability was also correlated to critical forest parameters such as biomass, stocking and crown area (Sampson *et al.* 2000).

In contrast, the degree of structural variability encountered across the12 study sites of the Bioindicators Project did not appear

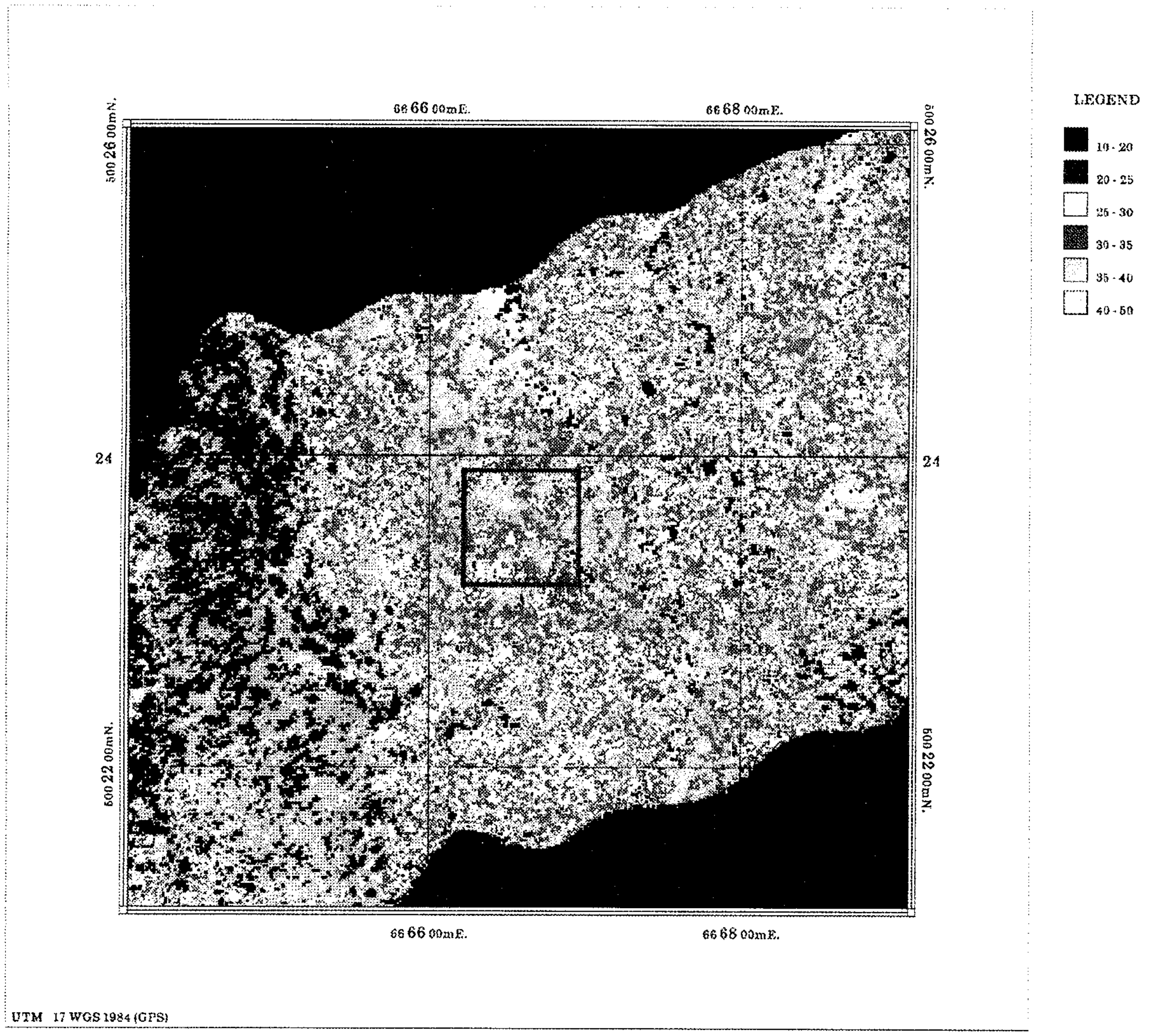

Fig. 4. Estimation of total chlorophyll ($a + b$) content from CASI data (72-channel data, 1.5 × 1.5m spatial resolution, obtained June 2000) for a predominately maple – beech study plot (shown within the 60 × 60m square) in Southcentral Ontario (Zarco-Tejada *et al.* 2000c). Six classes of total chlorophyll (μg/cm^2) were considered: i) <20 (very low chlorophyll); ii) 20–25 (low chlorophyll, suspected decline condition); iii) 25–30 (below average, possible decline symptoms); iv) 30–35 (average chlorophyll, usually healthy); v) 35–40 (average to above average chlorophyll, trees healthy); and vi) >40 (above average chlorophyll, trees healthy).

to affect the spectral indices found most effective in tracking bioindicators. However, further examination is necessary before this conclusion can be extended to other situations involving larger canopy openings. Nonetheless, the findings of this study complement the larger effort to develop physiological indicators by identifying a suitable scale or resolution for remote sampling and revealing influences of canopy opening.

Individual Tree Assessments

Ground-based physiological techniques, including the leaf-level methods discussed so far, can provide useful supplementary and diagnostic information to accompany remotely sensed information, or they can be used on their own for intensive field surveys. Many of these techniques have been available for a long time and are used fairly widely. In particular, tests that can integrate whole-tree responses may be very useful. An example is stem electrical resistance (SER), which has been shown in many studies with a variety of species to be correlated with tree vigour, growth rate, and visual symptoms of crown defoliation or dieback (e.g., Wargo and Skutt 1975, Newbanks and Tattar 1977, Kostka and Sherald 1982, Gagnon *et al.* 1987, Lindberg and Johansson 1989, Lekas *et al.