DROUGHT STRESS AND PAPER BIRCH *(BETULA PAPYRIFERA)* SEEDLINGS: EFFECTS OF AN ORGANIC BIOSTIMULANT ON PLANT HEALTH AND STRESS TOLERANCE, AND DETECTION OF STRESS EFFECTS WITH INSTRUMENT-BASED, NONINVASIVE METHODS

By Andrew D. Richardson[1], Melissa Aikens[2], Graeme P. Berlyn[3], and Philip Marshall[4]

Abstract. We conducted a progressive-drought greenhouse experiment, using potted paper birch (*Betula papyrifera*) seedlings in their third year of growth, to investigate whether a commercially available organic biostimulant improved plant health and stress tolerance, and to compare four noninvasive, instrument-based methods for monitoring plant stress. In the well-watered (no drought) plants, the biostimulant application significantly increased foliar nitrogen concentrations ($P = 0.01$) and led to marginally higher rates of photosynthesis ($P = 0.10$) and slightly higher F_v/F_m fluorescence ratios ($P = 0.14$). Reflectance indices further indicated that the biostimulant application resulted in increased chlorophyll content (Chl NDI, $P = 0.07$) and either (depending on interpretation) a significantly higher Chl:carotenoid ratio or a lower proportion of xanthophyll cycle pigments in the de-epoxidated state (PRI, $P = 0.02$). The PRI results suggest less oxidative stress in the treated plants, which may be related to the fact that the biostimulant used (Roots 3) contained ascorbate, an antioxidant. In the plants exposed to progressive drought, the biostimulant application had similar effects but did not appear to dramatically improve the drought stress tolerance of seedlings, in that impaired physiology occurred at about the same level of soil moisture in both treated and untreated seedlings. Photosynthesis responded to the drought treatment at about 12% to 15% soil moisture content (SMC), whereas PRI did not respond until about 9% to 10% SMC, and F_v/F_m did not respond until about 4% to 5% SMC. Chl NDI did not show a significant response to SMC.

Key Words. *Betula papyrifera*; biostimulant; leaf reflectance; paper birch; photosynthesis; progressive drought; stress.

To improve the health of urban and ornamental trees, it is important not only to have better tools available for treating stressed trees but also to have better tools available for detecting stress. Ideally, stress should be detected quickly, before its effects are visually apparent, so that a proactive treatment can be prescribed prior to the stress significantly weakening the tree.

Stress responses within a tree are coordinated by phytohormones, and, so in the end, every organ within an individual is to some degree affected by a given stress factor. Stress effects on trees include changes in leaf pigments (e.g., reduced chlorophyll content and decreased chlorophyll: carotenoid ratio) as well as altered physiology (e.g., impaired photosynthesis). Ultimately, stress may lead to poor growth, loss of vigor, and even death (Larcher 1995). Detecting and treating stress is, therefore, clearly an important task for arborists.

Using sophisticated modern instruments, it is possible to monitor changes in plant health over the course of a growing season. Based on these measurements, it may be possible to identify stressed individuals. In this study, we investigated the use of several techniques (direct measurement of photosynthesis, chlorophyll fluorescence, and leaf reflectance) to assess the level of drought stress in paper birch (*Betula papyrifera*) seedlings and to determine which method is able to first detect signs of stress. These instruments can be used on living leaves still attached to the tree; hence, they are described as "noninvasive" techniques.

Photosynthesis, the process by which plants use energy from the sun to fix atmospheric CO_2 into the complex organic molecules (sugars) that power the biosphere, is perhaps the most basic measure of productivity (Jones 1992). Infrared gas analysis is used to determine, in real time, the amount of CO_2 being taken up by an individual (but still intact) leaf, which is placed in a controlled-environment cuvette. In response to drought stress, stomatal closure typically occurs in order to minimize water loss (Hsiao 1973). The resulting decrease in stomatal conductance restricts the gas exchange necessary for photosynthesis to occur. It has been suggested that as drought stress becomes extreme, nonstomatal factors may become even more limiting to photosynthesis (Ögren and Öquist 1985). Therefore, there are a number of mechanisms whereby drought stress leads to reduced photosynthetic rates. The well-known midday shutdown of photosynthesis in many field-grown plants is an example of short-term drought stress caused by stomatal limitation.

Fluorescence refers to the re-emission of an absorbed photon, and chlorophyll fluorescence is one mechanism by

which excess excitation energy can be dissipated within the photosynthetic antenna complex. The kinetics of chlorophyll fluorescence are indicative of the overall health and functioning of the photosynthetic apparatus, especially photosystem II (PS II) (e.g., Bolhàr-Nordenkampf et al. 1989; Ball et al. 1994). Here we use the ratio of variable (F_v) to maximal (F_m) fluorescence, F_v/F_m, as a stress index. When measured on dark-adapted leaves, this ratio is equal to the potential quantum yield of photosynthesis. Depressed F_v/F_m ratios indicate chronic photoinhibition, a typical end-result of prolonged stress.

Leaf reflectance at visible wavelengths (400 to 750 nm) is determined in large part by pigmentation, in particular chlorophylls, carotenoids, and anthocyanins (Gamon and Surfus 1999). Reduced chlorophyll content, or chlorosis, is a common response to chronic stress (Carter and Knapp 2001) and can be detected by reflectance changes at ≈ 700 nm. The xanthophyll cycle pigments are important for the photoprotective dissipation of excess energy (Demmig-Adams and Adams 1996), and subtle changes in reflectance at 531 nm are related to changes in the epoxidation state of the xanthophylls (Gamon et al. 1992). Thus, reflectance can be used to quantify stress-related changes in leaf pigmentation.

