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# PHYSIOLOGICAL RESPONSES OF TWO POPLAR CLONES TO WATER AND NITROGEN AVAILABILITY

By

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# ABSTRACT

## PHYSIOLOGICAL RESPONSES OF TWO POPLAR CLONES TO WATER AND NITROGEN AVAILABILITY

By

Zhijun Liu

Two hybrid clones of the genus Populus were subjected to varied combinations of soil water and nitrogen (N) levels in a greenhouse. Gas exchange, water relations, and involvement of abscisic acid (ABA) were examined.

Clones Tristis and Eugenei maintained comparable photosynthesis and stomatal conductance during the initial days of flooding as compared to non-flooded plants. As flooding lengthened, significant declines in diurnal photosynthesis and conductance occurred. However, the declines were partially compensated by the addition of N and the emergence of adventitious rooting around the submerged portions of the stem, suggesting that both clones are flood-resistant. Flooding did not induce substantial changes of leaf ABA concentrations, indicating that the physiological changes induced by flooding were not associated with ABA.

Under minimum water stress, additions of N to the soil increased photosynthesis, leaf chlorophyll, and N contents. As soil progressively dried, however, high-N treated plants showed drastic decreases of photosynthesis. Drought induced substantial accumulation of ABA in the leaves, which was associated with the physiological changes. Nitrogen caused additional ABA accumulation beyond what was induced by drought alone. The experience of one drought period substantially reduced the accumulation of ABA when second drought followed a period of stress interruption.

Photosynthesis, conductance and internal $CO_2$ levels of Eugenei were less sensitive to ABA accumulation than Tristis, although Eugenei accumulated substantially more ABA. Tristis, on the other hand, was sensitive to ABA concentrations but accumulated a smaller amount. This difference may lead to contrasting physiological responses of the two clones in the face of a prolonged drought.

Dedicated to

My mother, and my parents in-law

In memory of my father

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# CHAPTER I

## INTRODUCTION

It is ideal for plants to grow in an environment well supplied with the resources that are required during their growth and development. In nature, however, plants often experience different kinds of stresses that occur because access to the resources required for optimal growth is limited. Even if one or more resources are adequate at one time, other resources may not be adequate, emphasizing the importance of interactions between these resources.

Among the resources required for plant growth and development, water and nitrogen (N) are often limiting (Pregitzer et al. 1990, Chapin et al. 1987), not only because these two resources are the most important components of plant composition, but also because they fluctuate in nature and frequently impose stress to plants (Chapin 1991, Mazzoleni and Dickmann 1988).

Water stress, either drought or flooding, could modify physiological processes of plants such that the growth potential is reduced. One of the common consequences of subjecting plants to soil water deficiency is the restriction of $CO_2$ diffusion into leaf chloroplasts because of stomatal closure, one of the first lines of defense

against desiccation (Chaves 1991). Concomitantly, because of the lack of $CO_2$ substrate, the photosynthetic process is reduced (Pezeshki and Chambers 1986, Ranney et al. 1990, Abrams et al. 1990, Sau and Minguez 1990, Ogren and Oquist 1985, Grieu et al. 1988). Drought stress also affects plant water relations and growth, causing embolisms of the xylem system which disturb water conduction (Sperry and Tyree 1990), reduction of leaf elongation rate (Saab and Sharp 1989) and leaf area (Seiler 1985), modification of carbon allocation patterns such that leaf to root ratios become larger (Ranney et al. 1990, Seiler 1985), and reduction of leaf, root, and stem biomass accumulation (Pregitzer et al. 1990).

Drought generally imposes water deficits to plants, thus disturbing physiological and allocation processes; flooding or waterlogging, on the other hand, does not necessarily alter leaf water relations (Zhang and Davies 1986, Bradford and Hsiao 1982), or can even slightly improve them (Jackson and Hall 1987, Bradford 1983a). In other cases flooding generates similar effects as induced by drought. For example, stomatal closure occurred in flooded plants (Van Der Moezel et al. 1989, Jackson and Hall 1987, Davies and Flore 1986, Neuman et al. 1990, Zhang and Davies 1986, Wadman-van Schravendijk and van Andel 1985, Wadman-van Schravendijk and van Andel 1986, Bradford 1983b), photosynthetic capacity was decreased (Bradford 1983a, 1983b), leaf growth was stunted (Schildwacht 1989, Zhang and

Davies 1986, Neuman et al. 1990), and root growth was reduced (Yamamoto et al. 1987, Schumacher and Smucker 1985, Vu and Yelenosky 1991).

Nitrogen availability around the root zone is crucial in determining plant growth and forest productivity under favorable water conditions. Ample N supply positively affects plant growth in two ways. One is to increase leaf area (Pregitzer et al. 1990, Moon et al. 1990), which allows the plants to maximize the capture of solar energy. The other way is to increase leaf chlorophyll content (Yamashita 1985) and leaf N concentration (Mulligan 1989), thus producing a high photosynthetic capacity (Evans 1983, Moon et al 1990, von Caemmerer and Farquhar 1981, Van Hove et al. 1989, Osmond 1983, Mulligan 1989, Sharkey 1985). High N nutrition can also induce morphological changes in root/shoot ratios, favoring shoot growth (Walters and Reich 1989).

Although high N improves gas exchange processes and growth under adequate water supply, high N plants appear vulnerable to declines of soil moisture. Stomatal conductance (Morgan 1984) and photosynthetic capacity (Walters and Reich 1989, Morgan 1986) in high-N plants were enhanced under well-watered conditions, whereas they dropped more rapidly than in low-N plants as soil dried, a strong indication of water and N interaction. There is little information, however, on the effects of N availability on physiological processes under flooded conditions.

Since water and N are so influential on physiological processes, and since the improvement of photosynthetic capacity with N nutrition depends to such an extent on water availability, it is important that plants experience an optimum zone (Hansen 1976) so these resources can be maximally sequestered and efficiently utilized to maximize photosynthetic capacity and biomass production. Finding this optimum zone is essential for crop managers, yet it is somewhat difficult since plants must be grown at a number of levels of the two factors before an optimum zone can be defined.

As plants often experience fluctuating water availability in the field, it is essential for plants to sense and regulate water consumption to maximize their survival and maintain growth. Thus, to physiologists, it is important to understand how plants respond to repeated progressive drought so that genotypic variations can be defined and corresponding cultural practices determined. Repeated progressive drought could lead to osmotic adjustment, a feature of drought resistance which allows plants to perform normal physiological functions, such as photosynthesis, under water deficiency (Morgan 1984, Seiler 1985, Seiler and Johnson 1985). As drought is prolonged and becomes severe, photosynthetic capacity would be reduced. This gradual reduction of photosynthesis could be induced through two independent mechanisms. Initial soil drying is sensed by the roots, where synthesis of abscisic acid (ABA)

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is initiated. ABA acts as a mediator to signal stomatal adjustment to control water loss independent of leaf water status (Davies et al. 1990). Subsequently under no sign of drought relief, hydraulic signals develop because of leaf water deficits (Abrams et al. 1990), adding to the negative effects on the photosynthetic process.

Diurnal performance of plants is a significant factor in total photosynthate production and, therefore, final yield. Water stress might be responsible for the midday depression of photosynthesis. Depending on the severity and length of a drought, this depression may occur earlier in the day and last longer. As a consequence, the effective hours for the photosynthetic process to function could be greatly shortened. Therefore, diurnal trends may provide a sensitive indication of physiological status of plants. Water stress causes gradual reduction of photosynthetic capacity, whereas effective hours determine how efficiently plants can function at this capacity.

Plants may possess a centralized system to respond to different types of stresses (Chapin 1991). The responses in physiological processes are presumably mediated by the imbalance of plant growth regulators. Indeed, the observations of reduced stomatal aperture under drought or flooding without apparent leaf water deficits (Bradford and Hsiao 1982, Zhang and Davies 1989 a&b, Jackson and Hall 1987, Massoleni and Dickmann 1988) provide evidence that a signal from the stressed roots was produced and translocated

to the leaves where stomatal behavior was regulated. Plants seem to be capable of sensing soil water status by their roots and communicating it to the shoots where physiological changes occur that maximize resistance to stress (Davies et al. 1990). More recent findings indicate that ABA could well be the chemical signal for stomatal change, as ABA accumulation during stress is closely associated with stomatal closure (Davies et al. 1990, Jackson and Hall 1987, Wadman-van Schravendijk and van Andel 1985). Therefore, it appears that investigation of ABA changes during water stress could provide further insight and understanding of the mechanisms of plant responses to water stress.

Soil N status influences physiological processes such as stomatal conductance and photosynthesis, presumably through its regulation of the sensitivity of stomata to ABA levels (Radin and Hendrix 1988). Low-N plants were reportedly sensitive to ABA accumulation, whereas high-N plants were less sensitive to ABA concentrations, requiring higher levels to attain a similar stomatal aperture (Radin et al. 1982). Unfortunately, there is little information regarding the effects of N on ABA production under flooded situations.

Populus, known for its diversity of distribution on earth, has received highest priority as a "model" species in wood energy plantations. These recent efforts are designed to find a feasible alternative that can be used to meet the anticipated need for large, renewable quantities of wood

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biomass for conversion to liquid and gaseous fuels. Such a commitment to Populus is justified by the exceptionally fast growth rate of this genera, its suitability as a model system for understanding and efficiently improving growth processes of wood energy species, and the suitability of its wood for liquid fuel conversion systems (Wright et al. 1987). Poplar also can be used for pulp, sawwood, plywood, chipboard, and other uses. Wood energy plantations emphasize maximum biomass production (Rawat and Nautiyal 1985, Ranney et al. 1987) under short-rotation intensive culture (SRIC), which mimics agricultural practices such as intensive site preparation, irrigation, weeding, and fertilization.

As a part of an on-going project that aims at the determination of responses of two poplar clones to varying levels of applied water and nitrogen, the study reported in this dissertation was designed, with a series of experiments, to address the question of how poplar plants respond to various combinations of water and N levels in terms of their physiological processes, ecological performance, and morphological adaptations. I also investigated how these physiological modifications were associated with the dynamics of ABA, a likely candidate for the chemical signal responsible for these changes. With variations of genotypic response in mind, two poplar clones, Eugenei and Tristis, with well-described differences in physiology, ecology, phenology, and morphology, were chosen

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as a case study. The major hypotheses behind this effort were 1) that both drought and flooding affect gas exchange and this effect is mediated by ABA accumulation; 2) that addition of N to the soil increases photosynthetic capacity and growth, and leads to higher shoot/root ratios; 3) that Eugenei is more responsive to N addition than Tristis; and 4) that high N decreases the sensitivity of stomata to ABA concentration. The results should help physiologists further understand how poplar plants respond under varying levels of water and N availability, and aid tree managers to find the optimal combinations of irrigation and fertilization under which maximum biomass production can be obtained.

# CHAPTER II

## SOIL WATER STATUS MODIFIES THE PHYSIOLOGICAL RESPONSES OF TWO POPLAR CLONES TO NITROGEN AVAILABILITY


### ABSTRACT

This study examined physiological and growth responses of two contrasting poplar clones, Tristis and Eugenei, which were subjected to various combinations of water and N availability. Supplemental N significantly increased net photosynthesis (Pn) and stomatal conductance (g), independent of flooding stress in both clones. Although the onset of flooding caused partial stomatal closure, photosynthetic responses varied with time and clone. Both Tristis and Eugenei showed unchanged Pn during the initial days of waterlogging, but Pn significantly declined as flooding lengthened. This negative effect disappeared with the emergence of adventitious roots in both clones. Photosynthesis in Eugenei fully recovered thereafter, whereas Pn in Tristis failed to completely recover as flooding continued.

Water supply was a major determinant of photosynthetic capacity in plants subjected to drought cycles. While supplemental N raised carboxylation efficiency and

photosynthetic capacity when water level was high, addition of N to droughted plants generated negligible photosynthetic $CO_2$ fixation, as indicated by $A/c_i$ analysis. Drought also resulted in significant reductions in g. Addition of N under drought led to further stomatal closure. Unlike flooding, which did not alter the internal $CO_2$ concentration ($c_i$), progressive drought lowered $c_i$. Eugenei displayed higher stomatal conductance (g) and transpiration (E) than Tristis, leading to a significantly lower water-use efficiency. Eugenei was more responsive to N, but vulnerable to drought, whereas high WUEs in Tristis were obtained within a narrower optimum zone of N yet a wider water level zone than in Eugenei.

Total leaf biomass of both clones was significantly reduced by prolonged drought but not by 27 days of flooding. Ample N led to greater leaf and stem mass, independent of water levels. Clones showed varied reactions to water stress. Height growth and stem mass accumulation of Eugenei was less affected by flooding, but significantly affected by prolonged drought; Tristis was significantly affected by flooding, but less by progressive drought.

# INTRODUCTION

Water and nitrogen (N) in the soil are the two most needed resources for growth of temperate forests (Pregitzer et al. 1990). Water stress, either drought or flooding, can modify physiological processes, causing a series of physiological, ecological, and morphological changes, the extent of these changes depending upon the severity and length of water stress. The ability of plants to respond to prolonged or periodic water stress by adjusting physiological and/or morphological processes often leads to the development of acclimations and adaptations. Variations in this ability are reflected in so called drought-resistant or intolerant genotypes.

Under natural conditions, plants frequently experience various unpredictable degrees of drought because of periodic and irregular rainfall. The most observable effect of drought is the restriction of $CO_2$ diffusion into the leaf because of stomatal closure, one of the first lines of defense against desiccation (Chaves 1991), and the resultant reduction of photosynthetic $CO_2$ fixation (Pezeshki and Chambers 1986, Ranney et al. 1990, Abrams et al. 1990, Sau and Minguez 1990, Ogren and Oquist 1985, Grieu et al. 1988). Reduced starch synthesis and activity of sucrose phosphate

synthase (Vassey and Sharkey 1989, Sharkey and Seemann 1989), decreased translocation velocity of assimilates out of the leaves (Deng et al. 1990), embolisms of the xylem system which disturb water conduction (Sperry and Tyree 1990), reduced leaf elongation rate (Saab and Sharp 1989) and leaf area accretion (Seiler 1985), modification in carbon allocation patterns such that leaf to root ratios become larger (Ranney et al. 1990, Seiler 1985), and reduced leaf, stem, and root biomass accumulation (Pregitzer et al. 1990) are other effects of drought. Despite stomatal closure in response to drought stress, intercellular $CO_2$ concentrations either remain relatively constant due to the close coupling between stomatal conductance and $CO_2$ assimilation (Cowan et al. 1982, Renou et al. 1990, and Burschka et al. 1985, Osmond 1983) or rise, tracing photosynthetic activity (Dickmann et al. 1991 unpublished manuscript).

Flooding shows varied effects on leaf water relations. Waterlogging caused either leaf water deficits (Schildwacht 1989, Wadman-van Schravendijk and van Andel 1985) and desiccation (Jackson and Kowalewska 1983, Wadman-van Schravendijk and van Andel 1986), or did not alter leaf water relations (Zhang and Davies 1986, Bradford and Hsiao 1982). Slight improvements in leaf water relations following flooding of the soil have even occurred (Jackson and Hall 1987, Bradford 1983a). Flooding imposes similar effects on plants as drought stress. Plants subject to

flooding display reduced stomatal conductance (Van Der Moezel et al. 1989, Jackson and Hall 1987, Davies and Flore 1986c, Neuman et al. 1990, Zhang and Davies 1986, Wadman-van Schravendijk and van Andel 1985, Wadman-van Schravendijk and van Andel 1986, Bradford 1982, Bradford 1983b), decreased photosynthetic capacity (Bradford 1983a, 1983b), reduced leaf chlorophyll content (Lorenzen et al. 1990, Jackson and Kowalewska 1983) and cell wall extensibility (Zhang and Davies 1986), stunted leaf growth (Schildwacht 1989, Yamamoto et al. 1987, Neuman et al. 1990, Zhang and Davies 1986) and height growth (Seliskar 1988), reduced root size (Yamamoto et al. 1987, Schumacher and Smucker 1985), and lower total plant biomass (Moon et al. 1990). Although flooding causes an immediate decrease in the concentration of dissolved $O_2$ in the soil water (Drew 1990), the mechanism(s) of the corresponding physiological and morphological modifications seems complex and unclear. Bradford and Hsiao (1982) speculated that accumulation of chemicals, especially abscisic acid, in the leaves with a concomitant reduced translocation of cytokinins induced a series of physiological changes, including reduced photosynthesis and leaf conductance.

It has been commonly recognized that N availability in the root zone is crucial in determining plant growth and forest productivity under favorable water conditions. The most apparent response to ample N supply is an increase in leaf area and biomass (Pregitzer et al. 1990, Moon et al.

1990). Nitrogen nutrition also increased light-dependent photosynthetic capacity (Evans 1983, Moon et al. 1990, Von Caemmerer and Farquhar 1981, Van Hove et al. 1989, Osmond 1983, Mulligan 1989, Sharkey 1985), leaf N content (Mulligan 1989), chlorophyll content (Yamashita 1985), stomatal conductance (Van Hove et al. 1989), shoot/root ratio (Walters and Reich 1989), height growth (Nakos 1979), and total plant biomass (Moon et al. 1990). Specific leaf weight responded to N increases inconsistently, showing either increases (Sau and Minguez 1990, Walters and Reich 1989), no change (Muller and Garnier 1990), or a decrease (Osmond 1983).

While high N imposed positive effects on gas exchange processes and growth, high-N plants have demonstrated vulnerability to declines in water supply. Stomatal conductance (Morgan 1984) and photosynthetic capacity (Walters and Reich 1989, Morgan 1986) in high-N plants were enhanced when soil water was adequate, whereas they dropped rapidly as soil dried, suggesting an increase in the sensitivity to water deficits by high-N plants. However, an earlier finding showed that low-N plants were more sensitive to the lowering of soil water potentials (Radin et al. 1982). In a more recent study, Radin and Hendrix (1988) observed similar reductions in stomatal conductance between high- and low-N plants during drought stress, suggesting no water and N interaction.

Since high water and N improve physiological processes, and since the effectiveness of N seems to be dependent on soil water status, it is crucial for cultured plants to experience an optimum zone (Hansen 1976) in which these resources can be maximally sequestered and efficiently utilized to maximize photosynthetic capacity and biomass production. Therefore, it is important to understand how plants respond physiologically when water is ample whereas N is limited, and vice versa.

This study examines water and N interactions in two poplar clones which differ physiologically, morphologically, ecologically, and phenologically (Michael et al. 1990, Mazzoleni and Dickmann 1988). The objectives of this experiment were (1) to determine the optimal combinations of water and N levels which would lead to best physiological performance and maximum biomass production; (2) to partition stomatal and non-stomatal limitations to photosynthesis; and (3) to compare and contrast the physiological responses of the two clones.

## MATERIALS AND METHODS

**Plant materials.** Two clones in the genus Populus were used: Tristis (Populus tristis x P. balsamifera cv. Tristis No.

1), a hybrid from section Tacamahaca, and Eugenei (P. x euramericana cv. Eugenei), a hybrid from section Aigeiros (Dickmann and Stuart 1983). Cuttings from both clones were planted in 22.7 l plastic pots (one cutting per pot) filled with a natural sandy-loam soil. Frequent watering was provided to cuttings to assure vigorous growth. Before they were used as experimental materials, all cuttings were allowed to grow until they possessed 30 leaves greater than 30 mm in length.

The experiment was conducted in September and October, 1989, in a greenhouse where day/night temperatures were maintained at 18/26$^{o}$C. Supplemental fluorescent lights were used during the day which extended the light period to 16 hours. Photosynthetic photon flux density ranged from 400 $\mu$mol $m^{-2}$ $s^{-1}$ on cloudy days at noon to 700 - 800 $\mu$mol $m^{-2}$ $s^{-1}$ on sunny days. Relative humidity was not controlled.

**Experimental design.** Cuttings were subjected to water stress using the dry down-recharge technique. One dry down-recharge cycle was equivalent to the length of time it took the severe drought stress treatment to dry to -0.5 MPa matric potential (see Soil water control section below for detail). During each cycle the less drought-stressed treatments were watered whenever they reached the target matric potential. There were two controlled factors in this study: soil water and N availability. Five soil water levels, 0 (flooding), -0.02 (field capacity), -0.05 (mild

stress), -0.1 (moderate stress), and -0.5 (severe stress) MPa of soil matric potential, as determined by a soil retention curve, were provided. Nitrogen treatments consisted of 3 levels: -N (no supplemental N), low N (equivalent to 200 kg $ha^{-1}$ of supplemental N, in the form of ammonium-nitrate), and high N (equivalent to 400 kg $ha^{-1}$ of supplemental N).

The experiment was designed as a randomized complete block with a three-factor (clone, water and N) factorial arrangement in three blocks. Analysis of variance with repeated measures was conducted for the plants subject to flooding. Analysis of covariance was taken for the growth data, using the initial growth values immediately preceding the treatments as covariates. Treatment means are regarded as significantly different at $P \leq 0.05$.

**Soil water control.** The simple dry down-recharge technique was used in imposing drought cycles. The control over each water level proposed in this study was monitored with a lysimetric technique. At the very beginning, pots with soil were weighed and subsampling from each pot was conducted to obtain dry soil weight of each pot. These pots were then saturated to field capacity. Soil moisture content was calculated on a dry weight basis. Cuttings were planted in pots, periodic determinations of soil moisture were made by weighing the pots. Adjustments in the weight of each pot was made based on the destructive harvest and weighing of

three extra plants twice during the whole experiment. Relative water content (RWC) could, therefore, be expressed as a percentage of total soil weight. A soil water retention curve developed with a pressure plate apparatus was used to determine the RWC at various matric potentials (APPENDIX A). These predetermined RWCs for each moisture treatment were employed as the threshold points at which re-watering was done. It took eight to ten days for the severe drought treatment to complete one dry down-recharge cycle. Zero MPa water potential (flooding) was maintained with the whole soil column immersed in a plastic bag of tap water.

**Nitrogen.** Nitrogen fertilizer (ammonium-nitrate) was applied with water at the beginning of each of four dry down-recharge cycles (based on severe drought stress). Therefore, N fertilization consisted of four applications at the rate of 0.77 g per pot for the high N treatment and 0.39 g per pot for the low N treatment. The -N (control) treatment received no N fertilizer.

**Growth measurement.** Measurements of growth of each cutting were taken at the end of each dry down-recharge cycle. These measurements included height, ground line diameter (GLD), number of leaves, and length of every leaf. At the end of the experiment, plants were harvested for the determination of biomass of leaves and stem. Due to logistical difficulties, root dry weight was not obtained.

**Gas exchange measurement.** Net photosynthesis (Pn), stomatal conductance (g), transpiration rate (E), and substomatal cavity $CO_2$ concentration ($c_i$) was measured on two new, fully expanded leaves for each plant with a portable leaf chamber and infrared gas analyzer (Analytical Development Co., Herts, England). Measurements were taken at 1000 hour immediately before and after the treatment applications. Water-use efficiency (WUE) was calculated as the ratio of Pn to E.