* 1990). SER measures the resistance to an alternating or pulsed direct current passing between two probes that are inserted into the bole of the tree and attached to a resistance bridge. In our study, pulsed direct current was passed between nails that were used as probes. This ground-based assessment method is rapid, the equipment portable, and requires minimal technical expertise.

Average SER of 15 trees from each of the 12 sugar maple stands was a quantitative measure of stand vigour when compared to average rankings of these same stands using hyperspectral remote imaging from 1998. Significant differences in average SER were observed between stands. There was also a significant correlation between average stand SER and several spectral indices. While SER could be correlated to stress conditions, others have cautioned (e.g., Newbanks and Tattar 1977, Carter and Blanchard 1978) that several other factors, such as thermal variation and seasonal changes, can influence results. It is important, therefore, to ensure that changes in SER are a true reflection of stress-related effects. Techniques such as SER may provide useful ground-based bioindicators if used in conjunction with other assessment techniques such as stable carbon isotopes (McNulty and Swank 1995) and dendrochemical analysis (Watmough *et al.* 1999).

Next Steps

Aerial photographs and intensive field surveys currently provide the majority of information for forest mapping and monitoring. But as expressed by Bernier *et al.* (1999), a more complete spatial representation of our forests is needed. Hyperspectral remote sensing offers potential for assisting traditional assessment methods (Treitz and Howarth 1999, Ustin and Trabucco 2000). When integrated with leaf-based studies addressing fundamental questions of spectral response of species and imposed stresses, hyperspectral data may provide another means of evaluating forest condition. As evident in this study, the integration of leaf-based and hyperspectral data has uncovered promising optical indices at the canopy level. In particular, the potential exists to use spectral measures of stand vigour, especially those related to chlorophyll pigment content and fluorescence.