In principle, these physiological measurements are all sensitive to plant stress. In this study, we test whether any of these approaches offers a *practical* means for the early detection of drought stress. We elected to use drought as the stress factor in this experiment because it is relatively straightforward to apply in a progressive manner (simply withhold water) and because the magnitude of the stress factor can be monitored easily with a soil moisture probe.

As a secondary objective, we treated half of the experimental plants with an organic biostimulant to investigate the viability of organic biostimulants as a low-cost means of promoting tree health and stress tolerance. Organic biostimulants (predominantly ascorbate, B-vitamins, vitamin E, and casein hydrolysate, with a carrier of humic substances and marine algal extracts) can be thought of as a stress vitamin mix for plants. Originally developed for tissue culture applications, they have also been shown to enhance the growth and stress tolerance of entire plants by increasing nutrient uptake and root development without heavy reliance on chemical fertilizers, herbicides, or pesticides (Berlyn and Sivaramakrishnan 1996). The formulation was also shown to decrease infestation of hemlocks (*Tsuga canadensis*) by the woolly adelgid (Sivaramakrishnan 2000). Here we test the effectiveness of a commercially available organic biostimulant in improving overall tree health and reducing the effects of progressive drought stress.

For this research, there were three reasons why paper birch provided an excellent model system: (1) it is a species on which we have already conducted significant ecophysiological research (e.g., Ashton et al. 1998; Richardson and Berlyn 2002; Richardson et al. 2002); (2) it is relatively susceptible to a variety of stressors, including drought (Li et al. 1996; Dirr 1998); and (3) it is an important ornamental and landscape tree, with a transcontinental distribution (Burns and Honkala 1990). We believe that greenhouse research on seedlings is an important first step before attempts are made to test the effectiveness of these techniques on mature trees in the urban forest.

METHODS

Two-year-old bare rootstock seedlings, 30 to 90 cm (12 to 36 in.) high, of paper birch (*Betula papyrifera* Marsh.) were purchased from a commercial nursery (Musser Forests, Inc., Indiana, PA) and transplanted to 28 cm (11 in.) pots filled with ProMix in early May 2002. A tablespoon of forest soil from a birch stand in northeastern Connecticut, U.S., was added to each pot as a source of mycorrhizal inoculum.

In mid June, dead seedlings were removed, and the remaining seedlings were randomly assigned to a bench position (blocks 1 through 15), watering treatment (well-watered vs. progressive drought), and biostimulant treatment (Roots [R] vs. No Roots [NR]). Within each block, there were three seedlings for each watering × biostimulant combination. Thus, a total of 180 seedlings were used in the experiment ($15 \times 2 \times 2 \times 3$).

The biostimulant (Roots 3, ROOTSinc, Independence, MO) was applied three times (mid June, late June, and late July) at a rate of 300 mL (10 fl oz) per pot per application. All plants were watered at least every other day until the drought treatment was started. Beginning August 1, measurements of soil moisture content (SMC) and leaf-level physiology were made on four plants (one for each watering × biostimulant combination) from each of five different randomly selected blocks. A total of 20 different plants were monitored each day. On August 5, the drought treatment pots were watered for the last time; measurements continued through September 6. During the course of the drought experiment, the mean daily maximum air temperature was 30.5°C (87°F), and the mean daily minimum was 23.9°C (75°F) (Table 1).

Measurements were started at 9 A.M. each day and were typically finished by 12 noon. Volumetric SMC was measured with a ThetaProbe (Type ML2x, Delta-T Devices Ltd.,

Table 1. Greenhouse air temperature and soil temperature during the course of the progressive drought experiment.

	Air temperature	Soil temperature
Overall mean	26.4°C	25.5°C
Mean daily maximum	30.5°C (2 P.M.)	27.4°C (5 P.M.)
Mean daily minimum	23.9°C (6 A.M.)	23.8°C (8 A.M.)
Absolute maximum	38.6°C	32.6°C
Absolute minimum	15.4°C	17.4°C

Cambridge, UK). For the physiological measurements, one fully expanded leaf per plant was selected for study. Photosynthesis was measured using infrared gas analyzer (IRGA) technology (LI-6400 Portable Photosynthesis System, LI-COR, Lincoln, NE). The CO_2 in the reference analyzer was held constant at 400 μmol CO_2/mol air, air temperature was held constant at 30°C (86°F), relative humidity was held above 50%, and the integral red + blue LED lamp was set at a PPFD of 300 μmol/m²/s. This quantum flux, equal to about 20% of full sunlight, was selected because it corresponded to the ambient light level inside the greenhouse, which had been whitewashed in May to minimize overheating during the summer. In this way, the photosynthetic rate of leaves inside the IRGA cuvette quickly stabilized, and it was not necessary to wait a long time for photosynthetic induction to occur. Analysis of photosynthetic light response curves (measured on leaves from five different plants at the start of the drought experiment) indicated that, at this quantum flux, these birches had reached 87 ± 4% (mean ± 1 S.D.) of their maximum photosynthetic rate.

The chlorophyll fluorescence ratio, F_v/F_m, was measured using a portable modulated fluorometer (Model OS-500, Opti-Sciences, Tyngsboro, MA). Prior to fluorescence measurements, all leaves were dark adapted for a minimum of 10 min using dark adaptation cuvettes (model FL-DC).