Carbon dioxide response curves were generated for well-watered and drought-stressed Eugenei and Tristis plants receiving no supplemental N and high N. $CO_2$ response analysis was conducted in a laboratory using an open gas exchange system described by Sams and Flore (1982) and modified as follows: (a) an ADC 225 MK3 Infrared Gas Analyzer (Analytical Development Company, Hoddesdon, UK) was used to measure differential $CO_2$ concentrations at the inlet and outlet of the leaf chambers; (b) air flow entering the chambers was regulated using the following Matheson equipment (Matheson Instruments, Horsham, Pennsylvania): 8100 series flow meters and 8200 series mass flow controllers connected to a model 8219 multichannel Dyna-Blender. Various levels of $CO_2$ concentration were provided in an increasing and consecutive order, at an interval of 0, 100, 180, 260, 350, 520, and 900 $\mu mol\ mol^{-1}$ ambient $CO_2$. Temperature and vapor pressure gradient were kept at 20 to

28°C and 1 kPa. Each level was stabilized for about 15 min before measurements were taken. All parameters were calculated based on the program developed by Moon and Flore (1986).

**Water relations.** Leaf water potential was measured with a pressure chamber (PMS Instruments) once on two fully mature leaves for each plant during 1200 to 1400 hours at the end of three drought cycles.

## RESULTS

### Effects of Flooding

Plants subject to 27 days of flooding did not show distinct reductions in their photosynthetic capacity when averaged over days (Table 2.1). During the 27 days of flooding stress, both clones displayed similar response patterns. No water and N interactions took place. Photosynthesis, however, did vary with days since the onset of the flooding treatment. Although flooding did not cause significant deviations in photosynthesis compared to the well-watered and well-drained plants over the experimental course, there was weak evidence (Adj. P values) that variation in N led to a different response.

It is of interest to examine on which day(s) the treatments differed once the water and N regimes were introduced. Therefore, further analyses were made on days 2, 4, 6, 8, 9, 14, 17, 20 and 27 independently; the probability of significance levels is presented in Table 2.2. Tristis displayed a higher Pn than Eugenei initially (2 days) but this superiority disappeared quickly as flooding lengthened (Figure 2.1A). Flooding did not significantly induce a reduction of Pn of both clones until day 14. Thereafter Eugenei recovered completely, whereas Pn of Tristis continued to be significantly lowered by flooding. It is notable that the commencement of recovery of Pn was accompanied by the emergence of adventitious roots from the portion of the stem that was submerged in water.

There was no immediate observable effects of N application on the Pn of flooded trees. However, N application raised the photosynthetic capacity beginning 17 days after application of flooding (Figure 2.1B), although there was no substantial difference between low- and high-N treatments. This delayed effect probably suggests a required length of time for incorporation of N-products into the photosynthetic apparatus.

Stomatal conductance was significantly affected by flooding (Table 2.3). There were different trends between Eugenei and Tristis, as well as between flooding and control water regimes. Clonal effects, flooding, and N applications generated much variation in g on most of the days, but no

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water and N interactions occurred on any day (Table 2.2). Eugenei displayed a higher g than Tristis, on the average, but this was primarily caused by the higher g of Eugenei under well-watered conditions (Figure 2.2A). Flooding led to decreases in g in Eugenei in the initial two weeks, whereas flooding was less effective in the induction of stomatal closure in Tristis. Similar to Pn responses, N applications increased g (Figure 2.2B). Although high N resulted in a higher g than low N, the increased g was not significant.

Clones demonstrated an overall difference in their transpiration along the 27 days of flooding stress (Table 2.4). Although water and N regimes did not generate substantial effects on water flux when averaged over days, water or N alone affected E on certain days, as indicated by the adjusted P values in Table 2.4 and Table 2.2. Eugenei displayed a higher E than Tristis and this trend was more obvious with time (Figure 2.3A). Flooding initially increased E but subsequently significantly reduced E up to the ninth day, followed by a slight recovery to the non-stressed level (Figure 2.3B). N enrichment increased E on the later days, but there was no difference between the added amounts (Figure 2.3C).

Neither flooding nor N additions altered $c_i$, although $c_i$ varied significantly with days (Table 2.5). There was some evidence that flooding caused $c_i$ changes at certain day(s) (Adj. P=0.0727), primarily the initial day of

flooding (Table 2.2). Flooding increased $c_i$ level by 8 $\mu$mol $mol^{-1}$. On day 17, addition of N at the lower amount resulted in a 13 $\mu$mol $mol^{-1}$ higher $c_i$ than the high N and -N treatments.

Water-use efficiency was not affected overall by the water and N regimes and was similar between the clones (Table 2.6). Again, the overall variations differed with days as the environment fluctuated. Although the differences between water levels and the interaction of water and N on day 14 reached significant levels, the primary difference in WUE resided between the two clones as the flooding prolonged (Figure 2.4), with Tristis being clearly superior to Eugenei.

## Effects of Drought

Drought stress imposed significant effects on gas exchange from the onset of first drought through the third drought cycle (Table 2.7). Results of the second drought cycle are not presented because the treatments did not reach their maximum water stress on the same day. Water level appeared to be the sole factor affecting gas exchange processes at the end of the first drought. Clones responded in a similar way. Nitrogen regimes did not induce any significant changes in Pn, $c_i$ and WUE. However, there was an obvious interaction of water and N in their effects on g and E.

Having experienced three drought cycles, clones showed deviations in g, E, $c_i$ and WUE, but not in Pn (Table 2.7). Eugenei displayed higher g, E, and $c_i$ than Tristis (Table 2.8). As a result, WUE was significantly lower in Eugenei due to the lack of change in Pn. Nitrogen regimes affected g, E, $c_i$ and WUE.

Mild drought stress did not cause Pn to decline, whereas Pn was significantly decreased as soil further dried (APPENDIX B.1). Eugenei (Figure 2.5A) resembled Tristis (Figure 2.5B), although the former seemed to respond more gradually than the latter. Nitrogen fertilization, however, showed little effect on Pn.

The effects of N levels on g were significantly altered by the severity of drought stress in both Tristis (Figure 2.5C) and Eugenei (Figure 2.5D). When plants experienced no water stress, N application did not induce apparent changes in g, resembling the effects on Pn. In contrast, under mild and moderate drought stress, high N substantially reduced g, whereas low N plants were relatively unaffected. When plants experienced severe drought conditions, any addition of N resulted in a significant decrease in g, with a strong interaction shown between water and N. Comparing -N plants with their high-N counterparts, g was increased by 17% under no water stress, but reduced by 27% under mild drought, 43% under moderate drought, and 52% under severe drought. There also was a slight variation between Eugenei and Tristis in demonstrating the effects of water and N interactions. It

appeared that Eugenei was more sensitive to declines of available water and less to N increases than Tristis in regulating stomatal behavior, whereas Tristis was more sensitive to N levels upon the onset of drought.

Transpiration of Tristis (Figure 2.5E) and Eugenei (Figure 2.5F) resembled g after three drought cycles. Drought stress alone induced little stomatal closure, particularly in Tristis, but addition of N to the droughted plants reduced E substantially. Again, Eugenei exhibited greater tolerance to soil drought at a higher N status, whereas Tristis displayed a greater sensitivity to increased N levels as the drought was prolonged.

Different water and N levels led to independent variations of $c_i$ in both clones (Figure 2.5G and 2.5H). As water availability decreased, $c_i$ became significantly lower in an approximately linear proportion. However, as severe drought developed, an apparent rise of $c_i$ occurred. When N levels increased, $c_i$ declined, independent of water availability.

The highest WUE was observed on plants with low-N supplement under mild drought conditions in both Tristis (Figure 2.5I) and Eugenei (Figure 2.5J). However, the slight differences between the two clones in the response patterns of E were also reflected in the patterns of WUE. The optimum zone for obtaining highest WUEs of Eugenei resided within a wider zone of supplemental N than Tristis, but only under mild drought. Tristis, on the other hand,

realized its optimal WUEs within a narrow zone of N but at a wider range of water availability, including mild and moderate drought.

Plants of both clones that had experienced three drought cycles were examined with the $CO_2$ response (or $A/C_i$ curve) analysis to explore and discriminate between stomatal and non-stomatal limitations to Pn. It was found that $CO_2$ compensation point was little changed by either drought or N addition, but significant interactions between drought and high N dramatically lowered this point in Eugenei (Table 2.9 iii). Carboxylation efficiency (Table 2.9 iv) was significantly affected by water and N regimes in a similar way in both clones, although Eugenei displayed lower values under well-watered conditions. Without N addition, drought itself did not significantly reduce carboxylation efficiency. With supplemental N, carboxylation efficiency showed variations with soil water status. Under no water stress, high N substantially increased this efficiency. On the other hand, high-N plants displayed little response to rise in $CO_2$ concentration under drought. Photosynthetic capacity was dramatically influenced by these treatments as indicated by the asymptotic values in Figure 2.6. Tristis exhibited a more apparent separation of the treatments than Eugenei. Nitrogen enrichment greatly raised the capacity for $CO_2$ fixation at an ample water supply. But $CO_2$ fixation was negligible under drought stress. Water deficiency alone lowered the photosynthetic capacity, but not substantially.

Residual (non-stomatal) resistance (Table 2.9 v) to $CO_2$ diffusion was much lower when plants were under both ample water and N, slightly higher under drought, and extremely high under drought and high N combinations.

In the well-watered plants, stomata imposed little limitations to photosynthesis, whereas supplementing N or withholding water from these plants increased stomatal limitations (Table 2.9 v). Specifically in Tristis, increases in $CO_2$ fixation responding to N enrichment under minimum drought stress were largely attributed to non-stomatal factors, i.e. biochemical capacity at the chloroplast level; only 13% of the increase was due to a stomatal contribution. In contrast, almost 54% of the decreases in photosynthesis because of drought were attributable to stomatal limitations.

Prolonged droughts resulted in significant decreases in midday leaf water potentials in both clones (Table 2.10). Supplemental N also led to decreases of midday leaf water potentials at all water levels except flooding.

## Effects of water stress and N on final yields

Total height was significantly affected following cyclic droughts or waterlogging (Table 2.11), but the responses differed in the two clones (APPENDIX B.2). In Eugenei, flooding caused little reduction in height, whereas restrictions in water supply led to decreases (Figure 2.7B).

In contrast, flooding significantly decreased height in Tristis, whereas only severe drought reduced height growth (Figure 2.7A). Effects of N significantly interacted with water levels. In Eugenei, supplemental N promoted height growth only when water supply was ample, not as soil dried (Figure 2.7B). In Tristis, however, addition of N promoted growth under no water stress and severe drought, yet did not generate a substantial influence at moderate drought (Figure 2.7A).

There was no significant difference in ground line diameter growth between Eugenei and Tristis, nor did flooding generate negative effects on GLD. Declines in soil matric potential, on the other hand, caused reductions in both Tristis (Figure 2.7C) and Eugenei (Figure 2.7D). Supplement of N increased diameter slightly in Tristis, but not at all in Eugenei.

On the average, leaf biomass was significantly higher in Eugenei than in Tristis. Flooding did not significantly reduce leaf biomass, whereas drought did (Figures 2.7E and 2.7F). Addition of N increased leaf biomass, independent of water levels. However, unlike the diameter responses, plants treated at the lower N level displayed substantially greater leaf growth than those treated at the higher N level.

Stem biomass accumulation resembled the responses of height to the water and N regimes, except that clones did not differ (Table 2.11). Eugenei was more affected by

drought than by flooding (Figure 2.7H). On the contrary, Tristis was less affected by drought but more so by flooding (Figure 2.7G).

## DISCUSSION

The short-term maintenance of photosynthetic capacity following initial waterlogging in this experiment probably indicated that both Tristis and Eugenei possessed a certain inherent ability to withstand or adjust to $O_2$-deficiency in the root zone (Figure 2.1). As flooding extended to two weeks, this ability weakened, and photosynthetic activity declined. This result is in agreement with findings with tomato plants subjecting to flooding (Bradford 1983a), but differs from the report on rabbiteye blueberry plants in which one day of flooding significantly lowered $CO_2$ fixation capacity (Davies and Flore 1986c), suggesting little inherent flooding resistance. As waterlogging continued, Eugenei and Tristis responded by means of a modification of their root morphology, exhibiting copious production of adventitious roots. These newly emerged roots from the submerged portion of the stem spread and stretched up towards the water surface. As a consequence, the decline in photosynthesis was reversed. This phenomenon has been observed in a variety of species, including Eucalyptus,

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where adventitious roots emerged after five weeks of waterlogging (Van Der Moezel et al. 1989), and with Phaseolus vulgaris where adventitious roots appeared after a week of flooding (Wadman-van Schravendijk and van Andel 1985). The newly emerged roots may be responsible for the recovery of photosynthesis because they may serve as new sinks for photosynthates, thus avoiding possible feedback inhibition due to weakened sink strength. The new roots also may synthesize plant growth regulators, especially cytokinins (Bradford and Hsiao 1982).

In contrast to the photosynthetic response, g was very sensitive to flooding (Table 2.2, Figure 2.2). Flooding led to partial stomatal closure and suppressed full opening until adventitious roots appeared, after which a slight recovery of g occurred. Stomatal closure is a common response of plants to flooding (Bradford 1983a), regardless of their variations in flooding resistances, e.g. in two contrasting flood-resistant Eucalyptus species (Van Der Moezel et al. 1989), sensitive pea plants (Jackson and Hall 1987), Phaseolus vulgaris and a Populus trichocarpa x P. deltoides hybrid (Neuman et al. 1990), and tomato (Bradford 1983b). The insensitivity of Pn to flooding and the contrasting sensitivity of g suggests that g is a sensitive indicator of root stress, regardless of the strength of flood-resistance. Reduced g was not the result of abscisic acid accumulation in leaves of flooded plants (Chapter 4). Other inhibitors such as CN ions or the reduction of

cytokinins in the flooded roots may be involved. The ability to generate adventitious roots accounts for the variations between sensitive or tolerant genotypes (Jackson and Kowalewska 1983, and Van Der Moezel et al. 1989).

The role of N in raising photosynthetic capacity has long been recognized. Ample N insures the buildup of the photosynthetic apparatus and enzymes for $CO_2$ assimilation (Evans 1989). In flooded plants in the current study, supplemental N caused significant increases in photosynthetic capacity, indicating that flooding did not interfere with N distribution and utilization in the leaves. Nitrogen addition to the plants subject to drought cycles, however, raised photosynthetic capacity slightly but not significantly. This result is contradictory to the findings by Morgan (1986) and Walters and Reich (1989). The ineffectiveness of N in increasing photosynthetic capacity in the current study may be due to the limited light resources in the greenhouse. Nitrogen-deficient and high-N plants started to deviate in their photosynthetic responses above 600 $\mu$mol $m^{-2}$ $s^{-1}$ (data not shown), but light levels in the greenhouse did not exceed 800 $\mu$mol $m^{-2}$ $s^{-1}$.

Under the circumstances of progressive drought, the positive role of N in improving stomatal openings observed in the flooded plants was changed, demonstrating a strong interdependence between the two most prominent resources to plants. As soil progressively dried, N-treated plants closed stomata to a greater extent, whereas N-deficient

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plants displayed relatively unchanged g, in agreement with findings in wheat (Morgan 1984). The drastic drop of g in high-N plants was correlated with substantial declines of midday leaf water potentials, as high N supply caused substantially more leaf biomass production. Since high-N nutrition often results in relatively large plants (Morgan 1984, 1986, and Walters and Reich 1989) and greater transpiratory surfaces, they deplete water from the root zone more quickly, leading to desiccation if the depleted water is not replaced. Whether high N in the leaves of water-stressed plants imposes any unfavorable direct effect on biochemical or metabolic processes is not clear.

Water level in the soil was a major determinant of physiological performance. When drought was prolonged, leaf water potentials decreased and corresponding stomatal closure occurred. With the restriction of $CO_2$ diffusion into the leaves under mild drought, $c_i$ was significantly lowered. An obvious rise of $c_i$ was observed as soil further dried (Figure 2.5G and 2.5H). Findings in the current study are in agreement with previous results showing that if water stress is severe enough, chloroplast-level inhibition of Pn occurs and $c_i$ can rise (Dickmann et al. 1991, unpublished manuscript), but does not support the findings that $c_i$ remains relatively constant in the face of drought. This hypothesis holds that reduced g is balanced by decreased Pn caused by increased diffusion resistance from substomatal vicinity to chloroplast to maintain a constant $c_i$

concentration (Renou et al. 1990). Unlike the drought stress, flooding rarely changed $c_i$ in the present experiment (Table 2.2), indicating different mechanisms by which plants perceive drought and flooding stresses.

Physiological, phenological and morphological differences between Eugenei and Tristis have been previously reported (Michael et al. 1990, Mazzoleni and Dickmann 1988, Dickmann et al. 1990, Pregitzer et al. 1990, Nguyen et al. 1990). However, both clones displayed similar responses to flooding; they both were able to maintain their photosynthetic capacity for a number of days despite declines in g and a relatively constant $c_i$. As flooding was prolonged, causing obvious declines in Pn, adventitious roots emerged, a characteristic of flooding-tolerance (Jackson and Kowalewska 1983, Drew 1990). The flood-resistant features of these hybrids could originate from their parent species which are naturally adapted to grow in wetlands or riverbanks (Dickmann and Stuart 1983). The two clones, on the other hand, started to deviate in their photosynthetic responses after a temporary relief of flooding. Eugenei displayed a full recovery, whereas Tristis was unable to reverse the negative effects of waterlogging, indicating that Eugenei is a more flood-resistant hybrid.

Eugenei and Tristis also demonstrated differences in other physiological responses. Eugenei, a hybrid with high resource demands (Pregitzer et al. 1990), was indeed more

responsive to N increases, incorporating more assimilates into leaf and height growth. However, because of its relatively high g and E, WUE in Eugenei was significantly lower than in Tristis. As a consequence, in face of progressive drought Eugenei was more vulnerable to desiccation. Tristis, on the other hand, was a more drought-resistant clone, for it possesses lower g and E, as well as a smaller total leaf mass. Tristis also has a larger allocation to roots and more fine roots (Pregitzer et al. 1990). In addition, a planophile leaf display in Tristis (Dickmann et al. 1990) creates shading of the lower leaves, thus effectively reducing the water transpiring area on a whole-plant basis. With its lower demand for N resources, Tristis seems to be a physiologically "conservative" type.

These physiological differences offer significant implications for the selection of clones for establishment of plantations, particularly in the short-rotation intensive culture systems. On a site where drought occurs frequently and irrigation is not feasible, fertilization should be employed with extreme caution. In places where soil water supply can be maintained, uses of fertilizer will be favorable to biomass production.

Table 2.1 Analysis of variance of photosynthesis with repeated measures during 27 days of flooding.

| Source | df | MS | F | P > F | Adj. P > F. G-G | Adj. P > F. H-F |
|---|---|---|---|---|---|---|
| Clone | 1 | 0.010 | 0.01 | 0.9334 | | |
| Water | 1 | 1.478 | 1.04 | 0.3206 | | |
| N | 2 | 2.643 | 1.86 | 0.1829 | | |
| Clone*Water | 1 | 0.995 | 0.70 | 0.4131 | | |
| Clone*N | 2 | 0.268 | 0.19 | 0.8294 | | |
| Water*N | 2 | 1.257 | 0.88 | 0.4291 | | |
| Clone*Water*N | 2 | 2.018 | 1.42 | 0.2662 | | |
| Error(treatment) | 19 | 1.421 | | | | |
| Day | 8 | 16.187 | 28.34 | 0.0001 | 0.0001 | 0.0001 |
| Clone*Day | 8 | 0.306 | 0.54 | 0.8279 | 0.7411 | 0.8279 |
| Water*Day | 8 | 0.717 | 1.26 | 0.2711 | 0.2910 | 0.2711 |
| N*Day | 16 | 1.046 | 1.83 | 0.0317 | 0.0688 | 0.0317 |
| Clone*Water*Day | 8 | 0.678 | 1.19 | 0.3102 | 0.3218 | 0.3102 |
| Clone*N*Day | 16 | 0.651 | 1.14 | 0.3235 | 0.3424 | 0.3235 |
| Water*N*Day | 16 | 0.608 | 1.06 | 0.3944 | 0.3977 | 0.3944 |
| Clone*Water*N*Day | 16 | 0.375 | 0.66 | 0.8331 | 0.7557 | 0.8331 |
| Error(Day) | 152 | 0.571 | | | | |

NOTE: Adj. P are probabilities associated with the Greenhouse-Geisser (G-G) and Hyynh-Feldt (H-F) adjusted F-tests. MS, mean square. Mauchly's sphericity test: $P > X^2 = 0.03$.