While the research findings have been encouraging, the issues of robustness of algorithms and approaches need to be addressed. These issues are the focus of our current work. In particular, to interpret spectral response requires some baseline thresholds to be defined, i.e., FCR system levels need to be established. Of interest will be optical indices and models (leaf and canopy) related to chlorophyll content and fluorescence. Our preliminary analysis has identified five classes of health, based on chlorophyll content. These will be refined for other species. A similar breakdown of classes is possible for chlorophyll fluorescence, using published and our own baseline research values (Mohammed *et al.* 2000). The basic premise is that fluorescence may not be subject to species effects (Bjorkman and Demmig 1987, Mohammed *et al.* 1995), while providing a measure of photosynthetic function – itself an integrative measure of physiological condition. The challenge is in evaluating the confounding effect of chlorophyll content.

The validation and evaluation phase will involve continued acquisition of hyperspectral imagery over established forest sites to provide an on-going database of average responses and also to serve as a database for physiological change detection. The latter is seen as the real strength of forest condition rating, because it provides an evaluation of forest responses over time, rather than single snapshot evaluations that would likely be too susceptible to natural fluctuations.

The question of operational feasibility is an important one. The cost and limitations of airborne hyperspectral imagery must be considered. For instance, the utility of hyperspectral imagery increases when one considers its broad application – from forest health to mapping and inventory. If the same information is eventually obtainable from hyperspectral satellite data and the costs are not prohibitive, operational integration will be greatly facilitated. We will be investigating the potential of spaceborne platforms in upcoming studies.

Operational application will be influenced by the comprehensiveness of these validation exercises. Pilot projects involving industry, forest managers and the research community may provide a suitable stepping stone. It is through this collaborative effort that interested parties can validate and develop physiologically based measures of condition. As well, the basic research and experience gained during this project should be of benefit if launching pilot studies elsewhere.

The integration of information across scales supports initiatives elsewhere, such as the ECOLEAP project, a federal research effort focussed upon understanding forest ecosystem processes (Bernier *et al.* 1999). The thrust of recent initiatives is to provide pertinent and timely information to forest managers regarding the state of the forest and productivity. To this end, the Bioindicators Project has advanced promising measures of condition, and has also given priority to developing on-screen visualization tools. For instance, we are developing a PC-based tool that would allow the forest manager to view an area as if positioned within the aircraft; scrolling to points of interest and selecting spectral measures of vigour to identify areas of concern. Having such an automated approach provides flexibility and facilitates decision-making.

Conclusion

The Bioindicators Project has advanced the development of objective measures of forest physiological condition. Through a multidisciplinary approach, fundamental studies at the leaf and individual tree level have provided insight into physiological and hyperspectral responses to stress. This basic knowledge has allowed canopy-level relationships to be established and refined, such as those relating hyperspectral reflectance to chlorophyll content and fluorescence. In addition, spatial patterns have been characterized in managed stands to identify suitable spatial resolutions and effects of canopy structure.

Before meaningful application of remote physiological response data can be widely implemented, attention must be given to the issues of validation and feasibility. Refining critical thresholds, while addressing the operational feasibility of remote sensing sensors, is the current focus of our project. The challenge lies in providing pertinent information in a timely and cost-effective manner. The hope is that as technological advancements in hyperspectral remote sensing are being realized, forest practitioners can capture the benefit of both the technology and the fundamental science presented here.

Acknowledgements

The authors gratefully acknowledge the financial support provided for this research through: the Ontario Ministry of Natural Resources; the Centre for Research in Earth and Space Technology (CRESTech, and Geomatics for Informed Decisions (GEOIDE) – a Canadian Centre of Excellence. We gratefully acknowledge our colleagues for their valuable contributions: John Harron (CRESTech), for the development of leaf measurement apparatus; Lawrence Gray, Paul Shepherd, Phil Brasher, and Heidi Beck whose dedication and skill made the airborne CASI field campaigns a success, and Brian Brown, Maara Packalen, Christy Lyons, Nick Seymour, Desmond Hickie and Wanda Nott at OFRI who planned and executed complex site-sampling campaigns. A note of appreciation is also extended to our Technical Advisory Team, the Canadian Forest Service, Ontario's Growth and Yield Program, and Dave McLaughlin, Ministry of Environment and Energy.

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