Leaf reflectance (300 to 1,100 nm wavelengths, at ≈ 3 nm increments) was measured using a portable spectrometer (UniSpec Spectral Analysis System, PP Systems, Haverhill, MA). Five separate reflectance readings were made on each leaf. The reflectance spectrum for each leaf was calculated as R_λ = (leaf radiance at wavelength λ)/(reflectance standard radiance at wavelength λ). Two reflectance indices were calculated from each spectrum: the chlorophyll normalized difference index (Chl NDI), an excellent indicator of chlorophyll content (Gitelson and Merzlyak 1994; Richardson et al. 2002), was calculated as $(R_{750} - R_{705})/(R_{750} + R_{705})$; and the photochemical reflectance index (PRI), a dynamic index correlated with photosynthetic radiation use efficiency, the epoxidation state of xanthophyll cycle pigments, and, more generally, the chlorophyll:carotenoid ratio, was calculated as $(R_{531} - R_{570})/(R_{531} + R_{570})$ (Gamon et al. 1997; Sims and Gamon 2002; Stylinski et al. 2002).

At the conclusion of the measurement period, five leaves were harvested (well-watered plants only) from each of the three seedlings in each biostimulant treatment in each block. The aggregate fresh mass of all 15 leaves in each sample was immediately determined, and then the corresponding (one-sided) leaf area was measured using a LI-3100 area meter (LI-COR, Lincoln, NE). Leaves were then oven-dried at 60°C (140°F) for 48 h, and then re-weighed to determine dry mass. The leaf mass-to-area ratio (LMA) was calculated on both fresh and dry mass bases as (aggregate leaf mass)/(aggregate leaf area). Oven-dried samples were then ground to a fine powder in a coffee grinder. Samples were analyzed for C and total N on a Carlo-Erba gas chromatograph (NA 1500 Series 2, CE Instruments, Lakewood, NJ) at Dartmouth College (Hanover, NH), and for the stable carbon isotope ratio $\delta^{13}C$ on a Europa Scientific ANCA-GSL elemental analyzer by a commercial laboratory (Iso Analytical Ltd., Cheshire, UK).

To determine whether the biostimulant treatment had a statistically significant effect on the measured leaf properties (well-watered plants only), a paired *t*-test (with samples paired by block) was conducted for each trait (n = 15 for each treatment). For the physiological data, all results from the daily physiological measurements (well-watered plants only; a total of ≈ 125 measurements per treatment) were pooled for each block × biostimulant treatment combination, and then a paired *t*-test was conducted on the resulting means (n = 15 for each treatment).

RESULTS

Effect of Biostimulant Application on Leaf Properties of Well-Watered Seedlings

There were no statistically significant differences between R (Roots) and NR (No Roots) treated seedlings (all $P \geq 0.10$) for fresh leaf mass, dry leaf mass, leaf area, or LMA_{Dry} (Table 2). In spite of this, LMA_{Fresh} was marginally lower (P = 0.09) in leaves of R-treated seedlings compared to those in the NR treatment. R seedlings had significantly higher ($P \geq 0.01$) foliar N concentrations (1.4%) than did NR seedlings (1.3%), but neither foliar C nor $\delta^{13}C$ differed between the two biostimulant treatments.

Photosynthesis of the R-treated seedlings (7.0 μmol CO_2/m²/s) was somewhat higher than that of the NR seedlings (6.4 μmol CO_2/m²/s); the difference was just barely significant at the $P \leq 0.10$ level (Table 2). F_v/F_m, Chl NDI, and PRI were also higher in the R-treated seedlings, but the difference between treatments was significant only for the reflectance indices (Table 2). The reflectance indices indicated that R-treated seedlings had higher chlorophyll contents, and higher chlorophyll:carotenoid ratios, than the NR seedlings.

Over the course of the experiment, R-treated seedlings grew 6% more than NR seedlings, but this difference in growth between biostimulant treated and control plants was not significant (P = 0.35).

Progression of the Drought Treatment

Over the course of the drought treatment, mean soil moisture content (SMC) of the well-watered plants remained similar (47 ± 6%) for both the R and NR treatments. For the well-watered plants, there was no apparent time trend in SMC (Figure 1; see p. 56). The droughted plants, on the other hand, showed a steady decline in SMC beginning after the final watering (day 5). The pattern of soil drying was similar for both the R and NR treatments (Figure 1). By day 20, SMC of the droughted plants was about 10% to 15%, and from day 30 through the end of the experiment, the

figure was about 4 ± 3% for droughted seedlings in both R and NR treatments.

Detection of Drought Stress

The four physiological measures used as noninvasive methods to detect drought stress were plotted against SMC, with separate series plotted for the two biostimulant treatments (Figure 2; see p. 57). A three-parameter model with an exponential rise to maximum (specified as $f(x) = y_0 + a \times [1 - e^{-b \times x}] + \varepsilon$) was fit to these data. The parameters y_0 and a control the minimum and range of the data, respectively, while parameter b controls the curvature of the relationship, and ε indicates the stochastic regression residual. If b equals zero, then the relationship reduces to a flat line ($y_0 + a$).