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Table 2.2 Probability of significance levels of analysis of variance on the effects of flooding and N on five gas exchange variables during a 27-day experiment.

| | | Days after waterlogging | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Treatment | Var. | 2 | 4 | 6 | 8 | 9 | 14 | 17 | 20 | 27 |
| Clone(C) | Pn | 0.02* | 0.91 | 0.22 | 0.35 | 0.71 | 0.75 | 0.78 | 0.09 | 0.80 |
| | g | 0.01* | 0.36 | 0.001* | 0.01* | 0.50 | 0.0001* | 0.009* | 0.02* | 0.04* |
| | E | 0.06 | 0.50 | 0.006* | 0.007* | 0.45 | 0.001* | 0.0005* | 0.04* | 0.002* |
| | Ci | 0.35 | 0.37 | 0.35 | 0.51 | 0.25 | 0.57 | 0.56 | 0.19 | 0.55 |
| | WUE | 0.88 | 0.81 | 0.68 | 0.76 | 0.62 | 0.13 | 0.28 | 0.002* | 0.03* |
| Water(W) | Pn | 0.69 | 0.86 | 0.73 | 0.89 | 0.37 | 0.03* | 0.96 | 0.09 | 0.14 |
| | g | 0.008* | 0.03* | 0.0001* | 0.0001* | 0.01* | 0.03* | 0.69 | 0.03* | 0.46 |
| | E | 0.01* | 0.04* | 0.0003* | 0.0001* | 0.04* | 0.13 | 0.30 | 0.06 | 0.14 |
| | Ci | 0.02* | 0.33 | 0.14 | 0.26 | 0.56 | 0.24 | 0.82 | 0.78 | 0.41 |
| | WUE | 0.06 | 0.39 | 0.23 | 0.32 | 0.69 | 0.04* | 0.82 | 0.98 | 0.43 |
| N | Pn | 0.95 | 0.86 | 0.61 | 0.89 | 0.48 | 0.34 | 0.003* | 0.03* | 0.01* |
| | g | 0.66 | 0.94 | 0.03* | 0.005* | 0.21 | 0.26 | 0.02* | 0.0008* | 0.003* |
| | E | 0.53 | 0.93 | 0.27 | 0.02* | 0.36 | 0.66 | 0.02* | 0.06 | 0.003* |
| | Ci | 0.72 | 0.43 | 0.85 | 0.70 | 0.93 | 0.36 | 0.002* | 0.59 | 0.94 |
| | WUE | 0.41 | 0.52 | 0.89 | 0.72 | 0.58 | 0.12 | 0.0002* | 0.36 | 0.67 |
| CxW | Pn | 0.17 | 0.22 | 0.62 | 0.99 | 0.91 | 0.95 | 0.87 | 0.23 | 0.04* |
| | g | 0.96 | 0.15 | 0.01* | 0.008* | 0.07 | 0.87 | 0.91 | 0.88 | 0.98 |
| | E | 0.59 | 0.43 | 0.79 | 0.07 | 0.44 | 0.53 | 0.33 | 0.51 | 0.27 |
| | Ci | 0.46 | 0.82 | 0.48 | 0.41 | 0.29 | 0.86 | 0.63 | 0.22 | 0.17 |
| | WUE | 0.46 | 0.26 | 0.59 | 0.55 | 0.42 | 0.80 | 0.78 | 0.47 | 0.14 |
| CxN | Pn | 0.84 | 0.64 | 0.11 | 0.59 | 0.27 | 0.67 | 0.68 | 0.13 | 0.72 |
| | g | 0.90 | 0.85 | 0.06 | 0.0002* | 0.67 | 0.44 | 0.39 | 0.58 | 0.34 |
| | E | 0.86 | 0.96 | 0.09 | 0.0003* | 0.69 | 0.53 | 0.32 | 0.75 | 0.24 |
| | Ci | 0.78 | 0.42 | 0.72 | 0.42 | 0.10 | 0.31 | 0.58 | 0.10 | 0.98 |
| | WUE | 0.49 | 0.60 | 0.13 | 0.60 | 0.13 | 0.45 | 0.32 | 0.14 | 0.89 |
| WxN | Pn | 0.47 | 0.85 | 0.49 | 0.93 | 0.40 | 0.14 | 0.50 | 0.11 | 0.22 |
| | g | 0.75 | 0.07 | 0.99 | 0.69 | 0.86 | 0.59 | 0.23 | 0.27 | 0.24 |
| | E | 0.46 | 0.12 | 0.76 | 0.84 | 0.77 | 0.98 | 0.19 | 0.73 | 0.18 |
| | Ci | 0.96 | 0.79 | 0.98 | 0.89 | 0.49 | 0.13 | 0.35 | 0.44 | 0.90 |
| | WUE | 0.99 | 0.99 | 0.65 | 0.89 | 0.75 | 0.03* | 0.15 | 0.13 | 0.36 |
| CxWxN | Pn | 0.10 | 0.90 | 0.36 | 0.84 | 0.46 | 0.37 | 0.59 | 0.67 | 0.61 |
| | g | 0.09 | 0.76 | 0.30 | 0.34 | 0.98 | 0.49 | 0.45 | 0.76 | 0.73 |
| | E | 0.04* | 0.73 | 0.15 | 0.42 | 0.96 | 0.23 | 0.08 | 0.48 | 0.11 |
| | Ci | 0.89 | 0.54 | 0.50 | 0.83 | 0.45 | 0.35 | 0.56 | 0.80 | 0.98 |
| | WUE | 0.69 | 0.71 | 0.44 | 0.86 | 0.24 | 0.46 | 0.42 | 0.87 | 0.32 |
| MSE[1] | Pn | 0.4165 | 1.2235 | 0.7691 | 2.1305 | 1.5437 | 2.2562 | 1.5026 | 0.6519 | 1.3855 |
| | g | 0.0053 | 0.0069 | 0.0039 | 0.0025 | 0.0040 | 0.0044 | 0.0054 | 0.0039 | 0.0035 |
| | E | 0.6224 | 0.7653 | 0.7528 | 0.2108 | 0.9864 | 1.3170 | 0.6115 | 1.0940 | 0.6326 |
| | Ci | 195.99 | 104.18 | 181.62 | 284.89 | 120.78 | 308.22 | 165.48 | 147.34 | 453.24 |
| | WUE | 0.0520 | 0.0756 | 0.0237 | 0.1804 | 0.1443 | 0.0512 | 0.0457 | 0.0288 | 0.0501 |
| (df)[2] | | (24) | (24) | (24) | (24) | (23) | (20) | (24) | (24) | (23) |

[1]Mean square of error.

[2]Degree of freedom associated with MSE.

Asterisks indicate probabilities that are less than or equal to 0.05.

Table 2.3 Analysis of variance of stomatal conductance with repeated measures during 27 days of flooding.

| Source | df | MS | F | P > F | Adj. P > F. G-G | Adj. P > F. H-F |
|---|---|---|---|---|---|---|
| Clone | 1 | 0.038 | 3.82 | 0.0656 | | |
| Water | 1 | 0.046 | 4.55 | 0.0463 | | |
| N | 2 | 0.028 | 2.81 | 0.0851 | | |
| Clone*Water | 1 | 0.016 | 1.62 | 0.2187 | | |
| Clone*N | 2 | 0.007 | 0.66 | 0.5289 | | |
| Water*N | 2 | 0.005 | 0.46 | 0.6351 | | |
| Clone*Water*N | 2 | 0.006 | 0.55 | 0.5852 | | |
| Error(treatment) | 19 | 0.010 | | | | |
| Day | 8 | 0.053 | 38.35 | 0.0001 | 0.0001 | 0.0001 |
| Clone*Day | 8 | 0.013 | 9.06 | 0.0001 | 0.0001 | 0.0001 |
| Water*Day | 8 | 0.010 | 7.09 | 0.0001 | 0.0001 | 0.0001 |
| N*Day | 16 | 0.003 | 2.38 | 0.0034 | 0.0145 | 0.0034 |
| Clone*Water*Day | 8 | 0.004 | 2.84 | 0.0057 | 0.0193 | 0.0057 |
| Clone*N*Day | 16 | 0.003 | 1.82 | 0.0327 | 0.0665 | 0.0327 |
| Water*N*Day | 16 | 0.003 | 2.09 | 0.0113 | 0.0323 | 0.0113 |
| Clone*Water*N | 16 | 0.001 | 0.88 | 0.5927 | 0.5545 | 0.5927 |
| Error(Day) | 152 | 0.00139 | | | | |

NOTE: Adj. P are probabilities associated with the Greenhouse-Geisser (G-G) and Hyynh-Feldt (H-F) adjusted F-tests. MS, mean square. Mauchly's sphericity test: P > $X^2$ = 0.0066.

Table 2.4 Analysis of variance of transpiration with repeated measures during 27 days of flooding.

| Source | df | MS | F | P > F | Adj. P > F G-G | Adj. P > F H-F |
|---|---|---|---|---|---|---|
| Clone | 1 | 8.682 | 4.41 | 0.0493 | | |
| Water | 1 | 5.358 | 2.72 | 0.1154 | | |
| N | 2 | 2.654 | 1.35 | 0.2834 | | |
| Clone*Water | 1 | 0.031 | 0.02 | 0.9010 | | |
| Clone*N | 2 | 1.362 | 0.69 | 0.5128 | | |
| Water*N | 2 | 0.699 | 0.36 | 0.7057 | | |
| Clone*Water*N | 2 | 4.508 | 2.29 | 0.1285 | | |
| Error(treatment) | 19 | 1.968 | | | | |
| Day | 8 | 22.229 | 101.99 | 0.0001 | 0.0001 | 0.0001 |
| Clone*Day | 8 | 1.606 | 7.37 | 0.0001 | 0.0001 | 0.0001 |
| Water*Day | 8 | 0.910 | 4.18 | 0.0002 | 0.0026 | 0.0002 |
| N*Day | 16 | 0.445 | 2.04 | 0.0138 | 0.0434 | 0.0138 |
| Clone*Water*Day | 8 | 0.451 | 2.07 | 0.0420 | 0.0831 | 0.0420 |
| Clone*N*Day | 16 | 0.322 | 1.48 | 0.1153 | 0.1688 | 0.1153 |
| Water*N*Day | 16 | 0.315 | 1.45 | 0.1270 | 0.1800 | 0.1270 |
| Clone*Water*N*Day | 16 | 0.185 | 0.85 | 0.6307 | 0.5763 | 0.6307 |
| Error(day) | 152 | 0.2179 | | | | |

NOTE: Adj. P are probabilities associated with the Greenhouse-Geisser (G-G) and Hyynh-Feldt (H-F) adjusted F-tests. MS, mean square. Mauchly's sphericity test: $P > X^2 = 0.0003$.

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Table 2.5 Analysis of variance of substomatal $CO_2$ level with repeated measures during 27 days of flooding.

| Source | df | MS | F | P > F | Adj. P > F G-G | Adj. P > F H-F |
|---|---|---|---|---|---|---|
| Clone | 1 | 4.583 | 0.01 | 0.9278 | | |
| Water | 1 | 5.022 | 0.01 | 0.9245 | | |
| N | 2 | 195.629 | 0.36 | 0.7025 | | |
| Clone*Water | 1 | 576.329 | 1.06 | 0.3162 | | |
| Clone*N | 2 | 87.384 | 0.16 | 0.8527 | | |
| Water*N | 2 | 191.007 | 0.35 | 0.7083 | | |
| Clone*Water*N | 2 | 285.806 | 0.53 | 0.5996 | | |
| Error(treatment) | 19 | 543.870 | | | | |
| Day | 8 | 3643.665 | 59.41 | 0.0001 | 0.0001 | 0.0001 |
| Clone*Day | 8 | 74.765 | 1.22 | 0.2915 | 0.3086 | 0.2915 |
| Water*Day | 8 | 133.322 | 2.17 | 0.0324 | 0.0727 | 0.0324 |
| N*Day | 16 | 88.207 | 1.44 | 0.1310 | 0.1866 | 0.1310 |
| Clone*Water*Day | 8 | 37.596 | 0.61 | 0.7660 | 0.6700 | 0.7660 |
| Clone*N*Day | 16 | 73.080 | 1.19 | 0.2807 | 0.3118 | 0.2807 |
| Water*N*Day | 16 | 52.778 | 0.86 | 0.6154 | 0.5616 | 0.6154 |
| Clone*Water*N*Day | 16 | 37.121 | 0.61 | 0.8762 | 0.7860 | 0.8762 |
| Error(day) | 152 | 61.335 | | | | |

NOTE: Adj. P are probabilities associated with the Greenhouse-Geisser (G-G) and Hyynh-Feldt (H-F) adjusted F-tests. MS, mean square. Mauchly's sphericity test: P > $X^2$ = 0.0054.

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Table 2.6 Analysis of variance of water-use efficiency with repeated measures during 27 days of flooding.

| Source | df | MS | F | P > F | Adj. P > F G-G | Adj. P > F H-F |
|---|---|---|---|---|---|---|
| Clone | 1 | 0.141 | 3.73 | 0.0685 | | |
| Water | 1 | 0.001 | 0.02 | 0.9034 | | |
| N | 2 | 0.088 | 2.34 | 0.1233 | | |
| Clone*Water | 1 | 0.058 | 1.56 | 0.2268 | | |
| Clone*N | 2 | 0.074 | 1.96 | 0.1686 | | |
| Water*N | 2 | 0.080 | 2.12 | 0.1470 | | |
| Clone*Water*N | 2 | 0.018 | 0.47 | 0.6308 | | |
| Error(treatment) | 19 | 0.038 | | | | |
| Day | 8 | 0.758 | 20.83 | 0.0001 | 0.0001 | 0.0001 |
| Clone*Day | 8 | 0.028 | 0.76 | 0.6384 | 0.5683 | 0.6384 |
| Water*Day | 8 | 0.038 | 1.03 | 0.4128 | 0.3990 | 0.4128 |
| N*Day | 16 | 0.039 | 1.06 | 0.3937 | 0.3971 | 0.3937 |
| Clone*Water*Day | 8 | 0.028 | 0.76 | 0.6360 | 0.5665 | 0.6360 |
| Clone*N*Day | 16 | 0.033 | 0.91 | 0.5620 | 0.5228 | 0.5620 |
| Water*N*Day | 16 | 0.023 | 0.64 | 0.8507 | 0.7634 | 0.8507 |
| Clone*Water*Day | 16 | 0.025 | 0.70 | 0.7926 | 0.7088 | 0.7926 |
| Error(day) | 152 | 0.036 | | | | |

NOTE: Adj. P are probabilities associated with the Greenhouse-Geisser (G-G) and Hyynh-Feldt (H-F) adjusted F-tests. MS, mean square. Mauchly's sphericity test: P > $X^2$ = 0.0064.

Table 2.7 Probability of significance levels of analysis of variance on gas exchange after one and three drought cycles[1]

| | Source | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Parameter | Clone (C) | Water (W) | N | C*W | C*N | W*N | C*W*N | MSE(df)[2] |
| Cycle 1 | | | | | | | | |
| Pn | 0.6829 | 0.0022* | 0.7308 | 0.7767 | 0.4153 | 0.1779 | 0.6097 | 0.9928(31) |
| g | 0.3746 | 0.0001* | 0.3535 | 0.0517 | 0.8374 | 0.0408* | 0.9567 | 0.0029(31) |
| E | 0.1779 | 0.0001* | 0.3845 | 0.3453 | 0.8364 | 0.0380* | 0.9223 | 0.4942(31) |
| $c_i$ | 0.0811 | 0.0005* | 0.2521 | 0.3853 | 0.2508 | 0.0990 | 0.3450 | 127.59(31) |
| WUE | 0.0976 | 0.0255* | 0.1424 | 0.8316 | 0.3460 | 0.2645 | 0.2239 | 0.0633(31) |
| Cycle 3 | | | | | | | | |
| Pn | 0.2173 | 0.0001* | 0.1161 | 0.0985 | 0.7339 | 0.3007 | 0.5023 | 1.9175(57) |
| g | 0.0141* | 0.0001* | 0.0199* | 0.7886 | 0.2728 | 0.0001* | 0.6793 | 0.0041(57) |
| E | 0.0103* | 0.0001* | 0.0080* | 0.3662 | 0.3378 | 0.0001* | 0.1312 | 0.8938(57) |
| $c_i$ | 0.0139* | 0.0336* | 0.0003* | 0.3540 | 0.9022 | 0.2813 | 0.7249 | 435.66(57) |
| WUE | 0.0039* | 0.0448* | 0.0002* | 0.5909 | 0.7388 | 0.1911 | 0.4793 | 0.1066(57) |

[1]Cyclic drought refers to the drying down of soil matric potential to approximately -0.5 MPa, occurring after 8 to 10 days of water withholding.
[2]Mean square of error (degree of freedom).
Asterisks indicate probabilities that are less than or equal to 0.05.

Table 2.8 Clonal responses after three drought cycles[1]

| Clone | Pn $\mu mol\ m^{-2}\ s^{-1}$ | g $mol\ m^{-2}\ s^{-1}$ | E $mmol\ m^{-2}\ s^{-1}$ | $c_i$ $\mu mol\ mol^{-1}$ | WUE $\mu mol\ mmol^{-1}$ |
|---|---|---|---|---|---|
| Eugenei | 3.5a[1] | 0.19a | 4.2a | 298a | 0.83b |
| Tristis | 3.7a | 0.17b | 3.8b | 290b | 0.98a |

[1]Means followed by different letters in each column indicate significant difference at $P \leq 0.05$. Each value represents mean of 87 to 90 observations.

Table 2.9. Gas exchange parameters calculated from the $CO_2$ response data shown in Figure 2.6

| | Water,-N | | Water,+N | | Drought,-N | | Drought,+N | |
|---|---|---|---|---|---|---|---|---|
| | Eugenei | Tristis | Eugenei | Tristis | Eugenei | Tristis | Eugenei | Tristis |
| i. Demand Functions | | | | | | | | |
| B(1)[1] | 8.58 | 8.56 | 12.04 | 15.68 | 5.64 | 7.88 | 5.22 | 1.80 |
| B(2) | 1.03 | 1.14 | 1.03 | 1.10 | 1.14 | 1.10 | 1.01 | 1.05 |
| B(3) | 0.0014 | 0.0045 | 0.0015 | 0.0035 | 0.0039 | 0.003 | 0.0003 | 0.0016 |
| ii. Supply Functions | | | | | | | | |
| g | 27.7 | 47.16 | 39.0 | 52.03 | 22.8 | 24.57 | 7.3 | 8.78 |
| $C_a$ | 353 | 353 | 354 | 353 | 351 | 351 | 351 | 351 |
| $C_i$ | 221 | 233 | 266 | 191 | 225 | 206 | 290 | 297 |
| A | 3.66 | 5.67 | 3.43 | 8.43 | 2.87 | 3.56 | 0.45 | 0.47 |
| iii. $CO_2$ Compensation Point | 21 | 29 | 20 | 27 | 34 | 32 | 4* | 30 |
| iv. Carboxylation Efficiency | | | | | | | | |
| k | 0.0124 | 0.0188 | 0.0260 | 0.0458 | 0.0160 | 0.0161 | 0.0017 | 0.003 |
| v. Stomatal Limitation | | | | | | | | |
| A | 3.66 | 5.67 | 3.43 | 8.43 | 2.87 | 3.56 | 0.45 | 0.47 |
| $A_o$ | 3.17 | 6.54 | 4.74 | 10.61 | 4.00 | 4.87 | 0.39 | 0.72 |
| $l_{g(i)}$ | 0.155 | 0.133 | 0.276 | 0.205 | 0.283 | 0.269 | 0.154 | 0.347 |
| $C_i$ | 418 | 270 | 243 | 248 | 216 | 232 | 400 | 219 |
| r* | 80.65 | 53.2 | 38.46 | 21.8 | 62.39 | 62.1 | 601.8 | 320.82 |
| $r_g$ | 17.76 | 14.6 | 32.36 | 12.5 | 47.04 | 33.4 | 108.9 | 280.9 |
| $l_{g(ii)}$ | 0.180 | 0.216 | 0.457 | 0.363 | 0.430 | 0.350 | 0.153 | 0.467 |

[1]Symbols: B(1),B(2) and B(3): coefficients of the model $Pn=B(1) \times (1.0 - B(2) \times e^{(-B(3) \times c_i)})$; Ca:ambient $CO_2$ concentration; A: calculated net $CO_2$ assimilation at the operating point; $A_0$: $CO_2$ assimilation at $c_i=Ca$; $l_g$:stomatal limitation; $r_g$: gas phase resistance to $CO_2$ fixation; r*:residual resistance to $CO_2$ diffusion.

*Due to the non-significant slope of this curve, it is difficult to accurately estimate this compensation point.

Table 2.10 Midday leaf water potential (-MPa) under different water and N regimes after three drought cycles.

| Water status | N availability | | |
|---|---|---|---|
| | -N | Low N | High N |
| Flooded | 0.88 ab[1] | 0.90 ab | 0.88 ab |
| Well-watered | 0.75 a | 0.85 d | 0.95 d |
| Mild drought | 0.80 abc | 0.93 def | 1.00 def |
| Moderate drought | 1.03 bc | 1.03 ef | 1.08 ef |
| Severe drought | 0.98 c | 1.20 f | 1.10 f |

[1]Means in a column or a row followed by different letters are significantly different at $P \leq 0.05$.

Table 2.11 Probability of significance levels of analysis of covariance[1] on growth affected by water and N regimes

| Variables | Clone(C) | Water(W) | N | C*W | C*N | W*N | C*W*N | Covariance | MSE(df) |
|---|---|---|---|---|---|---|---|---|---|
| Height(cm) | 0.0019* | 0.0001* | 0.0001* | 0.0011* | 0.2717 | 0.0249* | 0.4607 | Height | 0.0764 | 145.45(57) |
| GLD(mm) | 0.2735 | 0.0001* | 0.0001* | 0.1383 | 0.6304 | 0.1037 | 0.7188 | GLD | 0.7568 | 0.6787(57) |
| Leaf wt(g) | 0.0051* | 0.0001* | 0.0001* | 0.2510 | 0.1382 | 0.5759 | 0.9963 | No.Leaf | 0.7932 | 12.048(51) |
| Stem wt(g) | 0.3012 | 0.0001* | 0.0001* | 0.0268* | 0.2192 | 0.0065* | 0.8626 | Height | 0.0963 | 11.897(51) |

[1]Covariates are the initial growth immediately preceding the application of water and N regimes.