Across all four physiological variables, the R-treated seedlings had higher measured values across most of the entire range of SMC (0 to 50+%), which is in agreement with the above result (i.e., for the well-watered plants) that photosynthesis, F_v/F_m, Chl NDI, and PRI were all higher in the R-treated seedlings compared to the NR seedlings. This is particularly evident in Figure 2A, for example, from which it can be seen that there were far more R-treated seedlings with photosynthetic rates above 8 μmol/m²/s and far more NR-treated seedlings (at least at SMC > 10%) with photosynthetic rates below 6 μmol/m²/s. Thus, there was a clear tendency for the biostimulant treatment to improve the health of treated seedlings.

There was considerable scatter in the data, even at a given SMC, for each of the different physiological measurements. This scatter was especially pronounced for Chl NDI (Figure 2C), and in fact, for this variable, it was difficult to detect a clear relationship with SMC. For both the R- and NR-treated seedlings, the b coefficient was not significantly different from 0 (both $P > 0.10$), and R^2 values were very low (both models had $R^2 \leq 0.10$, Table 3). On this basis, it is suggested that leaf chlorophyll content is not very sensitive to progressive drought stress.

In comparison, the other three physiological measures exhibited a far tighter relationship with SMC. For the photosynthesis, F_v/F_m, and PRI models, b coefficients were all significantly different from 0 at $P \leq 0.001$. Model fits, as gauged by the coefficient of determination, R^2, were strongest for photosynthesis, intermediate for PRI, and weakest for F_v/F_m (Table 3). However, for all three measures, surprisingly low moisture contents were required for a functional response to be exhibited. For example, in all four cases, the modeled response $f(x)$ did not fall more than 1

Table 2. Comparison of paper birch (*Betula papyrifera*) leaf traits for control plants and plants treated with Roots 3, an organic biostimulant. Reported values are mean ± 1 S.D. *P*-values based on paired t-test (n = 15 independent samples, paired by block). Data are for well-watered plants only.

	Roots	Control (No Roots)	*P*-value
Leaf area (cm²/leaf)	28.2 ± 5.9	28.5 ± 7.3	0.89
Fresh leaf mass (g/leaf)	0.35 ± 0.08	0.36 ± 0.09	0.56
LMA_{Fresh} (g/m²)	122 ± 8	127 ± 6	0.09*
Dry leaf mass (g/leaf)	0.12 ± 0.03	0.13 ± 0.03	0.47
LMA_{Dry} (g/m²)	43 ± 4	45 ± 3	0.15
%N	1.40 ± 0.08	1.30 ± 0.09	0.01***
%C	45.8 ± 1.0	45.8 ± 1.6	0.99
$\delta^{13}C$ (‰)	–30.5 ± 0.5	–30.3 ± 0.5	0.28
Photosynthesis (μmol CO_2/m²/s)	7.0 ± 0.8	6.4 ± 0.9	0.10*
F_v/F_m	0.805 ± 0.008	0.800 ± 0.011	0.14
Chl NDI	0.327 ± 0.024	0.307 ± 0.026	0.07*
PRI	0.013 ± 0.005	0.009 ± 0.004	0.02**

Significance levels:
*, $P \leq 0.10$
**, $P \leq 0.05$
***, $P \leq 0.01$

Table 3. Comparison of model parameters (± 1 S.E.) relating soil moisture content (SMC, %) to different physiological measurements conducted on paper birch (*Betula papyrifera*) seedlings subjected to a progressive drought. The three-parameter model with an exponential rise to maximum was specified as $f(x) = y_0 + a \times (1 - e^{-b \times x}) + \varepsilon$. Results are illustrated in Figure 2.

Model parameters	y_0	a	b	R^2
Photosynthesis				
Roots	–1.23 ± 0.62	9.10 ± 0.63	0.17 ± 0.02	0.69
No Roots	–0.46 ± 0.70	6.87 ± 0.70	0.18 ± 0.03	0.55
Fluorescence (F_v/F_m)				
Roots	0.71 ± 0.02	0.10 ± 0.02	0.41 ± 0.11	0.31
No Roots	0.70 ± 0.02	0.11 ± 0.02	0.59 ± 0.17	0.26
Chlorophyll Normalized Difference Index (Chl NDI)				
Roots	0.28 ± 0.02	0.05 ± 0.02	0.13 ± 0.10	0.08
No Roots	0.28 ± 0.01	0.20 ± 2.20	0.01 ± 0.06	0.06
Photochemical Reflectance Index (PRI)				
Roots	–0.007 ± 0.003	0.021 ± 0.003	0.161 ± 0.051	0.29
No Roots	–0.019 ± 0.004	0.031 ± 0.004	0.185 ± 0.045	0.40

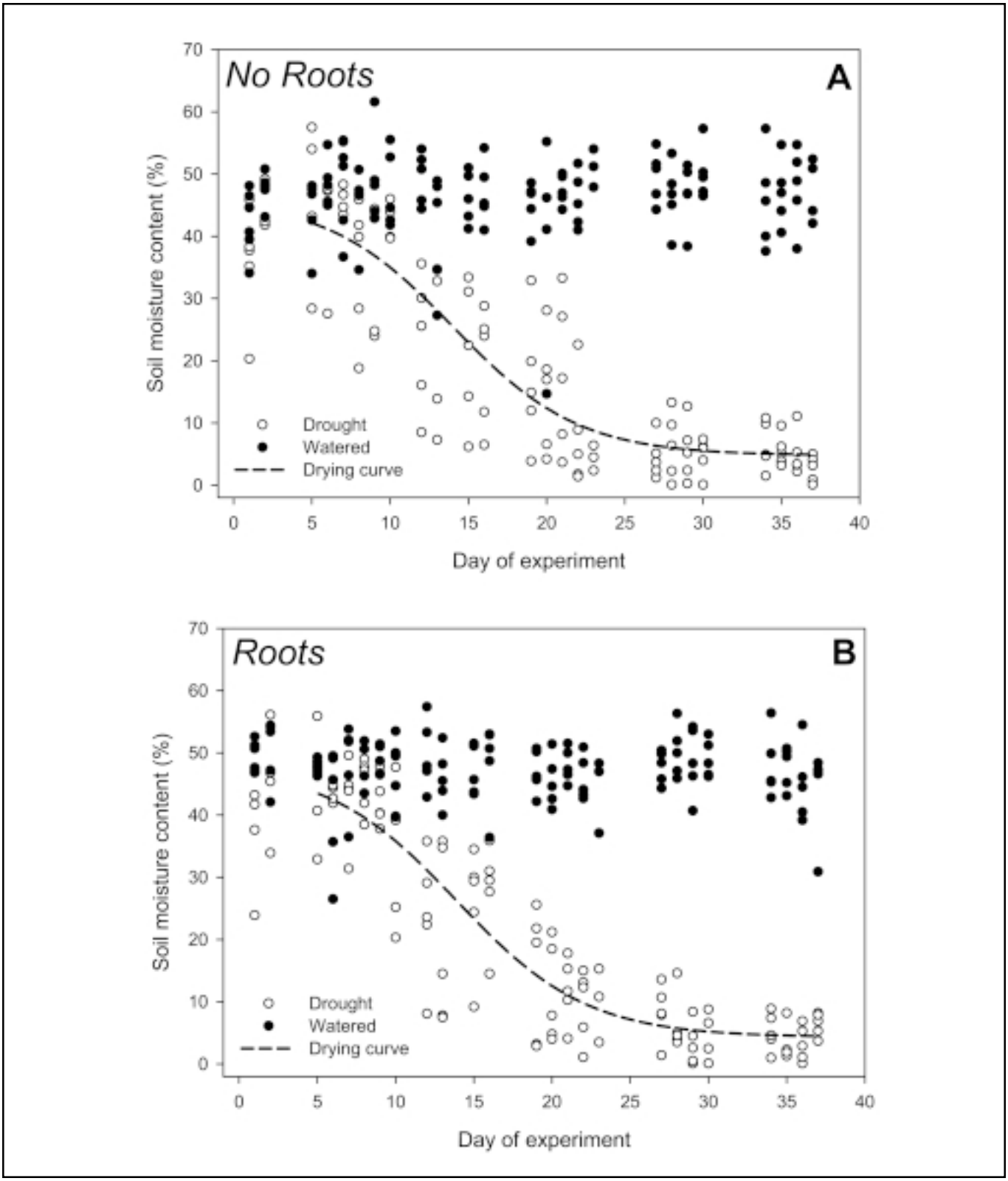

Figure 1. Time trend of volumetric soil moisture content over the course of the experiment. Plants in the "drought" treatment were watered for the last time on day 5 of the experiment. The soil drying curve depicts the progressive drought that resulted. Panel (A) shows data for pots without an organic biostimulant (Roots) treatment; (B) is for pots treated with the biostimulant. The soil drying curves are nearly identical in panels (A) and (B).