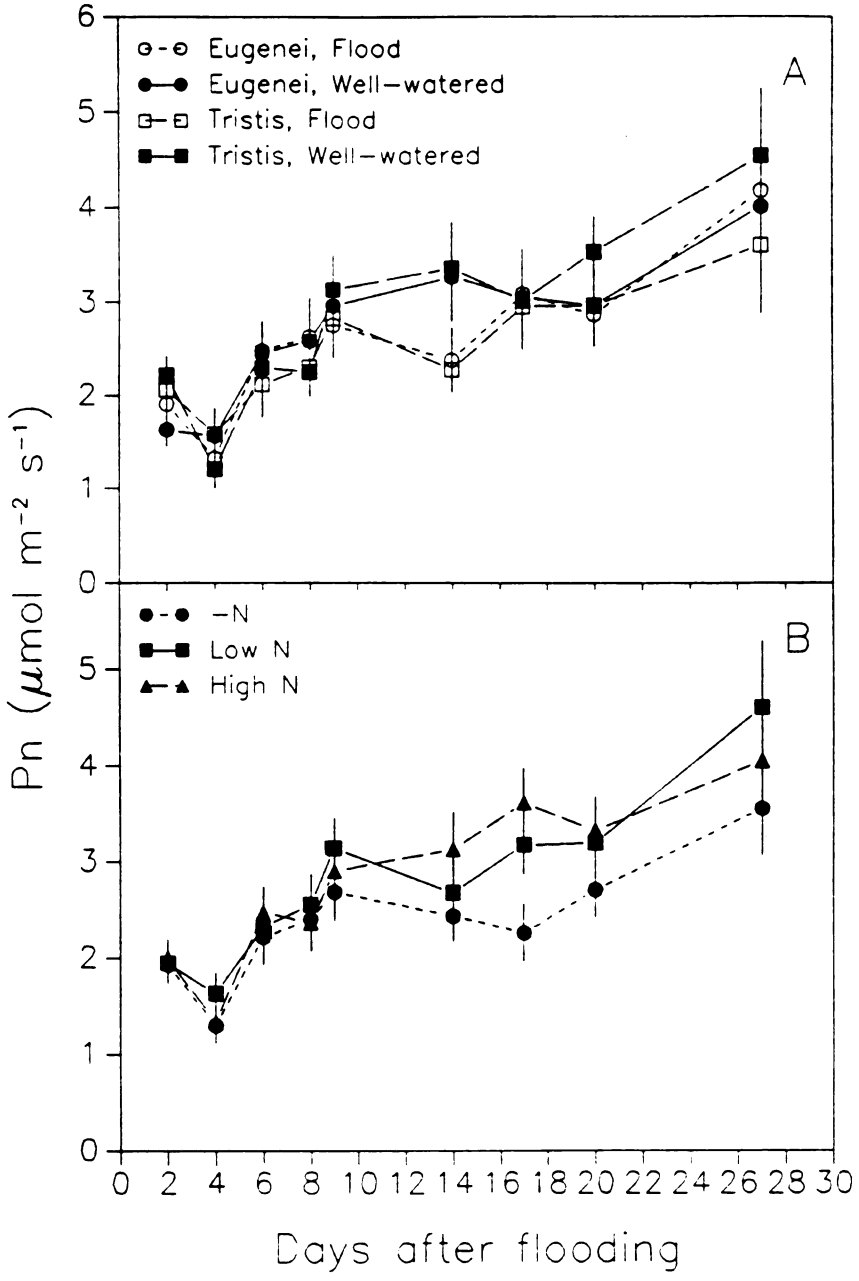



**Figure 2.1** **Effects of flooding on photosynthesis. (A) Clonal differences; and (B) Nitrogen effects during the course of flooding. Vertical lines represent the standard error of means.**

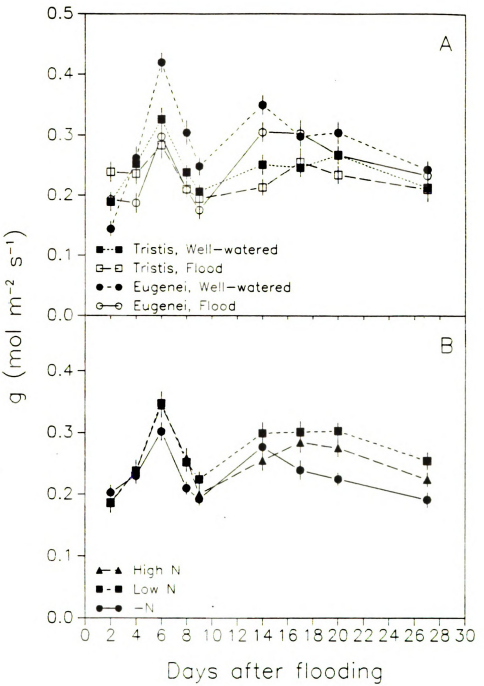



**Figure 2.2 Effects of flooding on stomatal conductance. (A) Clonal differences; and (B) Nitrogen effects during the course of flooding. Vertical lines represent the standard error of means.**

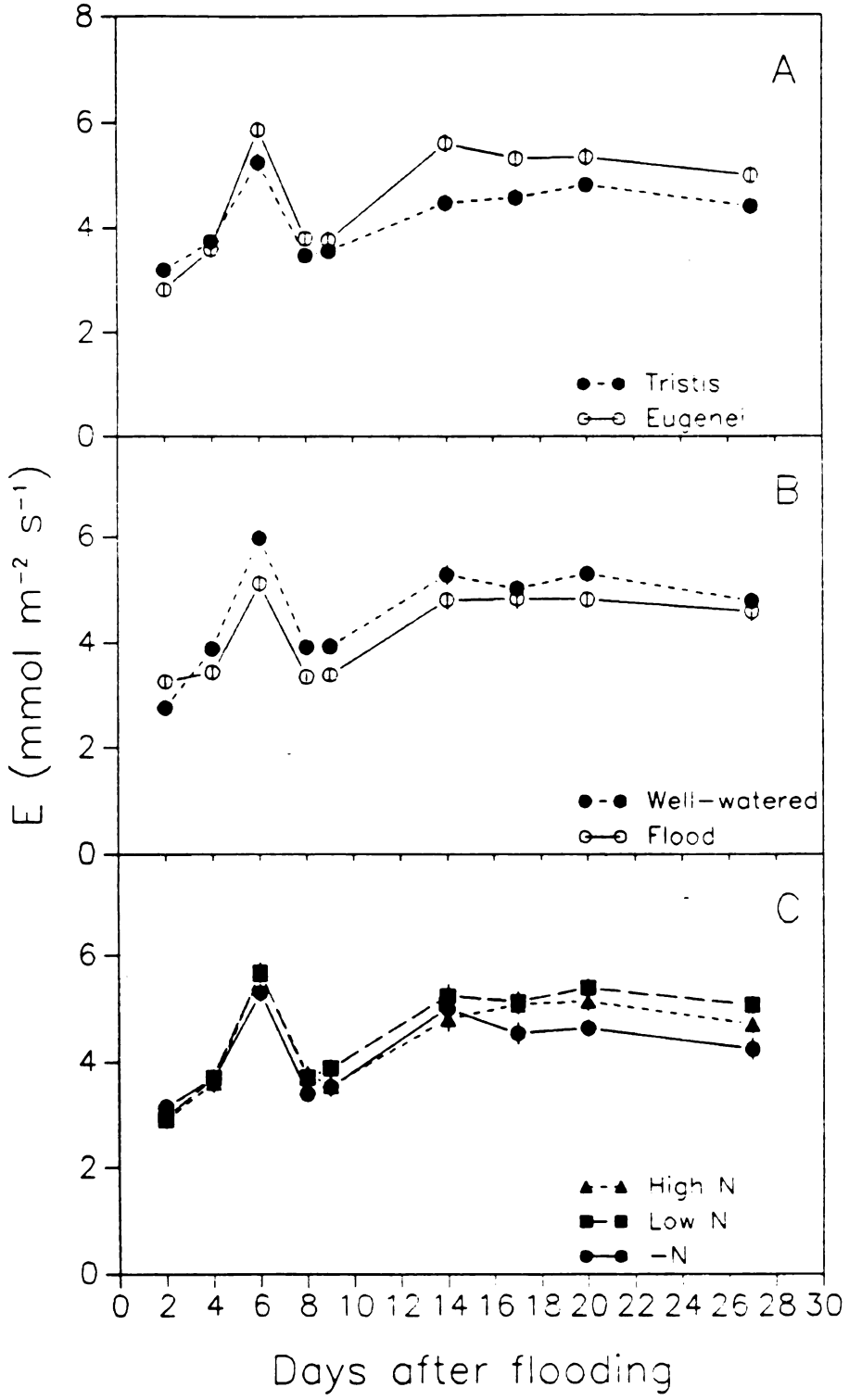



**Figure 2.3** **Effects of flooding on transpiration. (A) Clonal differences averaged over water and N treatments; (B) Flooding effects averaged over clones and N; and (C) Nitrogen effects averaged over clones and water along the course of flooding. Vertical lines represent the standard error of means.**

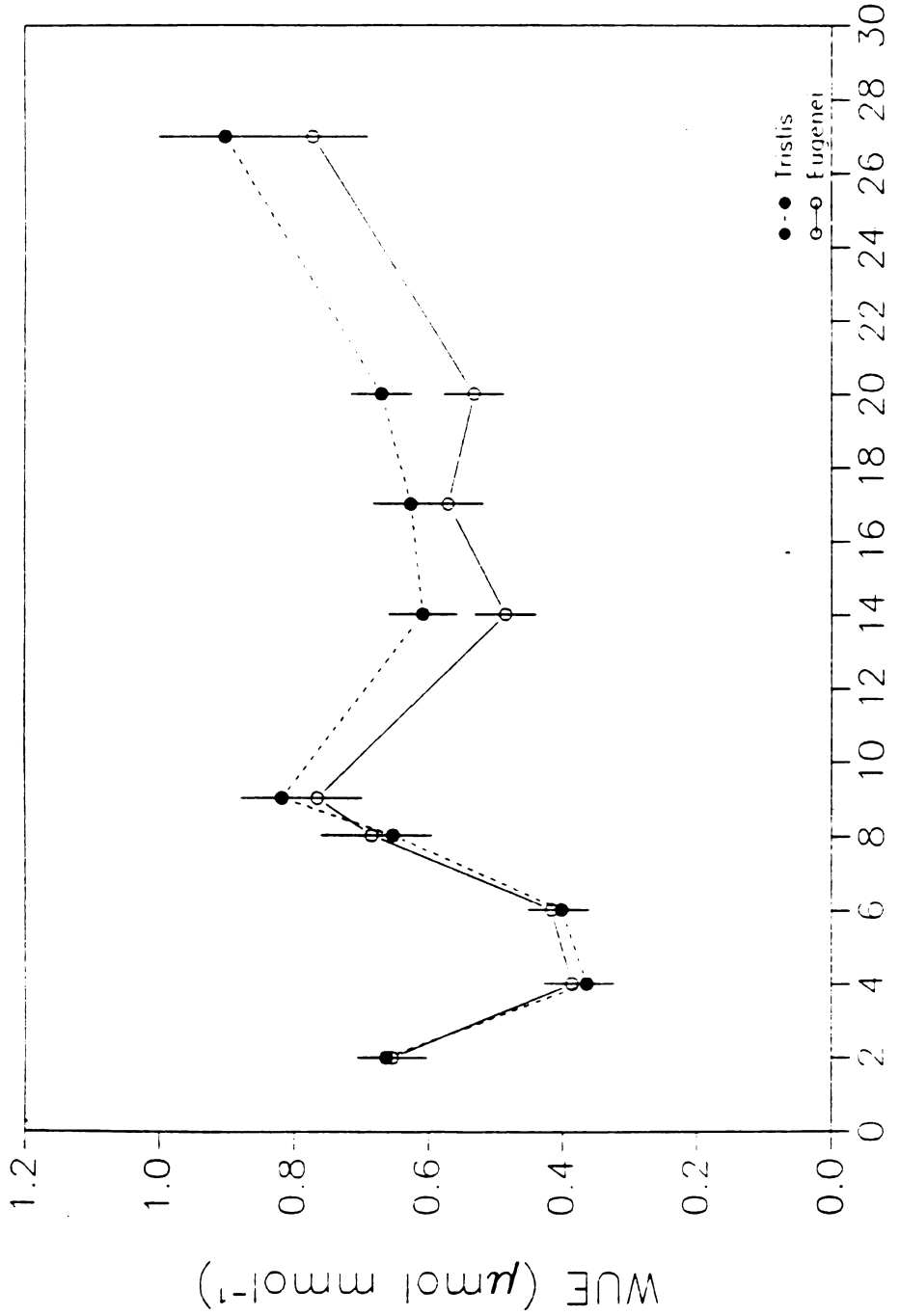
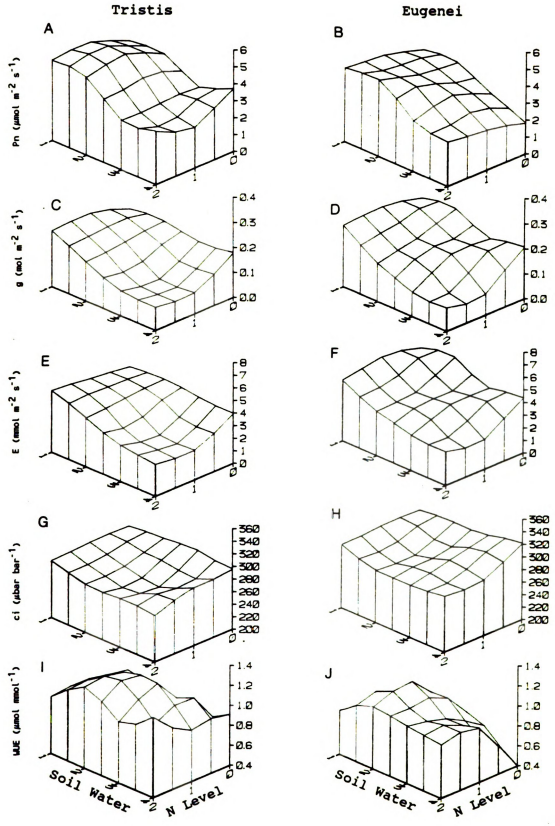



**Figure 2.4 Water-use efficiency of Tristis and Eugenei under flooded conditions. Vertical lines represent the standard error of means.**

Figure 2.5 Water and N interactions affecting gas exchange following three drought cycles. Numerical labels of soil water status, 1, 2, 3, and 4, stand for well-watered, mild drought, moderate drought, and severe drought, respectively, which represent soil matric potentials at -0.02, -0.05, -0.1, -0.5 MPa, respectively; 0, 1, and 2 along the N level represent -N, low N (+1.5 g N), and high N (+3.08 g N).


Tristis
Eugenei
A
B
C
D
E
F
G
H
I
J
Pn (μmol m-2 s-1)
g (mol m-2 s-1)
E (mmol m-2 s-1)
ci (μbar bar-1)
WUE (μmol mmol-1)
Soil Water
N Level

Figu

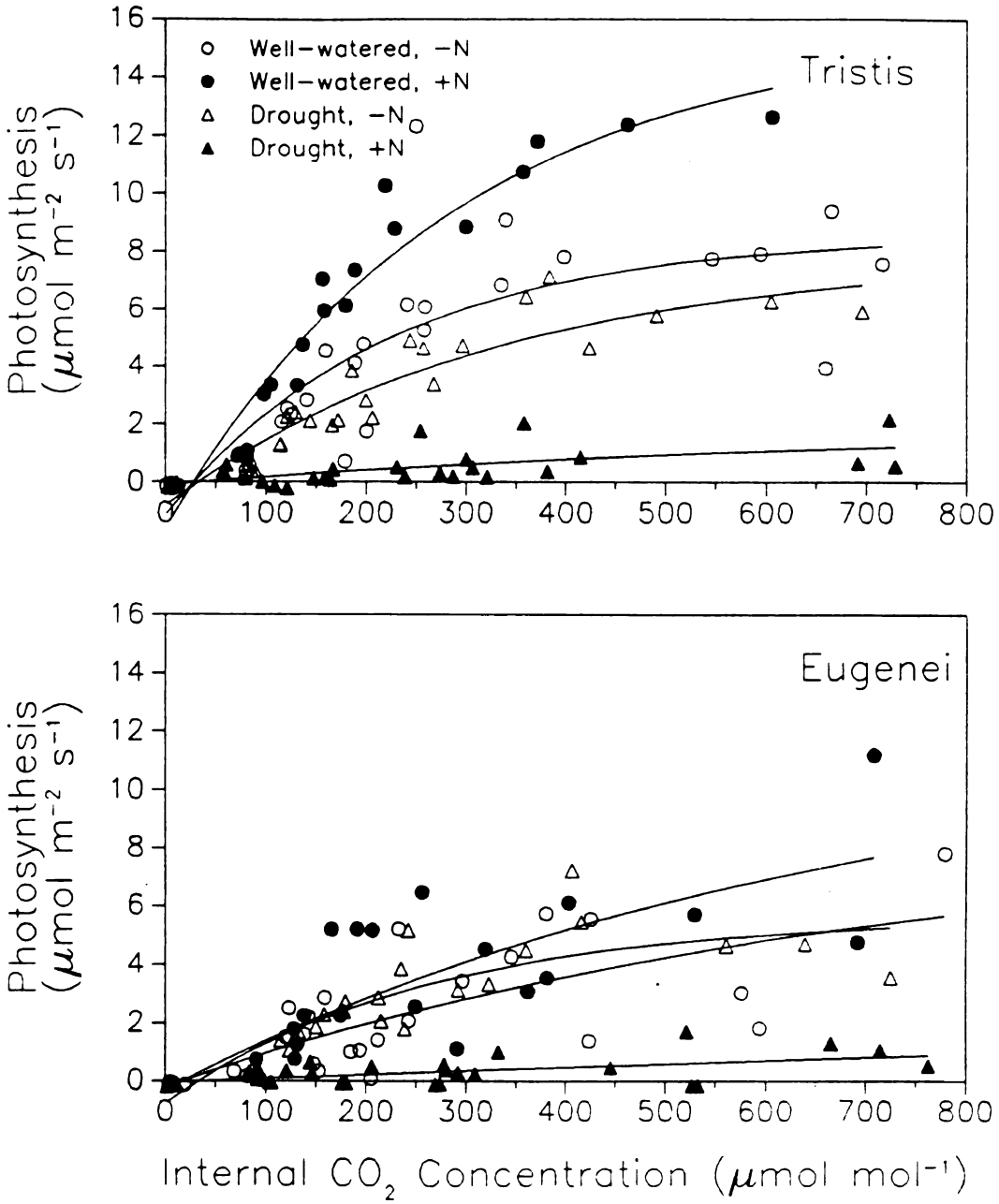
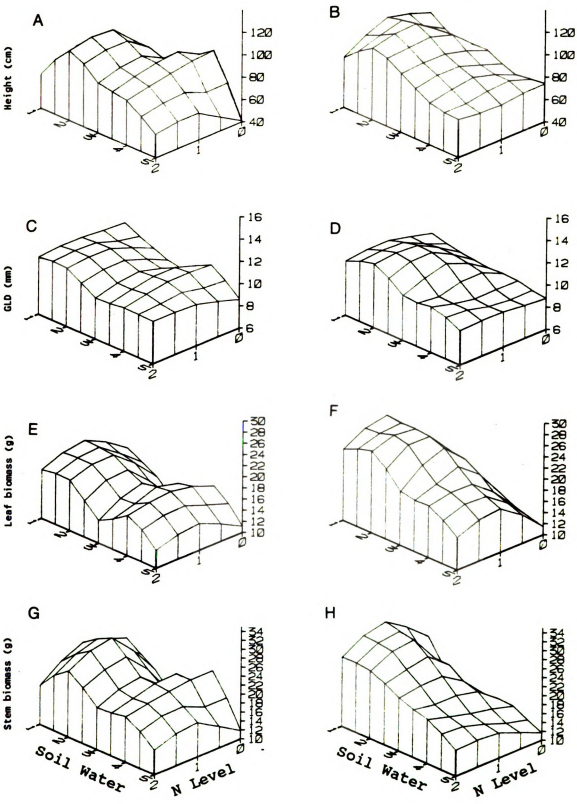



**Figure 2.6 A/ci relationships in Tristis (above) and Eugenei (below).**

Figure 2.7 Water and N interactions affecting height, GLD, leaf biomass, and stem biomass following three drought cycles. Numerical labels of soil water status, 1, 2, 3, and 4, stand for well-watered, mild drought, moderate drought, and severe drought, respectively, which represent soil matric potentials at -0.02, -0.05, -0.1, -0.5 MPa, respectively; 0, 1, and 2 along the N level represent -N, low N (+1.5 g N), and high N (+3.08 g N).


Tristis
Eugenei
A
B
C
D
E
F
G
H
Height (cm)
GLD (mm)
Leaf biomass (g)
Stem biomass (g)
Soil Water
N Level

# CHAPTER III

# PHYSIOLOGICAL AND MORPHOLOGICAL MODIFICATIONS OF TWO HYBRID POPLAR CLONES INDUCED BY NITROGEN AVAILABILITY UNDER FLOODING AND SOIL WATER DEFICITS

## ABSTRACT

Repeated progressive drought and flooding stress were imposed on two hybrid poplar clones grown under two nitrogen levels. Diurnal responses of Pn, g, E, and $c_i$ were measured periodically during the experiment. Over a period of 18 days of flooding, plants displayed no reduction in photosynthesis during the initial days, followed by a midday depression, and finally whole-day declines as flooding was prolonged. Supplemental N dramatically changed this response by reversing the initial declines in photosynthesis and maintaining matching rates in Eugenei and significantly higher rates in Tristis, compared to low-N counterparts. Internal $CO_2$ levels ($c_i$) mirrored diurnal photosynthesis. Low $c_i$ was associated with adequate water and N resources, especially during midday hours when light intensity became high.

Additions of N enhanced photosynthesis, and increased leaf N and chlorophyll contents, given minimum drought;

When soil water was restricted, however, high-N plants showed a drastic decrease in photosynthesis. Experience of one cycle of progressive drought substantially improved the capacity of plants to maintain a stable rate during another drought cycle, whereas a period of stress interruption appeared to increase the physiological vulnerability of high-N plants during another drought cycle.

Drought-stressed plants gained full and quick recovery of Pn upon relief from stress, suggesting stomatal restriction of $CO_2$ as a major determinant on reduced photosynthesis. In contrast, flooded plants did not recover until 9 days after the removal of stress, indicating strong residual effects, possibly involvement of growth regulators.

Stomatal conductance showed a similar response to photosynthesis. However, it appeared that N played a dominant role in determining photosynthetic capacity, whereas water seemed a major determinant of stomatal conductance.

Flooding did not invoke leaf water deficits. Rather, leaf water potential was increased. Progressive soil drying up to 10 days did not induce leaf water deficits. However, after a period of water stress interruption, stomata of Eugenei tended to lose their sensitivity to declining soil moisture, resulting in substantial leaf water deficits. Tristis, in contrast, appeared able to maintain the adaptation produced by the previous drought, showing leaf

water potentials comparable to well-watered plants during another drought cycle.

Sufficient N significantly changed leaf morphology and carbon allocation patterns, leading to a thinner leaf, and a lower root/shoot ratio.

# INTRODUCTION

To achieve maximum biomass production, plants must not only be able to access sufficient resources, but also utilize them efficiently. Among the resources required for plant growth, water and nitrogen are most critical (Pregitzer et al. 1990, Chapin et al. 1987). In particular, the irregular intervals of rainfall in the natural environment often impose severe water stress (Chapin 1991) and N is deficient in many soils. The capacity for utilizing available resources differs among species and with physiological status within a species. For example, the poplar clone Eugenei showed a high intrinsic capacity to sequester nitrogen when it was ample in the soil, whereas poplar clone Tristis was less responsive to high nitrogen availability. Under prolonged drought, the reduction of photosynthesis and growth led to pronounced reductions of nitrogen utilization, even if high amounts of nitrogen were present in the root zone (Chapter 2).

Effects of water stress and nitrogen as single factors have been extensively studied in various species. It has been shown that water stress alone, either flooding or drought, modifies plant physiology and morphology. The common symptoms of water stress are retarded leaf growth

(Saab and Sharp 1989, Seiler 1985, Seiler and Johnson 1984) and reduced stomatal opening (Harrison et al. 1989, Frederick et al. 1989, Pezeshki and Chambers 1986, Ni and Pallardy 1991). These observable changes probably indicate that leaf growth and stomatal behavior are the most sensitive indicators of stress.

Nitrogen is a primary determinant of plant productivity, as the amount of available N during leaf development determines the size of the photosynthetic machinery and the capacity or efficiency of this apparatus (Evans 1989). Therefore, if a plant possesses an intrinsic potential for growth, ample N would raise the actual growth closer to this potential (Sinclair and Horie 1989).

Plants in nature rarely experience optimal environmental conditions (Chapin 1991, Chapin 1987). In fact, during a growing season, fluctuations or deficiencies of any of the environmental factors, including light, temperature, water, and nutrients, can lead to stress. These factors also interact. The previous study (Chapter 2) showed that stomatal conductance (g) of high-N plants was significantly reduced as soil water potential became more negative, whereas low-N plants were slightly affected.