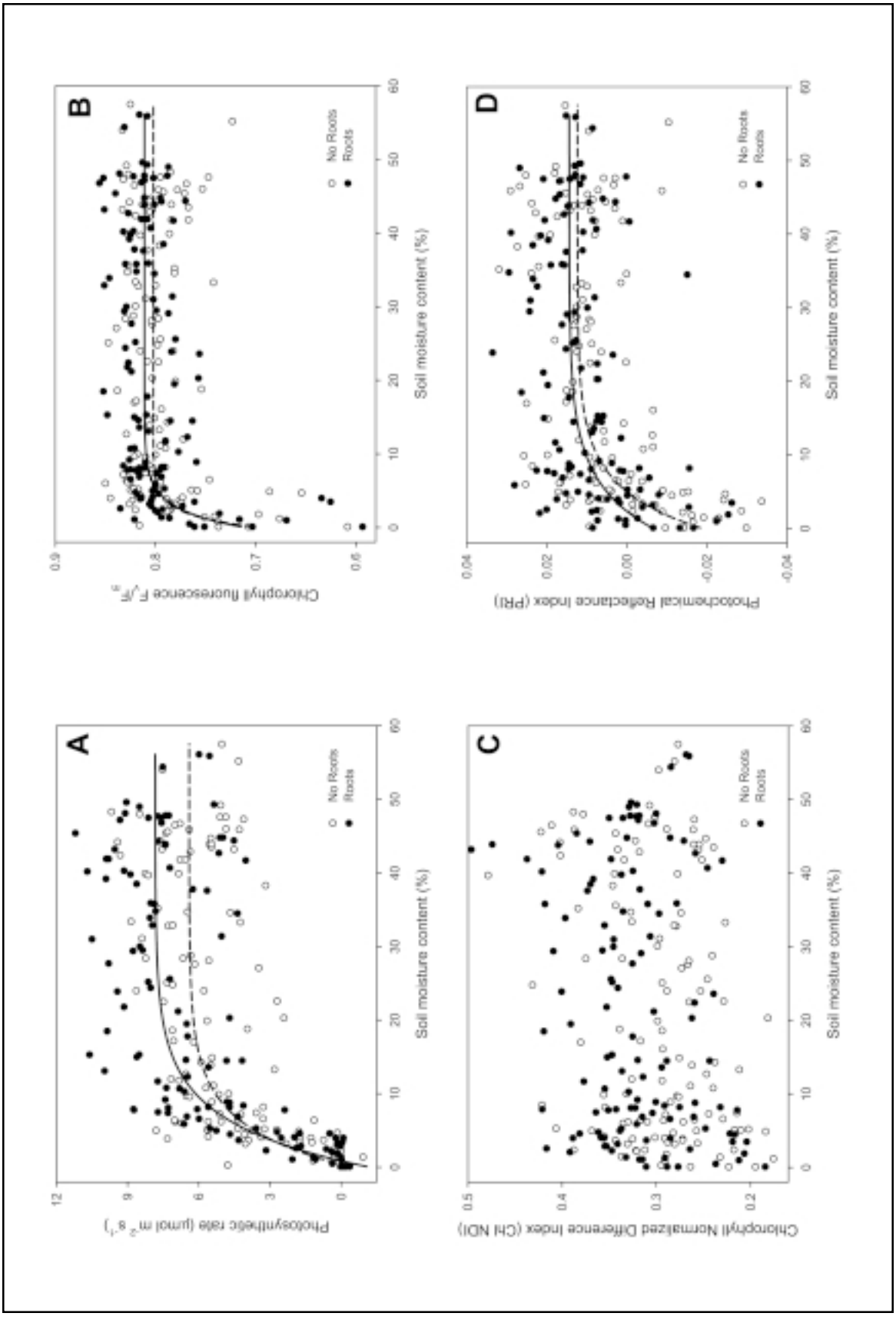

Figure 2. Relationship between volumetric soil moisture content and four physiological variables over the course of a progressive drought. (A) net photosynthesis; (B) chlorophyll fluorescence F_v/F_m ratio; (C) chlorophyll normalized difference index (Chl NDI), an index of foliar chlorophyll content; and (D) photochemical reflectance index (PRI), an index based on xanthophyll cycle pigmentation. Data are plotted separately for the two biostimulant treatments (Roots, No Roots). Curves are based on a three-parameter model with an exponential rise to maximum (specified as $f(x) = y_0 + a \times [1 - e^{-b \times x}] + \varepsilon$) fit to the data. No curve is shown for panel (C) because the coefficient *b* was not significantly different from 0. A dashed line is used to depict the curve for the No Roots plants, whereas a solid line is used for Roots-treated plants.

S.D. below the modeled maximum ($y_0 + a$) until SMC was below 15%. Photosynthesis of R-treated seedlings hit this threshold at 14.3%, whereas for NR seedlings the corresponding SMC was somewhat lower (11.9%). For PRI, SMCs of 9% to 10% were required for a similar magnitude of response, and for F_v/F_m the figures were even lower (4% to 6%). Although the R-treated seedlings had higher mean photosynthesis, F_v/F_m, and PRI, there was little or no evidence that the R treatment enabled seedlings to tolerate more severe drought before physiology was impaired (Figure 2).

DISCUSSION

Interpretation of Drought Effects on the Measured Physiological Variables

Results suggested that photosynthetic declines in the droughted seedlings were first triggered by stomatal closure, which led to reduced stomatal conductance. For both R-treated and NR seedlings in the drought treatment, conductances of below 0.05 mol $H_2O/m^2/s$ were associated with the lowest photosynthetic rates (≤ 4 μmol $CO_2/m^2/s$). Stomatal effects on photosynthesis were probably larger than those of secondary physiological effects, and this may explain why F_v/F_m, PRI, and Chl NDI were all comparatively less sensitive indicators of drought stress.

Ögren (1990) found that drought-stressed and unstressed *Salix* leaves could be accurately differentiated on the basis of their fluorescence induction curves. Indeed, induction kinetics were of more use in this regard than measurements of photosynthetic capacity. However, results further demonstrated that the F_v/F_m ratio was of little use in identifying drought stress (see also Di Marco et al. 1988; Epron and Dreyer 1993; Lu and Zhang 1998; Tambussi et al. 2002), and Ögren (1990) concluded that drought stress does not affect photochemistry unless the stress becomes severe. Similarly, studying *Quercus*, Epron et al. (1992) found that photosynthesis of water-stressed trees was lower throughout the day than that of the control trees, but F_v/F_m differed by only a modest amount between treatments, and even then differences between treatments were only evident for several hours around midday. Thus, because the midday declines in F_v/F_m of drought-treated plants were found to be more or less fully reversible, Epron et al. (1992) suggested that this decline represented photoprotection and not photodamage to the PS II reaction center. In the present experiment, it was only at very low SMCs (i.e., extreme drought stress) that F_v/F_m began to show a nonreversible decline. On this basis, it is hypothesized that SMCs of 5% or less are required for drought-induced photodamage to occur in *Betula papyrifera.* This assumes the temperature and light regime of the present experiment—under higher irradiances (e.g., full sunlight, instead of the 20% full sun here), photodamage would likely occur under more mild drought stress (Lu and Zhang 1998). Furthermore, other experiments have demonstrated that even if PS II photochemistry does not show a drought response when fluorescence measurements are conducted on dark-adapted samples (i.e., the *potential* quantum yield, as given by F_v/F_m, does not change), there is still evidence of photosynthetic down-regulation in that the *actual* quantum yield of PS II ($\Phi_{PS\ II}$) is decreased (Lu and Zhang 1998). This stress response can be detected by studying the fluorescence kinetics of light-adapted samples, for which pulse-modulated fluorescence techniques are required.

Tambussi et al. (2002) demonstrated that although chlorophyll content did not differ between control and severely water-stressed durum wheat plants (see also Epron and Dreyer 1993 for two *Quercus* spp.), a transmittance-based version of PRI was sensitive to even moderate drought. Results of that study confirmed that PRI is negatively correlated with nonphotochemical quenching (qN) of excess energy in the thylakoids through the xanthophyll cycle, and negatively correlated with the de-epoxidation state of the xanthophyll cycle pigment pool. It is likely that PRI performs better than F_v/F_m as a stress index precisely because it correlates with these photoprotective mechanisms, whereas nonreversible change in F_v/F_m requires that actual photodamage has occurred.

Effect of the Biostimulant Treatment

The biostimulant treatment appeared to have a modest effect on plant health (Table 2, Figure 2), but it did not result in greatly improved stress tolerance of treated plants (Figure 2), nor did it lead to a significantly enhanced growth rate. However, since carotenoid pigments are important for the de-excitation of harmful oxygen species (e.g., singlet oxygen radicals), and PRI indicated that the biostimulant-treated plants may have a higher chlorophyll:carotenoid ratio, it is suggested that the antioxidants in the Roots 3 formulation may help to reduce oxidative stress in the treated plants.

The effect of biostimulant treatment on plant growth and stress tolerance, as shown here, is weaker than that previously reported for a variety of different plant species [e.g., *Coffea*, *Alnus*, *Pinus*, and *Populus*, as well as several grasses (Berlyn and Sivaramakrishnan 1996)]. This may be due to the short duration of the present experiment and the comparatively large size of the birch seedlings relative to the dosage. Thus, experiments will be needed to determine the correct application rate for full-sized trees in a garden or landscape setting.

Detecting Stress with Noninvasive Physiological Measures

The photosynthetic rates we measured on well-watered plants (Table 2, for both R- and NR-treated seedlings) were somewhat lower than those previously measured on field-grown seedlings of the same species at a mid-elevation [550 m (1,800 ft) ASL] site in northern Vermont (≈ 10 μmol

$CO_2/m^2/s$) (Richardson and Berlyn 2002). Those same plants in Vermont also had higher mean Chl NDI (0.403) but lower PRI (–0.007) than the greenhouse-grown seedlings in the present experiment. The F_v/F_m of the well-watered plants in the present experiment was within the range considered "normal" (≈ 0.800) for dark-adapted, healthy, and unstressed leaves (Ball et al. 1994). F_v/F_m is the only one of the measures used here for which there is such a generally accepted standard against which measurements can be compared, but F_v/F_m was also less sensitive to drought stress than was photosynthesis or PRI. For photosynthesis and reflectance indices, site-specific differences in biotic and abiotic factors (e.g., microclimate, soil fertility, tree age) can have a significant effect on what is "normal" and hence what one considers to be "stressed." Clearly, then, stress ultimately has to be a relative concept: It requires that we have some standard of "normal." One-shot measurements of any physiological variable may tell the plant physiologist little about drought stress, since there is so much variation not only among species, but also within single species—i.e., across populations and even individuals (see, for example, the scatter apparent even for SMC > 30% in the different panels of Figure 2). Rather, monitoring plant health throughout the season, and detecting changes over time, may be the best way to implement an instrument-based stress monitoring program.

These results may be applicable to the detection of a wide range of stress agents or factors, since it has been previously noted that many stress factors produce similar stress responses (Larcher 1995). However, this also means that diagnosing, and prescribing treatment for, the causal stress factor can be exceptionally difficult, especially in the field. For example, Carter (1993) showed that a variety of stress factors all led to similar changes in the visible and near-infrared foliar reflectance spectra. Thus, particular stress agents do not appear to yield spectrally unique reflectance responses. A key may be distinguishing between the sometimes-unique primary effects of a stress factor (e.g., loss of turgor and resulting stomatal closure as a consequence of drought) from the resulting secondary effects (e.g., photoinhibition, chlorosis), which are more general responses to almost all stress factors. Unfortunately, at this stage, we don't have field instruments capable of detecting the primary effects of most stressors, and, as demonstrated here, it was necessary for the stress to be well-developed before secondary effects could be detected by either reflectance or fluorescence methods.

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[1,2,3,4]*Yale University*
School of Forestry & Environmental Studies
370 Prospect Street
New Haven, CT 06511, U.S.

Corresponding author:
Andrew Richardson
Complex Systems Research Center
University of New Hampshire
Morse Hall
39 College Road
Durham, NH 03824, U.S.