Experimental evidence indicates that plants demonstrate a similar physiological response to various sources of stress. For example, stomatal closure was observed under drought (Harrison et al. 1989, Frederick et al. 1989), flooding (Van Der Moezel et al. 1989, Jackson and Hall 1987,

Davies and Flore 1986c, Neuman et al. 1990), salinity (Klein and Itai 1989, Flanagan and Jefferies 1989), sudden darkness (Ceulemans et al. 1989), high vapor pressure deficits (Guehl and Aussenac 1987), and elevated $CO_2$ concentration (Hollinger 1987). This led to the proposal that plants may have a centralized system that responds to stresses in basically a same way, i.e. hormonal balance is upset (Chapin 1991, Radin 1984, Wadman-van Schravendijk and van Andel 1986, Neuman et al. 1990, Blackman and Davies 1985). It is this imbalance of hormonal levels that causes a series of physiological and morphological modifications. Despite the proposed possession of a centralized system, plants appear to adjust or resist stresses in different ways so that the nature of the stress can be realized according to the adjustments made. Some of the adjustments involve morphological changes, e.g. emergence of abundant lenticles and adventitious roots under waterlogging; leaf shedding or reductions of leaf size under drought to avoid further desiccation; root morphogenesis under N deficiency; and physiological changes such as osmotic adjustment, reduced g, and lower transpiration (E), thus increasing water-use efficiency (WUE). Such adjustments could enable plants to maximize their survival and maintain some growth under unfavorable environments.

The ability to maintain the integrity of the photosynthetic apparatus (Seiler and Cazell 1990) during stress is of significance and is a characteristic of stress-

resistance. This ability would allow plants to recover and fully utilize available resources upon the relief of the stress. Such a strategy would be particularly beneficial to those plants growing where resource fluctuations occur frequently. High-N wheat plants showed a greater recovery of g after relief of drought (Morgan 1984). Low recovery from salinity stress was found in Commelina communis plants associated with high level of proline (Klein and Itai 1989).

Repeated progressive droughts could lead to osmotic adjustment which allows the plant to function photosynthetically under water deficiency (Morgan 1984, Seiler 1985, Seiler and Johnson 1985). Photosynthetic capacity is initially reduced since roots transport abscisic acid to the leaves where it accumulates, independent of leaf water status (Davies et al. 1990). Subsequently hydraulic signals could develop (Abrams et al. 1990), adding to the negative effects on the photosynthetic process. It is still controversial whether there exists a functional linkage between stomatal conductance and photosynthesis (Wong et al. 1985, Grieu et al. 1988), or whether the photosynthetic process drives stomatal behavior. Much evidence has showed that photosynthesis and conductance were indeed tightly coupled during diurnal changes (Seiler and Cazell 1990, Michael et al. 1990, Mazzoleni and Dickmann 1988).

Diurnal performance of plants has significant implications to total photosynthate production, and thus final yield. Common observations of diurnal changes involve

the midday depression of photosynthesis under minimum soil water stress. This midday depression has been explained by the drop of leaf water potential, increased vapor pressure deficits sensed directly by stomata (Grantz 1990), or more recently feedback inhibition from the accumulation of photosynthates (Flore, personal communication). However, the absence of midday depression of photosynthesis has been observed, with photosynthesis closely following sunlight changes (Dickmann et al. unpublished manuscript). This result might indicate that plants were under low-stress environmental conditions. Under drought stress, depending on its severity, the depression might occur earlier, or remain for a long period diurnally. As a consequence, the effective hours for the photosynthetic process to function could be greatly shortened. Therefore, diurnal trends can provide sensitive indications of the physiological status of plants. Progressive drought causes gradual reduction of photosynthetic capacity, whereas effective hours determine how efficiently plants can function at this capacity.

The study reported in this chapter closely examined the physiological and growth responses to combinations of water and N resource levels, tracing the diurnal trends as water-stress intensified. The two hybrid poplar clones used in this study, Tristis and Eugenei, exhibit different physiological, phenological and morphological features (Michael et al. 1990, Mazzoleni and Dickmann 1988, Dickmann et al. 1990, Nguyen et al. 1990, Pregitzer et al. 1990, and

Chapter 2). Here I sought to further contrast their ability to recover from stress following repeated drought cycles and flooding.

## MATERIALS AND METHODS

Two clones representing two sections of the genus Populus were used: Tristis (Populus tristis x P. balsamifera cv. Tristis No. 1) from section Tachamahaca and Eugenei (P. x euramericana cv. Eugenei) from section Aigeiros (Dickmann and Stuart 1983). Cuttings from both clones were planted in 22.7 l plastic pots (one cutting per pot) filled with a natural sandy-loam soil containing 0.058 mol nitrogen per kilogram dry soil, and placed in a greenhouse in May, 1990. The temperature and relative humidity in the greenhouse were not regulated and they closely followed the changes of the surrounding natural environment. Frequent watering was provided to cuttings to assure vigorous sprouting and growth. Before they were used as experimental materials, all cuttings were allowed to grow until they possessed 30 leaves greater than 30 mm in length. Water and N treatments were initiated on June 24, 1990 and continued through August, 1990.

The whole experiment included three phases. The first phase consisted of two drought cycles. The second phase

comprised no water stress, followed by the third phase in which water stress was resumed. During the first water stress period, cuttings were subjected to water withholding for eight to ten days so that soil matric potential reached -0.5 MPa, based on a previously determined soil retention curve developed with a pressure plate technique (APPENDIX A). Once the soil matric potential reached the target value, watering brought the soil to field capacity and the second dry-down cycle began. Waterlogging lasted for the duration of the two drought cycles. Water stress was interrupted after two repeated drought cycles; all the plants received water to field capacity every 2 to 3 days for 10 days. Finally, water stress (drought and flooding) was resumed for another dry-down cycle before plants were harvested.

There were two controlled factors in this study, soil water status and N availability. The three soil water levels included flooding, abundant water, and drought. Nitrogen treatment consisted of low N (no supplemental N fertilizer) and high N (supplemental N in ammonium-nitrate form equivalent to 200 kg $ha^{-1}$). The amount of N fertilizer for the high-N treatments was divided into three equal parts and applied with water at the beginning of each drought cycle. Thus, this experiment was composed of six combinations of water and N levels for each of two clones and was designed as a randomized complete block with a three

factor (clone, water and N) factorial arrangement in three blocks.

On day 1, 3, 5, and 9 after each dry-down cycle began, net photosynthesis (Pn), stomatal conductance (g), transpiration rate (E), and substomatal cavity $CO_2$ concentration ($c_i$) were measured on two new fully expanded leaves for each plant with a portable leaf chamber and infrared gas analyzer (Analytical Development Co., Herts, England). Measurements were taken from 800 through 1600 hour (solar hours) at 2-hour intervals. Water-use efficiency (WUE) was calculated as the ratio of Pn to E. Leaf water potential of two fully mature leaves for each plant was measured with a pressure chamber (PMS Instruments) at the end of two water stress phases during 1100 to 1300 hours.

Growth and final yield were measured at the end of the experiment lasting 37 days. Height growth, number of leaves, and average leaf size were recorded. Biomass, partitioned into leaves, stem, and roots, was obtained after 48 hours in an oven at 70°C. Specific leaf weight (SLW), defined as leaf weight per unit of leaf area, and root/shoot ratio on a dry weight basis, were calculated from measurements.

Chlorophyll (Chl) contents of leaves were analyzed with the procedure of Hiscox and Israelstam (1979). Briefly, leaves were freeze-dried, digested with 80% dimethyl sulphoxide solution, and incubated for 24 hours at 65°C.

The supernatant was read on a spectrophotometer at wavelengths of 645 and 663 nm. The sum of the two readings were used to obtain the total chlorophyll content on a dry weight basis using the formula by Arnon (1949): Chl = (20.2 $D_{645}$ + 8.02 $D_{663}$) x a dilution factor.

Leaves were dried to their constant weights, ground, digested with concentrated $H_2SO_4$ and HgO as catalyst in a block digester, and analyzed for total N content with a Technicon Autoanalyzer (Technicon 1977).

Data were analyzed with analysis of variance (SAS), using days and hours as repeated measures for the gas exchange variables, to examine the response trends following water and N treatments. All treatment means were tested with least square difference (LSD) unless otherwise specified. Probability of difference between treatment means less than or equal to 0.05 was regarded significant.

## RESULTS

### Photosynthetic response

Water stress and N availability imposed strong effects individually and interacted together in influencing photosynthesis throughout the experiment (Table 3.1). The

two clones did not exhibit significant differences in response to the combined effects of water and N. However, photosynthesis varied substantially with days as soil progressively dried, and varied within diurnal patterns on a given day.

Photosynthesis was not affected at the initial day of waterlogging in cycle 1 (Figure 3.1A). Subsequently on the third day onwards, diurnal patterns deviated from the well-watered treatments. The deviations started at midday, with Pn in the flooded plants of both clones declining throughout the afternoon. Starting day 9, flooded plants with different N availability showed different diurnal trends in Pn. Flooded low-N plants showed significantly lower photosynthesis throughout the day, with an obvious plunge after midday. This trend extended through the first stress phase (Figure 3.1B). High-N Eugenei plants, on the other hand, seemed able to partially overcome the negative effects of flooding, demonstrating a similar diurnal pattern as non-flooded plants at the end of the first cycle. Tristis with high N, however, did not achieve full reversal of flooding effects until day 18 (cycle 2), although substantial increases of Pn were observed during both cycles.

Plants undergoing mild water deficiency showed similar diurnal patterns and comparable rates of photosynthesis in both clones during the first experience of drought (Figure 3.1A). High-N plants had obviously higher Pn under mild water stress. However, as drying progressed, high-N plants

showed dramatic decreases of Pn in the morning and negligible rates in the afternoon, whereas low-N plants displayed less reductions in Pn, although they were substantially lower than their well-watered counterparts.

Upon the relief of the first cycle of drought, Tristis was able to photosynthesize immediately at comparable rates to the well-watered plants in the morning hours, and was slightly higher than the well-watered, low-N plants (Figure 3.1B). Eugenei, on the other hand, did not gain full recovery in Pn until day 3 after re-watering.

At the onset of the second severe drought, both clones showed a certain degree of acclimation of Pn compared to the first drought (Figure 3.1B). Low-N plants of both clones manifested comparable rates of photosynthesis, whereas high-N plants of Eugenei seemed able to maintain a matching Pn in the morning but drastic declines were observed in the afternoon. Similar to the first drought cycle, high-N plants of Tristis displayed dramatic continuous decreases in Pn from early morning on at the end of the second cycle.

When droughted plants were re-watered fully and flooded plants were kept well-drained in the stress interruption phase, the effects of water and N from the two drought cycles appeared significant, and varied with days as well as diurnally (Table 3.1). The significant after-effects of prior treatments were most obvious after the low-N flooding treatment (Figure 3.1C). Both clones showed quick and full recovery of Pn one day following re-watering in the drought

treatments, and high-N plants appeared to recover more completely. The high-N plants attained high levels of Pn, particularly during the midday hours when light intensity was high. Experience of two repeated drought cycles in both clones seemed to additively increase Pn of high-N plants and eliminated midday depressions.

Flooded high-N plants exhibited a quick recovery of Pn upon the release of stress (Figure 3.1C). Low-N plants of both clones, on the contrary, continued to show the residual effects of flooding. Diurnal patterns of Pn were low throughout the day, with a midday depression in the first three days of the stress interruption, followed by a disappearance of midday depression, still with significantly low rates. On the ninth day of release from flooding, the residual effects of flooding were completely eliminated, with a complete recovery in both the diurnal trends and rates of Pn.

When water stress subsequently resumed, photosynthetic responses again were apparent (Table 3.1). Photosynthesis was significantly affected by water and N levels as water stress intensified. Diurnal changes were typical quadratic curves with diurnal trends different among the three water levels but not in N regimes. Similar to the previous flooding, high-N plants were able to partially overcome the negative effects of waterlogging, whereas low-N plants showed significant reductions of Pn (Figure 3.1D). In contrast to previous drought cycles, where high-N plants

showed significant reductions of Pn only as severe drought developed, high N plants in both clones, having experienced a period of ample water supply, exhibited dramatic decreases of Pn upon the development of a mild drought.

## Stomatal Conductance

Stomatal conductance was significantly affected by water levels and differed in Eugenei and Tristis upon the first onset of water stress (Table 3.2). Diurnal trends primarily followed a quadratic change pattern, and varied among the three watering regimes. Eugenei and Tristis displayed significant differences in g, but the degree of difference varied with days and hours. Nitrogen did not show prominent effects on g during the initial nine days of water stress, although N interacted with water levels and with clones at certain hours diurnally.

Flooding did not reduce g in the initial three days in both clones (Figure 3.2A). Five days of submersion in water, however, caused partial stomatal closure diurnally. On the ninth day, dramatic reductions of g occurred after mid-morning in flooded Tristis regardless of N levels. In Eugenei, N availability appeared to influence g, with high-N flooded plants maintaining comparable gs with well-watered counterparts, but low-N plants displaying strong midday depression of g.

Nitrogen availability significantly modified stomatal behavior in the flooded treatments. Ample N supply greatly improved stomatal opening in Eugenei (Figure 3.2B). Starting approximately two weeks after being waterlogged, g in high-N plants was able to completely recover. Low-N plants, however, had considerably lower g throughout the first 18 days of continuous flooding. Flooded Tristis plants, on the other hand, were less responsive to supplemental N. Although g in high-N plants was enhanced, it was never as high as in plants not subjected to water stress.

Mild drought imposed no effects on g and this pattern was the same in both clones (Figure 3.2A). However, as drying continued, stomata of both clones closed during the day, although in Eugenei stomatal opening of low-N plants was not as strongly affected by severe drought.

Unlike Pn, relief from the first drought failed to immediately bring g back to the control levels in both clones (Figure 3.2B); it took three days for normal stomatal function to resume following severe drought. High N did not significantly improve g at minimum water deficiency. However, like the responses to the first drought, the second drought caused high-N plants to display decreases of g throughout most of the day. However, experience of a previous drought significantly improved stomatal behavior, especially in low-N plants. In both

clones low-N plants were superior to their high-N counterparts in conducting water vapor.

Interruption of water stress led to complete recovery of g from drought in both clones, but flooded plants were still dominated by the residual effects of oxygen deficiency (Figure 3.2C). High-N plants recovered more quickly than low-N plants, particularly in the waterlogged treatments. Stomata of low-N plants did not fully recover from flooding effects until day 9.

Previous encounter with flooding seemed beneficial to plants when their roots were again submersed in water (Figure 3.2D). Flooded plants of both clones with supplemental N were capable of sustaining normal stomatal function throughout the duration of flooding. Low-N plants displayed slight stomatal closure when waterlogged.

When water was withheld during a third drought cycle, high-N plants of both clones again became sensitive to progressive drought, displaying significant reductions of g under mild drought (Figure 3.2D). Low-N plants showed a significantly lower g only as severe drought neared, but this response was not as pronounced as in high-N plants.

## Transpiration

Responses of transpiration to water and N regimes resembled those of g in the first two progressive droughts and 18 days of continuous flooding (Table 3.3). Both clones

showed capability to maintain similar rates of water loss when the root zone was initially submersed (Figure 3.3A). Waterlogging for five days only reduced E in the afternoon. Flooding significantly reduced E on day 9, when clonal responses deviated. Eugenei was capable of utilizing ample N to somewhat resist changes of E induced by flooding, whereas Tristis failed to maintain rates comparable to the control, even with supplemental N. As flooding continued, E of low-N plants was substantially decreased in both clones (Figure 3.3B). However, E was less reduced in Eugenei than in Tristis.

Mild drought did not reduce E in either clone (Figure 3.3A), but severe drought did. With the onset of a second drought, E showed similar responses to the previous drought, but low-N plants of both clones seemed much less susceptible to water stress (Figure 3.3B).

Revival from drought was quick and complete in both clones (Figure 3.3C). The role of supplemental N in promoting the recovery from flooding was evident, mimicking that of g. However, unlike g, E displayed a higher degree of recovery from flooding, especially in Eugenei.

When water stress resumed, E of flooded plants was not affected (Figure 3.3D), despite reduced g (Figure 3.2D). Return of another drought had no effect on low-N plants. However, similar to responses of g, high-N plants were susceptible to mild water deficiency.

## Internal $CO_2$ levels

Internal $CO_2$ concentrations were not altered by the inception of water stress or N levels (Table 3.4). However, significant differences existed between the two clones. Eugenei had overall lower $c_i$ levels than Tristis. Diurnal changes of $c_i$ were typical quadratic curves, starting at high concentrations, gradually declining to the lowest levels at midday, and rising towards late afternoon (Figure 3.4A). These diurnal trends were similar but in opposite directions with Pn. Internal $CO_2$ levels remained largely unchanged by either flooding or progressive drought. In the case of extreme drought, however, a significant rise of $c_i$ during midday hours occurred in both clones.

Upon re-watering, droughted plants returned to normal in both clones (Figure 3.4B). Continuous flooding, however, led to considerable elevation of $c_i$. Extreme drought, in contrast, resulted in a substantial plunge of $c_i$ in high-N plants.

When water stress was interrupted, the variations in $c_i$ were primarily caused by N (Table 3.4). The lowest $c_i$ was typically around noon (Figure 3.4C). However, effects of N regime were less obvious in the previously flooded plants. Unlike Pn, g, and E, $c_i$ of previously flooded plants was comparable to the controls.

Low $c_i$ was generally observed in plants grown with high N levels under adequate water supply. However, as water was

withheld for the third time, high-N plants showed an elevated $c_i$ (Figure 3.4D). Resumption of flooding also caused a slight rise of $c_i$.

## Water-use efficiency

Water-use efficiency remained relatively unaffected upon the commencement of water stress. Highest WUEs occurred at midday and lowest at either end of the day (Figure 3.5A). At the conclusion of the first drought cycle, where extreme stress was reached and flooding had lasted 9 days, apparent deviations emerged among the three water levels. Absence of water stress resulted in high WUEs, drought led to low WUEs and flooding was in between. Both clones showed similar response patterns, although Tristis had, on the average, higher WUEs than Eugenei (Table 3.5).

Prolonged flooding led to unceasing declines in WUE in both clones (Figure 3.5B). High N levels produced high WUEs, but this enhancement varied with clones. Eugenei was highly responsive to supplemental N, whereas Tristis showed less separation between the two N levels. Experience of one progressive drought enabled plants of both clones to maintain comparable WUEs when another drought occurred.

Nitrogen level dominated WUEs when water stress was interrupted, but its effects depended on the water levels previously experienced (Table 3.5). Plants maintained

without water stress showed greatest effects of N, whereas those water stressed were not strongly affected (Figure 3.5C).

As water stress resumed, water and N regimes again became major determinants of WUEs, with interactions between the two occurring (Table 3.5). Resumption of flooding did not cause significant decreases in WUEs (Figure 3.5D). Under minimum water stress, high-N plants showed WUEs superior to low-N plants in both clones. However, as mild drought developed, WUEs of high-N plants dramatically dropped. With further drying of the soil, clear separation among water levels was readily seen in Tristis, with WUEs highest in non-stressed plants, lower in flooded ones, and lowest in droughted ones. Eugenei, on the other hand, showed less variation in WUEs.

## Water relations

At the end of first stress phase, in which two repeated drought cycles and continuous flooding were imposed, midday leaf water potential was significantly affected by water and N regimes and their interactions (Table 3.6). There were no clonal differences. Flooding did not produce leaf water deficits (Table 3.7). Instead, flooded plants displayed significantly higher leaf water potential. In comparison to their well-watered counterparts, restriction of water supply caused no disturbance of leaf water potential if no

supplemental N was added, whereas addition of N led to higher leaf water potential.

Interruption of water stress seemed to have altered this water potential pattern upon the introduction of another cycle of water stress. Clonal deviations emerged, and effects of water levels differed in the two clones (Table 3.6). In Tristis, both flooding and progressive drought created similar leaf water deficits (Table 3.8). In Eugenei, only restricted water supply caused leaf water deficits, whereas flooded plants maintained a comparable leaf water potential to non-stressed plants.

## Morphological responses

Number of leaves was significantly affected by water and N regimes and differed in the two clones (Table 3.9). However, the effects of water were modified by N status. Without supplemental N, water stress did not reduce the emergence of new leaves (Figure 3.6A). In contrast, high-N plants displayed increased leaf production when well-watered, whereas water stress, either deficiency or excess, significantly decreased the number of leaves. Tristis produced two more leaves, on the average, than Eugenei.

Average leaf area was significantly affected by water regimes and the effects differed in the clones (Table 3.9). In Tristis repeated droughts did not significantly reduce leaf growth, but flooding produced substantial reductions of

leaf expansion. In contrast, Eugenei showed an apparently smaller leaf due to both types of water stress. Water and N interactions took place with a similar way in the clones. High N significantly increased leaf area across the water levels (Figure 3.6B). However, the effects of N were overwhelmingly stronger in the non-stressed plants than in water-stressed counterparts, showing the significant interactions of water and N.

Varied availability of water and N during plant growth dramatically changed carbon allocation patterns, indicated by the modifications of root/shoot ratios (Table 3.9). Water, in particular, and N as individual factors imposed strong effects on root/shoot ratios. The intensity of N effects was dependant upon water status. Flooded plants displayed relatively low root/shoot ratios, but supplemental N had little effect on this pattern (Figure 3.6C). In well-watered and repeatedly droughted plants, addition of N substantially decreased root/shoot ratios, causing considerably more fixed carbon to be allocated into above-ground plant parts. However, the strength of ample N supply in switching carbon allocation towards the shoot was stronger when sufficient water was supplied than in water-restricted treatments.

Leaf specific weight was also affected by N regimes and water levels, which interacted with clones (Table 3.9). High-N leaves had a significantly lower SLW (Figure 3.6D). Tristis developed higher SLWs than Eugenei under flooding

and well-watered conditions, whereas there was no significant difference under drought. In Tristis, flooding led to a thicker leaf, but drought induced a thinner leaf. In contrast, waterlogged Eugenei plants produced a thicker leaf, but SLW was not modified by drought.

The leaves of fertilized plants were darker green than those on their non-fertilized counterparts. Leaf chlorophyll and N contents were significantly influenced by water and N regimes, independent of clones (Table 3.9). Water and N availabilities substantially changed leaf chlorophyll content independently, resulting in high chlorophyll content in high-N and droughted plants (Figure 3.6E). Effects of supplemental N on leaf N content, however, depended on soil water status. At any water level, addition of N significantly increased leaf N content (Figure 3.6F). However, even in the high-N treatments, flooding considerably reduced influx of N into leaves.

## Growth responses

Water and N were two major determinants of height growth, as well as leaf, stem, and total biomass (Table 3.10). In most cases, these two resources interacted. However, water appeared the sole factor affecting root growth. The clones differed in height growth, stem and total biomass productions, but not in leaf and root growth despite fluctuations of water and N resources.

Height growth was enhanced by addition of N (Figure 3.7A). However, the positive effect of ample N was greatly reduced under water stress, either flooding or drought. Tristis was taller than Eugenei across the water and N regimes.