Résumé. Nous avons mené une expérience progressive de sécheresse en serre au moyen de semis de bouleaux à papier (*Betula papyrifera*) empotés lors de leur troisième année de croissance, et ce afin d'investiguer si un bio-stimulant commercial pouvait améliorer la santé des végétaux et la tolérance au stress, et aussi afin de comparer quatre méthodes instrumentales de base non invasives pour suivre le stress des végétaux. Chez les végétaux bien irrigués (aucune sécheresse), l'application du bio-stimulant a permis d'accroître significativement la concentration foliaire en azote (P = 0,01), et a produit des taux de photosynthèse marginalement plus élevées (P = 0,10) ainsi que des ratios de fluorescence F_v/F_m légèrement plus élevés (P = 0,14). Les indices de réflexion ont indiqué par après que l'application de bio-stimulant résultait en un accroissement en contenu chlorophyllien (Chl NDI, P = 0,07) et aussi soit – dépendant de l'interprétation – en un accroissement significatif dans la ratio Chl NDI : caroténoïdes, soit en une plus faible proportion de pigments cycliques de xanthophylles à l'état de dé-expoxidation (PRI, P = 0,02). Ce dernier résultat suggère un stress d'oxydation moindre chez les végétaux traités, ce qui pourrait être relié au fait que le bio-stimulant utilisé (Roots 3) contient de l'ascorbate, un anti-oxydant. Chez les végétaux exposés à une sécheresse progressive, l'application du bio-stimulant avait des effets similaires, mais n'apparaissait pas améliorer dramatiquement la tolérance au stress hydrique des semis; des processus physiologiques diminués se produisaient à des taux similaires d'humidité du sol à la fois chez les semis traités que ceux du groupe témoin. La photosynthèse répondait au traitement de sécheresse à un taux de contenu en humidité du sol de 12 à 15%, alors que le PRI ne répondait pas avant un taux de 9 à 10%, et le ration F_v/F_m ne répondait pas avant un taux de 4 à 5%. Le Chl NDI n'a pas montré de différence significative au contenu en humidité du sol.

Zusammenfassung. Um herauszufinden, ob ein kommerziell erhältliches organisches Wachstumsstimulans die Pflanzengesundheit und Stresstoleranz verbessert, leiteten wir ein Gewächshausexperiment über fortschreitende Trockenheit bei getopften Papierbirken in ihrem 3. Wachstumsjahr. Wir verglichen 4 nicht-invasive, auf Messinstrumenten basierende Methoden, um den Pflanzenstress zu beobachten. In den gut bewässerten Pflanzen bewirkte das Wachstumsstimulans einen deutlichen Anstieg der Blattstickstoffkonzentration (P = 0.01) und führte am Rande zu höheren Photosyntheseraten (P = 0.10) und leicht erhöhten Fv/Fm Fluoreszenzverhältnissen (P = 0.14). Die Reflektionen zeigten auch, dass das Biostimulans zu erhöhten Chlorophyllanteilen (Chl NDI, P = 0.07) und entweder (abhängig von der Interpretation) einem deutlich höheren chl:carotinoid-Verhältnis oder einem geringeren Anteil von xanthophyll-Zyklus-Pigmenten in dem De-expoxidations-Zustand (PRI, P = 0.02). Die PRI-Ergebnisse lassen auf weniger oxidativen Stress in den behandelten Pflanzen schließen, was in Beziehung gesetzt werden kann mit dem Umstand, dass das verwendete Biostimulans (Roots 3) Ascorbat, ein Anti-oxidans enthält. In den der Trockenheit ausgesetzten Pflanzen hatte das Biostimulans eine ähnliche Wirkung, aber es schien nicht so dramatisch die Trockenheitsstresstoleranz der Sämlinge zu verbessern. In unbehandelten und behandelten Sämlingen blieb der Bodenfeuchtigkeitsgehalt auf dem selben Level. Die Photosynthese reagierte auf die Trockenheitsbehandlung bei ungefähr 12-15% Bodenfeuchtigkeitsgehalt (SMC), während der PRI erst ab 9-10% SMC reagiert und der F_v/F_m Wert erst ab 4-5% SMC. Chl NDI zeigte keine Änderung gegenüber SMC.

Resumen. Se realizó un experimento en invernadero sobre resistencia a la sequía, usando brinzales de abedul (*Betula papyrifera*) en su tercer año de crecimiento, con el fin de investigar si un bioestimulante orgánico disponible comercialmente mejora la salud y la tolerancia al estrés de la planta. También para comparar cuatro métodos para monitorear el estrés de las plantas. En plantas bien regadas (sin sequía), la aplicación del bioestimulante incrementó significativamente la concentración de nitrógeno foliar (*P* = 0.01) y permitió tasas más altas de fotosíntesis (*P* = 0.10) y levemente más altas relaciones de fluorescencia F_v/F_m (*P* = 0.14). Los índices de reflectancia indican además que la aplicación del bioestimulante resultó en un incremento del contenido de clorofila (Chl NDI, *P* = 0.07) y también (dependiendo de la interpretación) una significativa más alta relación clorofila:carotenoide o una más baja proporción de pigmentos de xantofila en estado de-expoxidado (PRI, *P* = 0.02). Los resultados de PRI sugieren menos estrés oxidativo en las plantas tratadas, lo cual puede estar relacionado al hecho de que el bioestimulante usado (Roots 3) contiene ascorbate, un anti-oxidante. En las plantas expuestas a sequía progresiva, el bioestimulante tuvo efectos similares, pero no pareció mejorar tan dramáticamente la tolerancia al estrés por sequía de los brinzales, el mismo debilitamiento fisiológicamente ocurrido al mismo nivel de humedad del suelo en brinzales tratados y no tratados. La fotosíntesis respondió al tratamiento de sequía en cerca de 12-15% de contenido de humedad del suelo (SMC), mientras que el PRI no respondió hasta 9-10% de SMC, y F_v/F_m no respondió hasta cerca 4-5% de SMC. Chl NDI no mostró una respuesta significativa a SMC.