Leaf development was retarded by water stress. In Tristis flooding caused considerably more reductions in leaf biomass than drought, whereas flooding and drought initiated equal influence in Eugenei (Figure 3.7B). Water stress had little effect on leaf biomass accumulation in N-deficient plants. However, in high-N plants leaves were greatly reduced by water stress; drought led to approximately a third reduction, whereas flooding caused a two-thirds reduction. Leaf growth of Eugenei appeared more resistant to flooding than Tristis given ample N, although this variation was not significant.

Stem biomass accumulation was prominently enhanced by supplemental N at any water level in Eugenei (Figure 3.7C). Stem growth showed little response to the addition of N in Tristis under submersion.

Flooding led to much smaller roots, ca 25% of the non-stressed counterparts, independent of N status (Figure 3.7D). Repeated drought also significantly reduced root growth, but to a lesser magnitude.

Exposure to water stress dramatically reduced biomass production in Tristis (Table 3.11). Repeated, progressive drought resulted in a 30% reduction, whereas flooding

decreased biomass production by 60%. In contrast, both types of water stress decreased biomass equally in Eugenei. Under flooding conditions, total biomass was not different in the two clones. However, in well-watered and droughty conditions, Tristis outgrew Eugenei. Effects of N on biomass also varied with water levels. Flooded plants showed slight increases with high N, whereas non-flooded plants displayed a significant biomass increment under ample N. However, well-watered plants had much higher biomass production than droughted ones, provided high amounts of N were available.

## DISCUSSION

Flooding failed to induce immediate changes in Pn, g and $c_i$. Subsequently, only a midday depression in these variables occurred, followed by diurnal reductions as flooding was prolonged. Unlike the previous report (Chapter 2), adventitious roots did not emerge, and full reopening of stomata did not occur during the 18 days of flooding. This discrepancy was probably due to the length of flooding stress, and the physiological status of the cuttings. The delayed reductions of Pn indicated that the two clones under investigation were somewhat flood-tolerant, whereas immediate declines of Pn upon waterlogging were apparent in

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flood-sensitive species such as rabbiteye blueberry (Davies and Flore 1986c), Douglas-fir and Norway spruce (Zaerr 1983), pea (Jackson and Hall 1987), and sweet gum (Pezeshki and Chambers 1985). The occurrence of comparable Pn in the morning was probably a sign that photosynthetic capacity of flooded plants was not decreased. Midday depression of Pn is a typical phenomenon in drought-stressed plants, but flooding stress apparently produces the same response.

The major effect of drought is the restriction of $CO_2$ diffusion as a result of reduced stomatal opening, either caused by leaf water deficits (Loveys 1984, Hinkley et al. 1978, Abrams et al. 1990), or the upset of the balance of growth regulators leading to more abscisic acid accumulation in the leaves (Zhang and Davies 1987). In flooded plants, however, leaf water deficits did not develop, and this response has been reported in other studies (Jackson and Hall 1987, Bradford 1983a, Zhang and Davies 1986, Bradford and Hsiao 1982). Although flooding induced changes in Pn and g, leaf abscisic acid concentration was not substantially increased (Chapter 4), contradicting the findings of Jackson and Hall (1987). This observation may indicate that other inhibitors other than ABA might have been involved or that an imbalance of growth regulators, particularly a reduction of cytokinin/ABA ratio, might have been primarily responsible for the physiological changes in flooded plants.

However, it is not clear whether the reduced Pn in the present study was caused solely by decreased g or whether lower Pn regulated stomatal aperture; in other words, whether a functional linkage existed between Pn and g. Although it has been suggested that the reduction of pH in the stroma caused by water stress is responsible for the release of ABA from mesophyll cells to the apoplastic regions in the guard cells to promote stomatal closure (Cowan et al. 1982), which process was affected earlier still is a question. Midday depression of Pn may also be attributed to the obstruction of translocation of photosynthates because of reduced sink strength (Bradford and Hsiao 1982, Schumacher and Smucker 1985).

Unlike the drought stress, root growth was almost stopped by flooding as soon as the dissolved oxygen was consumed. Oxygen deficiency is probably the universal sign of flooding (Drew 1990) and this deficiency had tremendous effects on root development. The weakened sink strength of roots could produce additional limitations on Pn. The present study did not find reduced growth of above-ground plant parts. Leaves and stems still served as active sinks for photosynthetic products, possibly suggesting the small magnitude of feedback inhibition that occurred in flooded plants. It is conceivable that the balance of growth regulators of flooded roots must have been disturbed. Because flooding did not induce a significant accumulation of ABA in the leaves, the imbalance of plant growth

regulators may actually come from the reduced generation of growth-promoting regulators, such as cytokinin, which could reverse the negative effects of ABA on stomatal behavior (Bradford and Hsiao 1982).

As flooding was prolonged, the whole diurnal course of Pn was suppressed. This response could indicate that photosynthetic capacity was decreased at the chloroplast level. The diurnal depression of Pn and accompanying reductions of g in this study indicated that $CO_2$ supply may have been restricted. However, stomatal closure did not lower $c_i$ levels. Rather $c_i$ levels increased, probably indicating that non-stomatal inhibition of Pn was taking place. It is not clear, though, whether the imbalance of growth regulators, particularly the rise of ABA levels, imposed a direct interference with the photosynthetic process in addition to the indirect inhibition through reduced stomatal opening. Both carboxylation efficiency and quantum yield can be reduced as a result of increased ABA levels (Ward and Bunce 1987).

Flooded plants with supplemental N were capable of maintaining high Pn, emphasizing the role of N in reversing the detrimental effects of flooding. Although flooding decreased the ability of roots to actively uptake N, reflected by the low leaf-N content relative to non-flooded plants, N-enriched plants still had significantly higher total N concentration in the leaves, possibly because of passive uptake through the transpirational stream. High

total leaf-N led to the buildup of more photosynthetic machinery, such as chlorophyll and presumably soluble proteins, including photosynthetic enzymes (Evans 1989). It is also possible that high N supply can reduce ABA production and transfer it to the shoot (Drew 1990), therefore alleviating or avoiding the negative effects of flooding.

Flooded roots were sensitive to stress under limited N supply (Cornish and Radin 1990). This increased sensitivity may be the combination effects of reduced cytokinin production, which activates stomatal sensitivity to ABA, and an elevated effective concentration of ABA. Internal $CO_2$ levels of high-N flooded plants remained comparably low, reflecting the high photosynthetic activity. High N also led to more leaf initiation and elongation, more leaf biomass per plant in both clones, and more stem biomass in Eugenei, thus above-ground proponents continued as major active sinks. But even ample N failed to diminish the negative effects of flooding on root growth, reflecting the overwhelming effect of flooding: oxygen deficiency.

During progressive drought, the interdependence of water and N resources was clearly demonstrated. Under adequate water conditions, Pn was substantially raised when high amounts of N were available to the roots, as found in other studies (DeJone 1982, Evans and Terashima 1988, Mulligan 1989). High-N plants in this study displayed much higher leaf-N and chlorophyll contents, paralleling previous

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findings (Evans and Terashima 1988, Evans 1989, Sage and Pearcy 1987). High leaf-N content led to more incorporation of N into soluble proteins such as RuBPCase (Evans 1989, Terashima and Evans 1988) and high Rubisco activity (Sage et al. 1987, von Caemmerer and Farquhar 1981). This incorporation of N enables plants to utilize light energy much more efficiently, while ample water supply enables plants to fix $CO_2$ (Sharkey 1985).

Photosynthesis was not affected by mild drought in this experiment. Mild drought favored stomatal opening, supporting the findings with sweet cherry and plum where optimal g was obtained at moderate leaf water deficits (Yoon and Richter 1990). As soil severely dried, however, the positive effects of supplemental N turned into negative effects, resulting in dramatic reductions of Pn. Since N uptake did not appear to be affected by the limited water supply, indicated by high leaf-N and chlorophyll contents, the development of photosynthetic apparatus probably was not affected by progressive soil drying. However, limited water supply imposed harsh restrictions on $CO_2$ diffusion through stomatal closure. The extremely low $c_i$ in this case did not represent high Pn activity, rather it was a consequence of reduced $CO_2$ diffusion, producing a $c_i$ level which seemed below the threshold requirement for active photosynthesis.

Low-N plants were able to photosynthesize and conduct $CO_2$ at significantly higher rates than their high-N counterparts, particularly after experiencing one drought

cycle. This result probably suggests that N status in the leaves modifies stomatal sensitivity to the decline of available water (Radin 1984). But contrary to Radin's findings and to the flooding response shown in the present study, high-N plants appeared more sensitive to soil drying than low-N plants. This discrepancy could be accounted for by the relatively bigger size of high-N plants. As a result, high-N plants depleted water more quickly (Morgan 1984, Walters and Reich 1989, Morgan 1986). Moreover, high-N plants developed a low root/shoot ratio, further deepening the gap between water absorption and transpiratory water loss.

Diurnal trends of $c_i$ closely mirrored the changes of Pn; $c_i$ was high in the early morning when Pn was low, and became lowest around noon hours when Pn was high. The lowest $c_i$ levels were associated with plants receiving adequate water and N resources, in agreement with the findings that high leaf-N content led to linear decrease in $c_i$ (DeJone 1982). The plunge in $c_i$ was especially apparent around noon when light intensity and g were high, probably indicating that $c_i$ is best correlated with photosynthetic activity (Mott 1990), rather than stomatal conductance to $CO_2$. In spite of the close coupling between Pn and g during the diurnal changes, $c_i$ did not remain constant diurnally or within the water treatments, in contrast to many other studies (Wong et al. 1979, Kuppers et al. 1986, Osmond 1983).

Recovery from water stress greatly varied with the types of water stress. Plants subject to progressive drought gained full recovery upon the relief, indicating that $CO_2$ restriction was a major constraint to Pn. The quick recovery also suggested that the integrity of the photosynthetic apparatus was maintained (Seiler and Cazell 1990), a characteristic of drought resistance. The quick recovery after rehydration was observed in other species, including Pn of red spruce after 10 to 11 days of drying (Seiler and Cazell 1990) and g of faba bean following 17 days of progressive drought (Sau and Minguez 1990). High-N plants in the present study recovered fully and quickly, indicating that high N concentrations in the leaves imposed no damage to the photosynthetic machinery.

The residual effects of flooding on Pn, in contrast, were apparent, especially in Tristis. As a consequence, the recovery after the removal of flooding was slow. The slow recovery from flooding was also found with other species. Highbush and rabbiteye blueberries (Davies and Flore 1986a, Davies and Flore 1986b) took at least 18 days to return to preflood Pn rates after 24 days of submergence, while sweet gum recovered from flooding at day 10 after drainage (Pezeshki and Chambers 1985). Flooded leaves were associated with high levels of ABA (Jackson and Hall 1987, Wadman-van Schravendijk and van Andel 1985, Wadman-van Schravendijk and van Andel 1986) and ethylene (Yamamoto et al. 1987, Seliskar 1988). The residual effects of these

substances and their variations in catabolism were probably responsible for the slow recovery. Proline accumulation was found responsible for the slow recovery from salinity stress (Klein and Itai 1989); it did not induce stomatal closure but only prevented them from opening. It is not clear that the slow recovery from flooding was also involved with high proline levels. The distinct patterns of recovery in the two stress treatments lead to the speculation that the ratio of cytokinin to ABA in the leaves may have been different, although under both types of stress ABA levels could be high. Droughted plants may be capable of producing a certain amount of cytokinin in the roots and translocating it to leaves, whereas flooded plants probably generate cytokinins in extremely small quantities.

The two clones showed distinct response patterns of leaf water potential when stress resumed after a period of water stress interruption. Tristis was able to maintain close control of water loss, displaying little reduction in leaf water potential. Eugenei, on the other hand, showed sensitivity to declining soil water potential, demonstrating dramatic midday leaf water deficits, which confirms the finding by Mazzoleni and Dickmann (1988).

Soil moisture level was the determinant of root biomass. Nitrogen availability, on the other hand, had little effect on root biomass. Regardless of this, apparent morphological changes in the root were observed in high-N

plants. Additions of N to the soil induced more fine roots, particularly in the well-watered plants.

Water stress and N availability dramatically altered carbon allocation patterns. Flooded plants allocated most of their fixed carbon to leaves and stems due to reduced root activity. This response presumably was caused by oxygen deficiency, as aerated water can reverse the negative effects of flooding (Zaerr 1983). In a split-root experiment where half of the root system of Phaseolus vulgaris was flooded, carbon allocated to this half was reduced (Schumacher and Smucker 1985), an indication of reduced sink strength caused by anoxia. The stagnant growth of roots in the present study could also be the result of reduced cytokinin production and obstructed auxin translocation from above-ground. High N resources led to a favorable carbon allocation towards the shoot, in agreement with other studies (Agren and Ingestad 1987, Rendig and Taylor 1989, Axelsson and Axelsson 1986, Morgan 1986, Walters and Reich 1989). The mechanism of high N promoting shoot growth relative to roots, though, remains unclear. It can be speculated that ample N could be the stimulus for more auxin production in the leaves and growing shoot tips, whereas water status within the plants could modify the distribution of this growth promoting regulator.

Although high-N plants displayed greater declines of Pn and stomatal closure under severe drought as compared to their low-N counterparts, they still gained higher total

tree biomass than low-N plants under sublethal drought stress. The higher biomass production in high-N plants than low-N plants was probably related to the higher water-use efficiency and higher photosynthate production when soil moisture was available.

The results from the current study lead to the conclusion that plants might indeed possess a centralized system for responses to any type of stress, as proposed by Chapin (1991). This centralized system could reside in the balance of growth regulators, as their levels interact to mediate physiological and morphological responses. The balance of growth regulators probably heavily relies on the so-called stress hormones, such as ABA and ethylene, since their levels dramatically increase in face of stress. Reportedly, drought, flooding and salinity all imposed stresses to plants because of elevated levels of ABA, which in turn mediates stomatal behavior. This common response to different types of stress further raises the interesting question about the mechanisms of plants which integrate their strategies in the face of stress. Further investigations of hormonal changes in response to water stress, temperature extremes, nutrient deficiency etc. could reveal the strategies of different species which enable them to survive and grow in unfavorable environments.

Eugenei and Tristis are indeed two distinct hybrid clones in many aspects. Eugenei was able to utilize N available in the flooded soil in an unknown mechanism to

continue a normal photosynthetic activity, whereas Pn in Tristis was less responsive to N availability. Leaf water deficits occurred readily in Eugenei in a prolonged droughty condition, due to its loose stomatal control of transpiratory water loss, whereas Tristis was less subject to leaf water deficits, by means of tight stomatal regulation of water use (Chapter 4). Similar findings have been reported in Chapter 2 of this dissertation that identify Tristis as a more drought-resistant clone than Eugenei. Tristis appeared to "memorize" its drought experience, maintaining comparable leaf water potentials when another drought developed after a period of drought interruption, while Eugenei developed apparent leaf water deficits (Mazzoleni and Dickmann 1988). Tristis developed thicker leaves than Eugenei (Michael et al. 1990), and more root biomass and fine roots (Pregitzer et al. 1990). Tristis had more sugar but less starch in structural roots than Eugenei (Nguyen et al. 1990). Tristis also recovered from drought more rapidly and completely than Eugenei in my studies. These integrated findings support the conclusion that Tristis is a more drought-resistant clone.

The results of this study have silvicultural implications in the establishment and management of short-rotation intensive culture (SRIC) plantations. Since N fertilization promoted physiological performance and biomass production only when soil moisture was adequate, fertilization should be avoided on sites where soil water

supply fluctuates frequently or irrigation is not feasible. On such sites, Tristis is a better hybrid clone for its drought resistance. In contrast, on sites where soil moisture was sufficient and flooding is a frequent threat, Eugenei would be a better choice. Since flooding greatly reduces root growth, and since coppice is an essential requirement in the SRIC system and its success relies on the root growth potential, coppicing should be delayed if a prolonged flooding occurred in a previous growing season.

Table 3.1 Probability of significance levels[1] of ANOVA with repeated measures for photosynthesis subject to various length of water stress and levels of N.

| Source | df | MS[2] | F | P > F | Adj. P > F[3] G-G | H-F | df | MS | F | P > F | Adj. P > F G-G | H-F |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | CYCLE 1 | | | | | | CYCLE 2 | | | | |
| Clone(C) | 1 | 7.29 | 2.89 | 0.1033 | | | 1 | 9.20 | 1.95 | 0.1761 | | |
| Water(W) | 2 | 101.81 | 40.32 | 0.0001 | | | 2 | 305.70 | 64.93 | 0.0001 | | |
| N | 1 | 13.11 | 5.19 | 0.0327 | | | 1 | 179.03 | 38.03 | 0.0001 | | |
| W*N | 2 | 10.10 | 4.00 | 0.0330 | | | 2 | 66.87 | 14.22 | 0.0001 | | |
| Error | 22 | 2.53 | | | | | 22 | 4.71 | | | | |
| Day(D) | 2 | 232.06 | 76.30 | 0.0001 | 0.0001 | 0.0001 | 3 | 118.82 | 41.88 | 0.0001 | 0.0001 | 0.0001 |
| C*Day | 2 | | | | | | 3 | 12.17 | 4.29 | 0.0079 | 0.0183 | 0.0079 |
| W*Day | 4 | 120.47 | 39.61 | 0.0001 | 0.0001 | 0.0001 | 6 | 24.49 | 8.63 | 0.0001 | 0.0001 | 0.0001 |
| C*W*Day | 4 | | | | | | 6 | 12.65 | 4.46 | 0.0008 | 0.0035 | 0.0008 |
| Error(Day) | 44 | 3.04 | | | | | 66 | 2.84 | | | | |
| Hour(H) | 4 | 587.30 | 217.91 | 0.0001 | 0.0001 | 0.0001 | 4 | 772.88 | 154.30 | 0.0001 | 0.0001 | 0.0001 |
| W*Hour | 8 | 18.88 | 7.00 | 0.0001 | 0.0001 | 0.0001 | 8 | 30.95 | 6.18 | 0.0001 | 0.0001 | 0.0001 |
| Error(Hour) | 88 | 2.70 | | | | | 88 | 5.00 | | | | |
| Day*Hour | 8 | 33.45 | 37.56 | 0.0001 | 0.0001 | 0.0001 | 12 | 8.90 | 10.64 | 0.0001 | 0.0001 | 0.0001 |
| C*D*H | 8 | | | | | | 12 | 3.78 | 4.52 | 0.0001 | 0.0001 | 0.0001 |
| W*D*H | 16 | 5.65 | 6.34 | 0.0001 | 0.0001 | 0.0001 | 24 | 3.90 | 4.66 | 0.0001 | 0.0001 | 0.0001 |
| Error(Day*Hour) | 176 | 0.89 | | | | | 264 | 0.84 | | | | |
| | | STRESS INTERRUPTION | | | | | | CYCLE 3 | | | | |
| Clone(C) | 1 | 5.28 | 1.35 | 0.2572 | | | 1 | 0.11 | 0.06 | 0.8043 | | |
| Water(W) | 2 | 169.20 | 43.32 | 0.0001 | | | 2 | 9.42 | 5.42 | 0.0122 | | |
| N | 1 | 271.96 | 69.63 | 0.0001 | | | 1 | 65.38 | 37.60 | 0.0001 | | |
| W*N | 2 | 14.60 | 3.74 | 0.0400 | | | 2 | 13.08 | 7.52 | 0.0032 | | |
| Error | 22 | 3.91 | | | | | 22 | 1.74 | | | | |
| Day(D) | 3 | 12.60 | 22.62 | 0.0001 | 0.0001 | 0.0001 | 2 | 127.37 | 97.09 | 0.0001 | 0.0001 | 0.0001 |
| C*Day | 3 | | | | | | 2 | 5.96 | 4.55 | 0.0160 | 0.0325 | 0.0160 |
| W*Day | 6 | 5.19 | 9.32 | 0.0001 | 0.0001 | 0.0001 | 4 | 23.65 | 18.03 | 0.0001 | 0.0001 | 0.0001 |
| N*Day | 3 | 3.58 | 6.42 | 0.0007 | 0.0014 | 0.0007 | 2 | 5.28 | 4.03 | 0.0248 | 0.0444 | 0.0248 |
| W*N*Day | 6 | 2.41 | 4.33 | 0.0010 | 0.0020 | 0.0010 | | | | | | |
| Error(Day) | 66 | 0.56 | | | | | 44 | 1.31 | | | | |
| Hour | 4 | 1217.67 | 339.51 | 0.0001 | 0.0001 | 0.0001 | 1 | 81.15 | 38.22 | 0.0001 | | |
| W*Hour | 8 | 21.93 | 6.12 | 0.0001 | 0.0001 | 0.0001 | 2 | 9.64 | 4.54 | 0.0223 | | |
| N*Hour | 4 | 44.35 | 12.37 | 0.0001 | 0.0001 | 0.0001 | | | | | | |
| W*N*Hour | 8 | 13.36 | 3.73 | 0.0009 | 0.0021 | 0.0009 | | | | | | |
| Error(Hour) | 88 | 3.59 | | | | | 22 | 2.12 | | | | |
| Day*Hour | 12 | 5.18 | 10.60 | 0.0001 | 0.0001 | 0.0001 | | | | | | |
| Error(Day*Hour) | | 264 | 0.49 | | | | 44 | 0.82 | | | | |

[1]Effects of interactions that had probability levels greater than 0.05 are not included.
[2]Mean square.
[3]Adj. P are the probability associated with the Greenhouse-Geisser (G-G) and Hyynh-Feldt (H-F) adjusted F-tests.

Table 3.2 Probability of significance levels[1] of ANOVA with repeated measures for stomatal conductance subject to various length of water stress and levels of N.

| Source | df | MS[2] | F | P>F | Adj. P > F[3] G-G | H-F | df | MS | F | P>F | Adj. P > F G-G | H-F |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | CYCLE 1 | | | | | | CYCLE 2 | | | | |
| Clone(C) | 1 | 0.30 | 23.00 | 0.0001 | | | 1 | 0.60 | 27.59 | 0.0001 | | |
| Water(W) | 2 | 0.64 | 48.99 | 0.0001 | | | 2 | 1.05 | 48.59 | 0.0001 | | |
| N | 1 | 0.03 | 1.95 | 0.1765 | | | 1 | 0.12 | 5.35 | 0.0305 | | |
| C*W | 2 | | | | | | 2 | 0.09 | 4.12 | 0.0302 | | |
| W*N | 2 | | | | | | 2 | 0.25 | 11.75 | 0.0003 | | |
| Error | 22 | 0.0130 | | | | | 22 | 0.0217 | | | | |
| Day(D) | 2 | 0.97 | 111.94 | 0.0001 | 0.0001 | 0.0001 | 3 | 0.05 | 5.80 | 0.0014 | 0.0040 | 0.0014 |
| C*Day | 2 | | | | | | 3 | 0.05 | 5.57 | 0.0018 | 0.0049 | 0.0018 |
| W*Day | 4 | 0.81 | 93.85 | 0.0001 | 0.0001 | 0.0001 | 6 | 0.24 | 29.31 | 0.0001 | 0.0001 | 0.0001 |
| N*Day | 2 | | | | | | 3 | 0.04 | 4.71 | 0.0048 | 0.0105 | 0.0048 |
| C*W*Day | 4 | 0.03 | 3.63 | 0.0122 | 0.0152 | 0.0122 | 6 | 0.04 | 5.22 | 0.0002 | 0.0009 | 0.0002 |
| W*N*Day | 4 | | | | | | 6 | 0.03 | 3.33 | 0.0063 | 0.0137 | 0.0063 |
| Error(Day) | 44 | 0.0086 | | | | | 66 | 0.008 | | | | |
| Hour(H) | 4 | 0.49 | 47.53 | 0.0001 | 0.0001 | 0.0001 | 4 | 0.88 | 88.52 | 0.0001 | 0.0001 | 0.0001 |
| W*Hour | 8 | 0.07 | 6.70 | 0.0001 | 0.0001 | 0.0001 | 8 | 0.05 | 5.13 | 0.0001 | 0.0003 | 0.0001 |
| C*W*Hour | 8 | | | | | | 8 | 0.03 | 3.30 | 0.0024 | 0.0081 | 0.0024 |
| C*W*N*Hour | 8 | 0.02 | 2.41 | 0.0213 | 0.0401 | 0.0213 | 8 | | | | | |
| Error(Hour) | 88 | 0.01 | | | | | 88 | 0.01 | | | | |
| Day*Hour | 8 | 0.13 | 38.37 | 0.0001 | 0.0001 | 0.0001 | 12 | 0.10 | 49.39 | 0.0001 | 0.0001 | 0.0001 |
| C*D*H | 8 | 0.009 | 2.67 | 0.0008 | 0.0067 | 0.0008 | 12 | 0.007 | 3.28 | 0.0002 | 0.0043 | 0.0002 |
| W*D*H | 16 | | | | | | 24 | 0.01 | 5.11 | 0.0001 | 0.0001 | 0.0001 |
| N*D*H | 8 | | | | | | 12 | 0.005 | 2.53 | 0.0036 | 0.0220 | 0.0036 |
| C*W*D*H | 16 | | | | | | 24 | 0.004 | 1.93 | 0.0070 | 0.0343 | 0.0070 |
| W*N*D*H | 16 | | | | | | 24 | 0.005 | 2.53 | 0.0002 | 0.0043 | 0.0002 |
| Error(Day*Hour) | 176 | 0.003 | | | | | 264 | 0.002 | | | | |

Table 3.2 (Cont'd)

| Source | df | MS | F | P>F | Adj. P > F G-G | Adj. P > F H-F | df | MS | F | P>F | Adj. P > F G-G | Adj. P > F H-F |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | STRESS INTERRUPTION | | | | | | CYCLE 3 | | | | |
| Clone(C) | 1 | 0.72 | 26.69 | 0.0001 | | | 1 | 0.70 | 59.22 | 0.0001 | | |
| Water(W) | 2 | 0.45 | 16.66 | 0.0001 | | | 2 | 0.19 | 15.70 | 0.0001 | | |
| N | 1 | 0.55 | 20.58 | 0.0002 | | | 1 | 0.43 | 36.41 | 0.0001 | | |
| W*N | 2 | 0.32 | 11.76 | 0.0003 | | | 2 | 0.15 | 12.70 | 0.0002 | | |
| Error | 22 | 0.0269 | | | | | 22 | 0.01 | | | | |
| Day(D) | 3 | 0.35 | 90.51 | 0.0001 | 0.0001 | 0.0001 | 2 | | | | | |
| W*Day | 6 | 0.07 | 17.89 | 0.0001 | 0.0001 | 0.0001 | 4 | 0.20 | 25.23 | 0.0001 | 0.0001 | 0.0001 |
| W*N*Day | 6 | 0.02 | 3.88 | 0.0022 | 0.0070 | 0.0022 | 4 | 0.04 | 5.03 | 0.0020 | 0.0107 | 0.0022 |
| Error(Day) | 66 | 0.0039 | | | | | 44 | 0.008 | | | | |
| Hour(H) | 4 | 1.47 | 202.01 | 0.0001 | 0.0001 | 0.0001 | 1 | 0.83 | 55.80 | 0.0001 | | |
| C*Hour | 4 | 0.06 | 7.98 | 0.0001 | 0.0001 | 0.0001 | 1 | | | | | |
| W*Hour | 8 | 0.03 | 4.39 | 0.0002 | 0.0007 | 0.0002 | 2 | 0.07 | 4.56 | 0.0220 | | |
| N*Hour | 4 | 0.02 | 3.24 | 0.0157 | 0.0260 | 0.0157 | 1 | | | | | |
| W*N*Hour | 8 | 0.06 | 8.19 | 0.0001 | 0.0001 | 0.0001 | 2 | | | | | |
| Error(Hour) | 88 | 0.007 | | | | | 22 | 0.0149 | | | | |
| Day*Hour | 12 | 0.03 | 21.80 | 0.0001 | 0.0001 | 0.0001 | 2 | 0.56 | 114.11 | 0.0001 | 0.0001 | 0.0001 |
| C*D*H | 12 | 0.006 | 3.96 | 0.0001 | 0.0014 | 0.0001 | 2 | | | | | |
| W*D*H | 24 | 0.005 | 3.49 | 0.0001 | 0.0002 | 0.0001 | 4 | | | | | |
| N*D*H | 12 | 0.005 | 3.26 | 0.0002 | 0.0060 | 0.0002 | 2 | 0.02 | 4.78 | 0.0132 | 0.0276 | 0.0132 |
| W*N*D*H | 24 | 0.004 | 2.52 | 0.0002 | 0.0063 | 0.0002 | 4 | | | | | |
| Error(Day*Hour) | 264 | 0.001 | | | | | 44 | 0.0049 | | | | |

[1,2,3]Same as in Table 3.1.

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Table 3.3 Probability of significance levels[1] of ANOVA with repeated measures for transpiration subject to various length of water stress and levels of N.

| Source | df | MS[2] | F | P > F | Adj. P > F[3] G-G | H-F | df | MS | F | P > F | Adj. P > F G-G | H-F |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | CYCLE 1 | | | | | | CYCLE 2 | | | | |
| Clone(C) | 1 | 12.59 | 10.01 | 0.0045 | | | 1 | 68.70 | 18.46 | 0.0003 | | |
| Water(W) | 2 | 79.21 | 62.95 | 0.0001 | | | 2 | 121.40 | 32.62 | 0.0001 | | |
| N | 1 | 1.40 | 1.11 | 0.3037 | | | 1 | 13.56 | 3.64 | 0.0694 | | |
| C*W | 2 | | | | | | 2 | 14.81 | 3.98 | 0.0335 | | |
| W*N | 2 | 5.44 | 4.32 | 0.0261 | | | 2 | 52.79 | 14.19 | 0.0001 | | |
| Error | 22 | 1.2582 | | | | | 22 | 3.7213 | | | | |
| Day(D) | 2 | 90.50 | 86.34 | 0.0001 | 0.0001 | 0.0001 | 3 | 143.97 | 97.50 | 0.0001 | 0.0001 | 0.0001 |
| C*Day | 2 | | | | | | 3 | 6.55 | 4.44 | 0.0067 | 0.0155 | 0.0067 |
| W*Day | 4 | 96.89 | 92.44 | 0.0001 | 0.0001 | 0.0001 | 6 | 29.60 | 20.04 | 0.0001 | 0.0001 | 0.0001 |
| N*Day | 2 | | | | | | 3 | 7.57 | 5.13 | 0.0030 | 0.0085 | 0.0030 |
| C*W*Day | 4 | | | | | | 6 | 8.70 | 5.89 | 0.0001 | 0.0005 | 0.0001 |
| W*N*Day | 4 | | | | | | 6 | 4.75 | 3.22 | 0.0078 | 0.0185 | 0.0078 |
| Error(Day) | 44 | 1.048 | | | | | 66 | 1.4767 | | | | |
| Hour(H) | 4 | 280.42 | 299.39 | 0.0001 | 0.0001 | 0.0001 | 4 | 328.91 | 156.47 | 0.0001 | 0.0001 | 0.0001 |
| W*Hour | 8 | 8.88 | 9.48 | 0.0001 | 0.0001 | 0.0001 | 8 | 14.21 | 6.76 | 0.0001 | 0.0001 | 0.0001 |
| C*W*Hour | 8 | | | | | | 8 | 5.14 | 2.44 | 0.0194 | 0.0402 | 0.0194 |
| W*N*Hour | 8 | | | | | | 8 | 5.00 | 2.38 | 0.0227 | 0.0451 | 0.0227 |
| Error(Hour) | 88 | 0.9366 | | | | | 88 | 2.1020 | | | | |
| Day*Hour | 8 | 15.26 | 52.26 | 0.0001 | 0.0001 | 0.0001 | 12 | 19.55 | 51.74 | 0.0001 | 0.0001 | 0.0001 |
| C*D*H | 8 | | | | | | 12 | 0.90 | 2.38 | 0.0063 | 0.0345 | 0.0063 |
| W*D*H | 16 | 1.37 | 4.69 | 0.0001 | 0.0001 | 0.0001 | 24 | 2.54 | 6.72 | 0.0001 | 0.0001 | 0.0001 |
| C*W*D*H | 16 | | | | | | 24 | 0.75 | 1.98 | 0.0052 | 0.0330 | 0.0052 |
| W*N*D*H | 16 | | | | | | 12 | 1.21 | 3.20 | 0.0001 | 0.0006 | 0.0001 |
| Error(Day*Hour) | 176 | 0.2919 | | | | | 264 | 0.4834 | | | | |
| | | STRESS INTERRUPTION | | | | | | CYCLE 3 | | | | |
| Clone(C) | 1 | 45.30 | 25.68 | 0.0001 | | | 1 | 16.53 | 41.83 | 0.0001 | | |
| Water(W) | 2 | 36.87 | 20.90 | 0.0001 | | | 2 | 3.14 | 7.94 | 0.0025 | | |
| N | 1 | 40.09 | 22.73 | 0.0001 | | | 1 | 6.74 | 17.05 | 0.0004 | | |
| W*N | 2 | 40.25 | 22.82 | 0.0001 | | | 2 | 4.51 | 11.42 | 0.0004 | | |
| Error | 22 | 1.7638 | | | | | 22 | 0.3952 | | | | |
| Day(D) | 3 | 112.67 | 345.45 | 0.0001 | 0.0001 | 0.0001 | 2 | 191.69 | 883.11 | 0.0001 | 0.0001 | 0.0001 |
| W*Day | 6 | 5.01 | 15.37 | 0.0001 | 0.0001 | 0.0001 | 4 | 4.46 | 20.52 | 0.0001 | 0.0001 | 0.0001 |
| N*Day | 3 | 1.12 | 3.46 | 0.0211 | 0.0349 | 0.0211 | 2 | 1.44 | 6.63 | 0.0030 | 0.0124 | 0.0032 |
| W*N*Day | 6 | 1.40 | 4.30 | 0.0010 | 0.0035 | 0.0010 | 4 | | | | | |
| Error(Day) | 66 | 0.3262 | | | | | 44 | 0.2171 | | | | |
| Hour(H) | 4 | 358.36 | 438.64 | 0.0001 | 0.0001 | 0.0001 | 1 | 468.95 | 1445.9 | 0.0001 | | |
| W*N*Hour | 8 | 8.53 | 10.44 | 0.0001 | 0.0001 | 0.0001 | 2 | 1.19 | 3.67 | 0.0420 | | |
| Error(Hour) | 88 | 0.817 | | | | | 22 | 0.3243 | | | | |
| Day*Hour | 12 | 12.34 | 79.15 | 0.0001 | 0.0001 | 0.0001 | 2 | 16.85 | 139.99 | 0.0001 | 0.0001 | 0.0001 |
| C*D*H | 12 | 0.46 | 2.98 | 0.0006 | 0.0150 | 0.0013 | 2 | | | | | |
| W*D*H | 24 | 0.51 | 3.25 | 0.0001 | 0.0011 | 0.0001 | 4 | | | | | |
| N*D*H | 12 | 0.43 | 2.78 | 0.0014 | 0.0215 | 0.0026 | 2 | | | | | |
| W*N*D*H | 24 | 0.33 | 2.11 | 0.0024 | 0.0304 | 0.0044 | 4 | | | | | |
| Error(Day*Hour) | 264 | 0.1559 | | | | | 44 | 0.1204 | | | | |

[1,2,3]Same as in Table 3.1.

Table 3.4 Probability of significance levels[1] of ANOVA with repeated measures for intercellular $CO_2$ concentration subject to various length of water stress and levels of N.

| Source | df | MS[2] | F | P > F | Adj. P > F[3] G-G | H-F | df | MS | F | P > F | Adj. P > F G-G | H-F |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | CYCLE 1 | | | | | | CYCLE 2 | | | | |
| Clone(C) | 1 | 2642.7 | 27.38 | 0.0001 | | | 1 | 2929.8 | 14.74 | 0.0009 | | |
| Water(W) | 2 | 295.1 | 3.06 | 0.0673 | | | 2 | 3513.0 | 17.68 | 0.0001 | | |
| N | 1 | 351.9 | 3.65 | 0.0693 | | | 1 | 5669.5 | 28.53 | 0.0001 | | |
| Error | 22 | 96.5087 | | | | | 22 | 198.7 | | | | |
| Day(D) | 2 | 13196.4 | 96.63 | 0.0001 | 0.0001 | 0.0001 | 3 | 4077.7 | 52.24 | 0.0001 | 0.0001 | 0.0001 |
| C*Day | 2 | | | | | | 3 | 350.5 | 4.49 | 0.0063 | 0.0067 | 0.0063 |
| W*Day | 4 | | | | | | 6 | 736.3 | 9.43 | 0.0001 | 0.0001 | 0.0001 |
| Error(Day) | 44 | 136.7 | | | | | 66 | 78.05 | | | | |
| Hour(H) | 4 | 31499.7 | 170.28 | 0.0001 | 0.0001 | 0.0001 | 4 | 71864.9 | 288.16 | 0.0001 | 0.0001 | 0.0001 |
| W*Hour | 8 | 1465.8 | 7.92 | 0.0001 | 0.0001 | 0.0001 | 8 | 928.9 | 3.72 | 0.0009 | 0.0027 | 0.0009 |
| Error(Hour) | 88 | 184.98 | | | | | 88 | 249.4 | | | | |
| Day*Hour | 8 | 1123.4 | 19.33 | 0.0001 | 0.0001 | 0.0001 | 12 | 2856.6 | 56.75 | 0.0001 | 0.0001 | 0.0001 |
| C*D*H | 8 | | | | | | 12 | 129.7 | 2.58 | 0.0030 | 0.0203 | 0.0030 |
| W*D*H | 16 | 510.6 | 8.79 | 0.0001 | 0.0001 | 0.0001 | 24 | | | | | |
| Error(Day*Hour) | | 58.111 | | | | | 264 | 50.34 | | | | |
| | | STRESS INTERRUPTION | | | | | | CYCLE 3 | | | | |
| Clone(C) | 1 | 6496.5 | 37.03 | 0.0001 | | | 1 | 739.7 | 16.44 | 0.0005 | | |
| Water(W) | 2 | 546.7 | 3.12 | 0.0643 | | | 2 | 274.3 | 6.09 | 0.0078 | | |
| N | 1 | 1902.4 | 10.84 | 0.0033 | | | 1 | 338.4 | 7.52 | 0.0119 | | |
| W*N | 2 | 1621.4 | 9.24 | 0.0012 | | | 2 | 271.8 | 6.04 | 0.0081 | | |
| Error | 22 | 175.4 | | | | | 22 | 45.0035 | | | | |
| Day(D) | 3 | 17866.2 | 464.72 | 0.0001 | 0.0001 | 0.0001 | 2 | 17328.4 | 516.19 | 0.0001 | 0.0001 | 0.0001 |
| C*Day | 3 | | | | | | 2 | 248.0 | 7.39 | 0.0017 | 0.0059 | 0.0017 |
| W*Day | 6 | | | | | | 4 | 310.4 | 9.25 | 0.0001 | 0.0002 | 0.0001 |
| N*Day | 3 | 148.3 | 3.86 | 0.0132 | 0.0209 | 0.0132 | 2 | 135.0 | 4.02 | 0.0249 | 0.0419 | 0.0249 |
| C*W*Day | 6 | | | | | | 4 | 113.4 | 3.38 | 0.0170 | 0.0342 | 0.0170 |
| Error | 66 | 38.4452 | | | | | 44 | 33.57 | | | | |
| Hour(H) | 4 | 127528 | 729.71 | 0.0001 | 0.0001 | 0.0001 | 1 | 33701.5 | 651.30 | 0.0001 | | |
| W*Hour | 8 | 883.6 | 5.06 | 0.0001 | 0.0001 | 0.0001 | 2 | 465.5 | 9.00 | 0.0014 | | |
| N*Hour | 4 | 2042.0 | 11.68 | 0.0001 | 0.0001 | 0.0001 | 1 | 682.5 | 13.19 | 0.0015 | | |
| C*W*N*Hour | 8 | | | | | | 2 | 192.3 | 3.72 | 0.0407 | | |
| Error(Hour) | 88 | 174.7657 | | | | | 22 | 51.7449 | | | | |
| Day*Hour | 12 | 1172.8 | 45.51 | 0.0001 | 0.0001 | 0.0001 | 2 | 1582.8 | 66.11 | 0.0001 | 0.0001 | 0.0001 |
| N*D*H | 12 | 86.0 | 3.34 | 0.0002 | 0.0040 | 0.0002 | 2 | | | | | |
| C*N*D*H | 12 | 10.7 | 0.41 | 0.9572 | 0.8719 | 0.9572 | 2 | 48.2 | 2.01 | 0.1457 | 0.1523 | 0.1457 |
| Error(Day*Hour) | 264 | 25.7689 | | | | | 44 | 23.9441 | | | | |

[1,2,3]Same as in Table 3.1.

Table 3.5 Probability of significance levels[1] of ANOVA with repeated measures for water-use efficiency subject to various length of water stress and levels of N.

| Source | df | MS[2] | F | P > F | Adj. P > F[3] G-G | H-F | df | MS | F | P > F | Adj. P > F G-G | H-F |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | CYCLE 1 | | | | | | CYCLE 2 | | | | |
| Clone(C) | 1 | 0.86 | 22.64 | 0.0001 | | | 1 | 0.48 | 8.12 | 0.0093 | | |
| Water(W) | 2 | 0.51 | 13.34 | 0.0002 | | | 2 | 2.45 | 41.53 | 0.0001 | | |
| N | 1 | 0.18 | 4.82 | 0.0390 | | | 1 | 2.87 | 48.58 | 0.0001 | | |
| W*N | 2 | | | | | | 2 | 0.24 | 4.08 | 0.0310 | | |
| Error | 22 | 0.0381 | | | | | 22 | 0.0590 | | | | |
| Day(D) | 2 | 7.44 | 153.92 | 0.0001 | 0.0001 | 0.0001 | 3 | 8.98 | 293.02 | 0.0001 | 0.0001 | 0.0001 |
| C*Day | 2 | | | | | | 3 | 0.10 | 3.17 | 0.0299 | 0.0410 | 0.0299 |
| W*Day | 4 | 0.52 | 10.86 | 0.0001 | 0.0001 | 0.0001 | 6 | 0.12 | 3.76 | 0.0028 | 0.0060 | 0.0028 |
| C*W*Day | 4 | | | | | | 6 | 0.10 | 3.27 | 0.0071 | 0.0128 | 0.0071 |
| Error(Day) | 44 | 0.0483 | | | | | 66 | 0.0306 | | | | |
| Hour(H) | 4 | 6.92 | 112.45 | 0.0001 | 0.0001 | 0.0001 | 4 | 4.52 | 56.62 | 0.0001 | 0.0001 | 0.0001 |
| W*Hour | 8 | 0.40 | 6.52 | 0.0001 | 0.0001 | 0.0001 | 8 | | | | | |
| Error(Hour) | 88 | 0.0616 | | | | | 88 | 0.0799 | | | | |
| Day*Hour | 8 | 0.84 | 50.58 | 0.0001 | 0.0001 | 0.0001 | 12 | 0.42 | 27.21 | 0.0001 | 0.0001 | 0.0001 |
| C*D*H | 8 | | | | | | 12 | 0.06 | 3.73 | 0.0001 | 0.0011 | 0.0001 |
| W*D*H | 16 | 0.13 | 7.57 | 0.0001 | 0.0001 | 0.0001 | 24 | 0.03 | 1.81 | 0.0136 | 0.0446 | 0.0136 |
| Error(Day*Hour) | 176 | 0.0166 | | | | | 264 | 0.0154 | | | | |
| | | STRESS INTERRUPTION | | | | | | CYCLE 3 | | | | |
| Clone(C) | 1 | 1.55 | 34.70 | 0.0001 | | | 1 | 0.20 | 5.83 | 0.0245 | | |
| Water(W) | 2 | 1.10 | 24.68 | 0.0001 | | | 2 | 0.22 | 6.46 | 0.0062 | | |
| N | 1 | 2.19 | 49.20 | 0.0001 | | | 1 | 0.74 | 21.78 | 0.0001 | | |
| W*N | 2 | 0.20 | 4.56 | 0.0220 | | | 2 | 0.24 | 7.05 | 0.0043 | | |
| Error | 22 | 0.0445 | | | | | 22 | 0.0341 | | | | |
| Day(D) | 3 | 3.35 | 319.76 | 0.0001 | 0.0001 | 0.0001 | 2 | 3.83 | 129.35 | 0.0001 | 0.0001 | 0.0001 |
| C*Day | 3 | 0.05 | 4.85 | 0.0041 | 0.0051 | 0.0041 | 2 | 0.19 | 6.41 | 0.0036 | 0.0131 | 0.0036 |
| W*Day | 6 | | | | | | 4 | 0.40 | 13.60 | 0.0001 | 0.0001 | 0.0001 |
| N*Day | 3 | 0.08 | 7.60 | 0.0002 | 0.0003 | 0.0002 | 2 | 0.12 | 4.15 | 0.0223 | 0.0442 | 0.0223 |
| Error(Day) | 66 | 0.0105 | | | | | 44 | 0.0296 | | | | |
| Hour(H) | 4 | 7.75 | 147.97 | 0.0001 | 0.0001 | 0.0001 | 1 | 2.17 | 35.87 | 0.0001 | | |
| C*Hour | 4 | 0.24 | 4.51 | 0.0023 | 0.0039 | 0.0023 | 1 | | | | | |
| W*Hour | 8 | 0.19 | 3.71 | 0.0009 | 0.0018 | 0.0009 | 2 | | | | | |
| N*Hour | 4 | 0.62 | 11.91 | 0.0001 | 0.0001 | 0.0001 | 1 | | | | | |
| Error(Hour) | 88 | 0.0523 | | | | | 22 | 0.0604 | | | | |
| Day*Hour | 12 | 0.28 | 31.56 | 0.0001 | 0.0001 | 0.0001 | 2 | | | | | |
| Error(Day*Hour) | 264 | 0.0089 | | | | | 44 | 0.0185 | | | | |

[1,2,3]Same as in Table 3.1.

Table 3.6 Probability of significance levels of analysis of variance on leaf water potential at the end of phases one and three[1].

| | Source | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Parameter | Clone (C) | Water (W) | N | C*W | C*N | W*N | C*W*N | MSE(df)[2] |
| Phase 1 | 0.6749 | 0.0102* | 0.0060* | 0.1136 | 0.9925 | 0.0010* | 0.6161 | 2.2827(12) |
| Phase 3 | 0.0298* | 0.0001* | 0.0185* | 0.0066* | 0.2322 | 0.5109 | 0.7936 | 1.1806(24) |

[1]First stress phase consisted of two repeated drought cycles and one continuous flooding. The third phase involved one drought cycle and a flooding of equivalent time. Each drought cycle was completed after 8 to 10 days of water withholding.

[2]Mean square of error (defree of freedom).

Table 3.7 Midday leaf water potential (-MPa) after 18 days under different water and N regimes.

| | N availability | |
|---|---|---|
| Water status | Low N | High N |
| Flood | 0.58 a[1] | 0.99 bc |
| Well-watered | 0.89 b | 1.06 c |
| Severe drought[2] | 0.98 bc | 0.84 b |

[1]Means followed by different letters are significantly different at $P \leq 0.05$. Each value was the average of two clones, representing 8 observations.

[2]Measurements of all treatments were made at the end of two consecutive dry-down cycles in this treatment.

Table 3.8 Midday leaf water potential (-MPa) after water stress was resumed following a period of stress interruption.

| Water Status | Clone | |
|---|---|---|
| | Tristis | Eugenei |
| Flood | 0.87 bd[1] | 0.81 cde |
| Well-watered | 0.78 ce | 0.87 bd |
| Severe drought[2] | 0.94 b | 1.09 a |

[1]Means followed by different letters are significantly different at $P \leq 0.05$. Each value represents 8 observations.

[2]Measurements of all treatments were made at the end of two consecutive dry-down cycles in this treatment.

Table 3.9 Probability of significance levels[1] of analysis of variance on morphology affected by water and N regimes.

| Variables | Clone(C) | Water(W) | N | C*W | C*N | W*N | C*W*N | MSE(df)[2] |
|---|---|---|---|---|---|---|---|---|
| Number of Leaves | 0.0324* | 0.0036* | 0.0001* | 0.3154 | 0.6355 | 0.0001* | 0.1994 | 7.69(22) |
| Leaf size($cm^2$) | 0.0869 | 0.0002* | 0.0001* | 0.0103* | 0.0570 | 0.0001* | 0.8352 | 92.16(22) |
| Root/shoot ratio | 0.9050 | 0.0001* | 0.0001* | 0.6840 | 0.9111 | 0.0011* | 0.8320 | 0.04(22) |
| SLW ($g/m^2$) | 0.0080* | 0.0001* | 0.0001* | 0.0422* | 0.0850 | 0.5834 | 0.6687 | 792.38(22) |
| Chl (mg/g) | 0.4051 | 0.0017* | 0.0001* | 0.2597 | 0.0836 | 0.9374 | 0.7016 | 1.15(22) |
| Leaf N (%) | 0.2664 | 0.0001* | 0.0001* | 0.9024 | 0.1692 | 0.0001* | 0.2119 | 0.10(57) |

[1]Asterisks indicated $P \leq 0.05$.

[2]Mean square of error and associated degree of freedom in parenthesis.

Table 3.10 Probability of significance levels[1] of analysis of variance on growth affected by water and N regimes.

| Variables | Clone(C) | Water(W) | N | C*W | C*N | W*N | C*W*N | MSE(df)[2] |
|---|---|---|---|---|---|---|---|---|
| Height(cm) | 0.0017* | 0.0001* | 0.0001* | 0.0606 | 0.1899 | 0.0003* | 0.1850 | 56.49(22) |
| Leaf biomass (g) | 0.4148 | 0.0001* | 0.0001* | 0.0451* | 0.0740 | 0.0001* | 0.0672 | 4.19(22) |
| Stem biomass (g) | 0.0003* | 0.0001* | 0.0001* | 0.0283* | 0.5791 | 0.0001* | 0.0126* | 6.06(22) |
| Root biomass (g) | 0.1078 | 0.0001* | 0.5100 | 0.0989 | 0.7475 | 0.7949 | 0.7270 | 24.72(22) |
| Total biomass (g) | 0.0394* | 0.0001* | 0.0001* | 0.0233* | 0.3965 | 0.0005* | 0.1601 | 62.68(22) |

[1]Asterisks indicated $P \leq 0.05$.

[2]Mean square of error and associated degree of freedom in parenthesis.

Table 3.11 Total biomass production (g) showing clone and water, water and N interactions.

| | | Flood | Well-watered | Drought |
|---|---|---|---|---|
| Clone | Tristis | 27.0 d[1] | 66.5 a | 46.8 b |
| | Eugenei | 32.3 cd | 54.0 b | 36.6 c |
| N level | Low N | 25.1 d | 41.9 bc | 35.6 c |
| | High N | 34.2 dc | 78.6 a | 47.7 b |

[1]Means in each clone and N level category followed by different letters are significantly different at $P \leq 0.05$.

Figure 3.

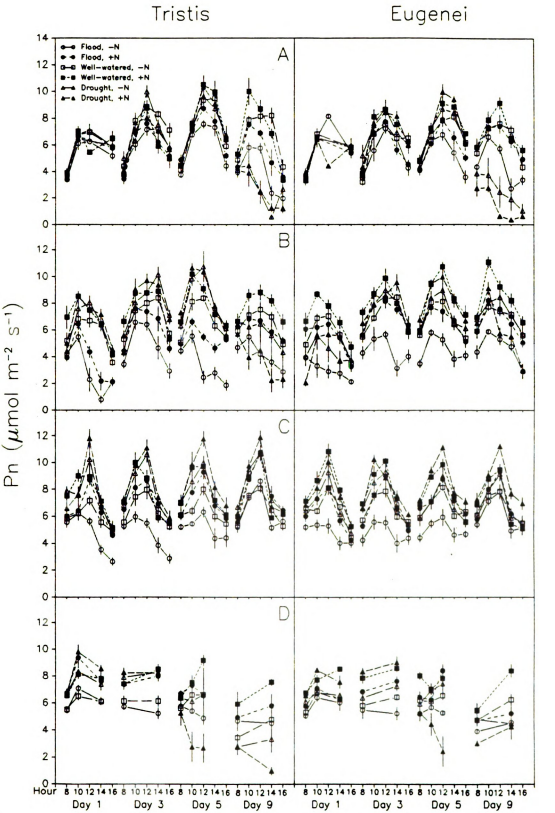


**Figure 3.1** Diurnal and daily changes of photosynthesis of two clones under six combinations of water and N. A: drought cycle 1; B: drought cycle 2; C: water stress interrupted; and D: water stress resumed, drought cycle 3.

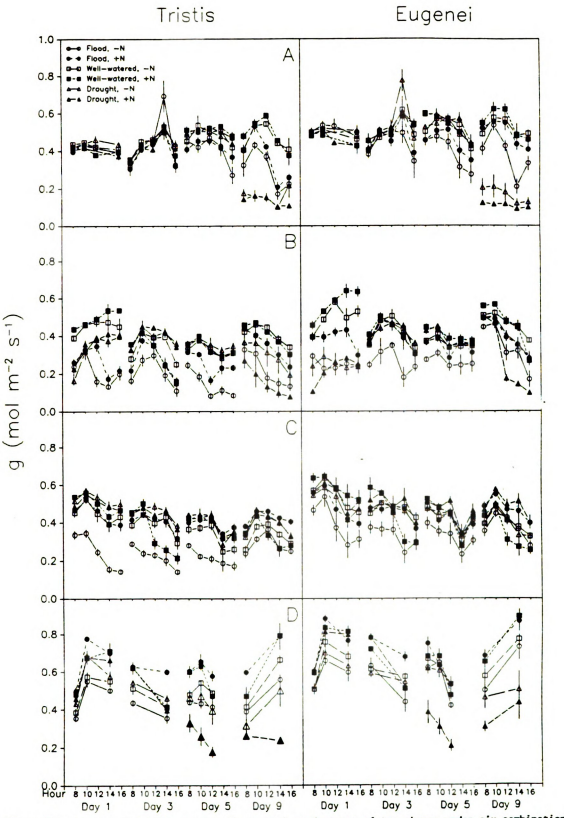


**Figure 3.2** Diurnal and daily changes of stomatal conductance of two clones under six combinations of water and N. A: drought cycle 1; B: drought cycle 2; C: water stress interrupted; and D: water stress resumed, drought cycle 3.

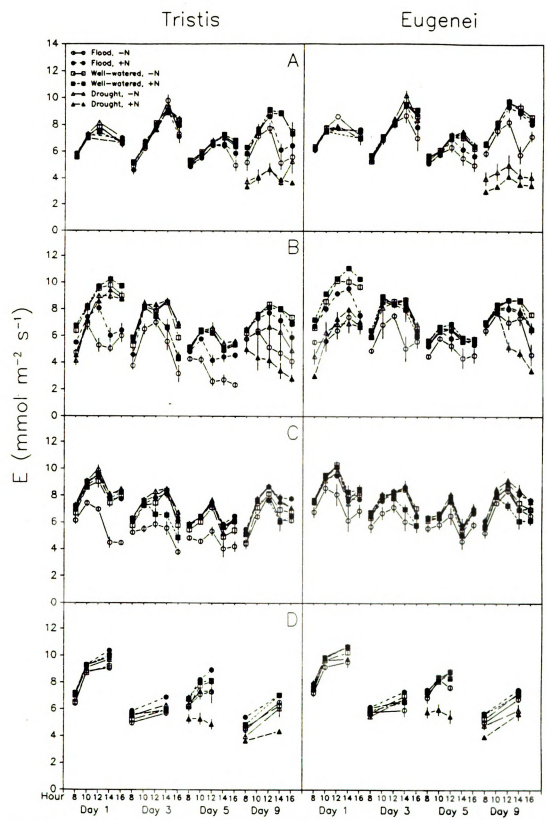


**Figure 3.3** Diurnal and daily changes of transpiration of two clones under six combinations of water and N. A: drought cycle 1; B: drought cycle 2; C: water stress interrupted; and D: water stress resumed, drought cycle 3.

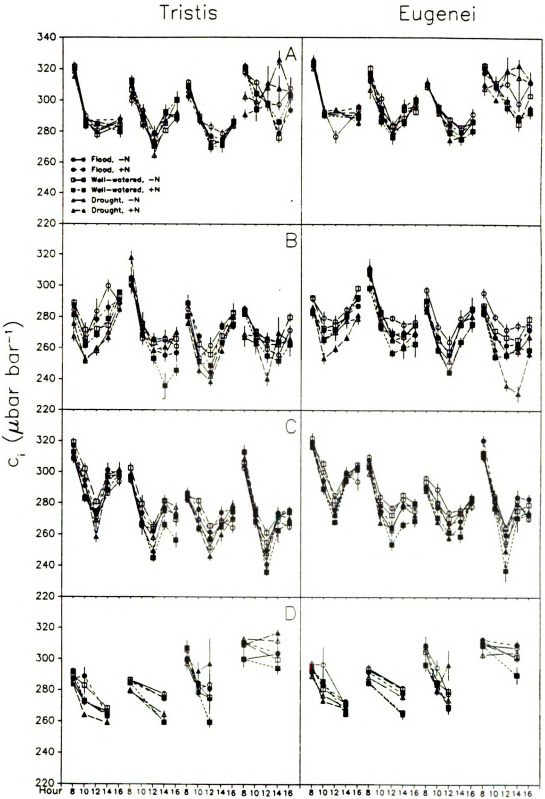


**Figure 3.4** Diurnal and daily changes of internal $CO_2$ concentration of two clones under six combinations of water and N. A: drought cycle 1; B: drought cycle 2; C: water stress interrupted; and D: water stress resumed, drought cycle 3.

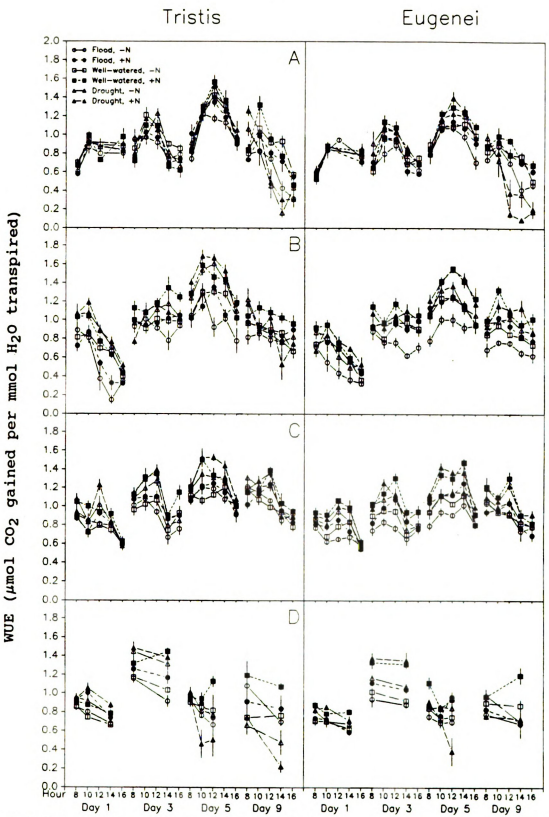


**Figure 3.5** Diurnal and daily changes of water-use efficiency of two clones under six combinations of water and N. A: drought cycle 1; B: drought cycle 2; C: water stress interrupted; and D: water stress resumed, drought cycle 3.

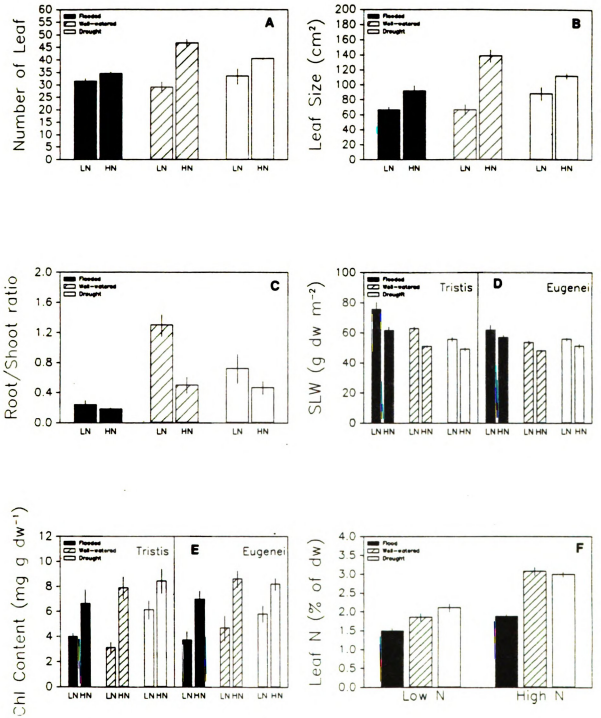


**Figure 3.6 Morphological changes induced by water and N regimes. (A) Number of leaves; (B) Leaf size; (C) Root/shoot ratio; (D) Specific leaf weight; (E) Chlorophyll content; and (F) Leaf N concentration.**

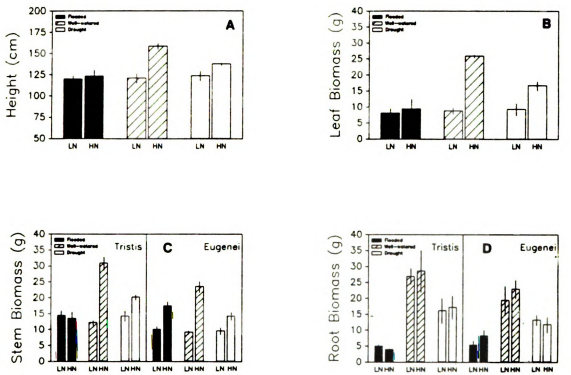


**Figure 3.7** **Effects of water and N regimes on height growth (A), and leaf (B), stem (C) and root (D) biomass accumulation.** LN: no N added; HN: 1.5 g N added.

# CHAPTER IV

## ABSCISIC ACID ACCUMULATION AS INDUCED BY WATER AND NITROGEN AVAILABILITY IN LEAVES OF TWO HYBRID POPLAR CLONES


## ABSTRACT

Cuttings of Populus clones Tristis and Eugenei growing in pots in a greenhouse were subjected to repeated water stress (drought and flooding) and were treated with two levels of nitrogen. Periodic sampling of recently mature leaves was done for abscisic acid (ABA) analysis by means of radioimmunoassay. Accompanying gas exchange variables were measured as physiological responses.

Photosynthesis and stomatal conductance were depressed five days after flooding, but ABA concentrations remained relatively unaltered. In contrast, soil water deficiency during an initial dry-down cycle resulted in a substantial ABA accumulation in the leaves, which closely correlated with changes of photosynthesis and conductance. Repeated drought during a second dry-down cycle led to the acclimation of gas exchange, in association with reduced ABA levels. Upon the relief of drought, gas exchange in Tristis was able to fully and quickly recover due to the quick turnover of accumulated ABA. Eugenei, however, showed a

slow recovery, which was associated with the retention of ABA. High N supply stimulated ABA production as soil dried.

Eugenei was insensitive to initial increases of ABA; gas exchange was affected only as ABA accumulated to over 100 ng g $dw^{-1}$. Tristis, on the other hand, was sensitive to elevated ABA, showing immediate declines of stomatal aperture and photosynthesis when leaf ABA concentration was as low as 10 ng g $dw^{-1}$. Internal $CO_2$ concentration was responsive to the increase of ABA concentration in Tristis, but less in Eugenei, probably accounting for the difference in photosynthetic capacity. In the face of prolonged drought the sensitive stomata of Tristis exercise tight control over water loss, improving its survival potential. However, reduced photosynthetic capacity as a result of ABA accumulation leads to reduced biomass production in Tristis as compared to Eugenei.

# INTRODUCTION

The observations of reduced stomatal aperture under drought or flooding without apparent leaf water deficits (Bradford and Hsiao 1982, Zhang and Davies a&b 1989, Blackman and Davies 1985, Jackson and Hall 1987, Mazzoleni and Dickmann 1988) provides evidence that a signal from the stressed roots is sent to the leaves, regulating stomatal behavior. These findings are contradictory to the conventional view of stomatal response to leaf water status, particularly leaf turgor pressure as a threshold signal for stomatal changes.

More recent findings indicate that plants are capable of sensing soil water status in their roots and communicating this condition to the shoots where compensatory physiological changes occur that maximize resistance to stress (Davies et al. 1990). Under water stress an imbalance of growth regulators, especially an increase of abscisic acid (ABA) (Davies et al. 1990, Jackson and Hall 1987, Wadman-van Schravendijk and van Andel 1985) and reduction of cytokinin (Blackman and Davies 1985), was well correlated with observed stomatal changes. Abscisic acid has been regarded as the signal responsible for

stomatal responses (Davies et al. 1990, Cornish and Radin 1990).

A common response of water stress is increased ABA concentrations in the leaves. However, the locations of ABA synthesis could vary with the type of water stress. Under progressive drought, roots were the sensitive measures of soil water status and ABA synthesized in the roots was translocated to the leaves where it imposed regulation over stomatal aperture (Zhang and Davies 1987, Zhang et al. 1987). On the other hand, elevated ABA levels in the leaves could be the consequence of obstructed ABA translocation out of leaves when plants are waterlogged (Setter and Brun 1981) or leaves are girdled at the lamina base (Henson 1984).

Not only the total amount of ABA accumulation, but also the partitioning of ABA between the active (apoplastic) and the inactive (chloroplastic) pools is important (Hartung et al. 1988). Abscisic acid is distributed in the leaves according to pH gradients (Cowan et al. 1982, Creelman 1989). Environmental factors such as light and water availability serve as stimuli for the formation of pH gradients in photosynthetic cells, resulting in a higher pH in the stroma. Chloroplasts become effective 'alkaline traps' for ABA because of its weak acid properties (Cowan et al. 1982). Water stress could cause a decrease in the pH gradient (Hartung et al. 1988); thus more ABA would be released into the apoplastic region and translocated to the outer surface of the guard cell plasmalema where the active

sites of ABA reside (Lahr and Raschke 1988, Hartung 1983, Creelman 1989).

Stomatal behavior was well correlated with ABA levels of the leaves under flooded conditions (Jackson and Hall 1987, Wadman-van Schravendijk and van Andel 1985), and under droughty conditions in xylem sap (Loveys 1984) and in leaves (Zhang and Davies 1987, Blackman and Davies 1985, Neales et al. 1989). The accumulation of ABA induced by water stress may also have direct effects on other physiological and growth processes. For example, leaf elongation appeared more sensitive to soil drying than stomatal behavior (Gowing et al. 1990), and possible direct interference of ABA on the photosynthetic process has been indicated (Ward and Bunce 1987, Burschka et al. 1985). On the other hand, the elevation of ABA is of significance in drought resistance through control of water loss through the reduction of stomatal aperture, and increases in root hydraulic conductivity (Lachno and Baker 1986).

Leaf N status seems to influence the sensitivity of stomata to ABA levels (Radin and Hendrix 1988). Low-N plants were sensitive to ABA accumulation, whereas high-N plants were less sensitive to ABA levels, requiring a higher concentration of ABA to attain the similar stomatal aperture (Radin et al. 1982). This contradicts the findings that high-N plants closed stomata earlier than their low-N counterparts during progressive drought (Chapters 2 and 3).

The major objectives of the study reported in this chapter were 1) to determine and differentiate between two types of water stress, excess or deficiency, in their ability to induce ABA accumulation; 2) to explore the roles of leaf-N status in modifying the responses of stomata under drought or flooding conditions, in particular, to address the question whether the rapid declines of stomatal conductance of high-N plants under stress were associated with ABA accumulation; and 3) to detect the changes in ABA levels when stress was removed, which could be correlated to the recovery capacity of physiological processes, such as stomatal functioning and photosynthesis.

## MATERIALS AND METHODS

Two clones representing two sections of the genus Populus were used: Tristis (Populus tristis x P. balsamifera cv. Tristis No. 1) from section Tachamahaca and Eugenei (P. x euramericana cv. Eugenei) from section Aigeiros (Dickmann and Stuart 1983). Cuttings from both clones were planted in 22.7 l plastic pots (one cutting per pot) filled with a natural sandy-loam soil containing 0.058 mol nitrogen per kilogram dry soil, and placed in a greenhouse. Frequent watering was provided to cuttings to assure vigorous growth. Before they were used as experimental materials, all

cuttings were allowed to grow until they possessed 30 leaves greater than 30 mm in length.

The whole experiment included three phases. The first phase included two drought cycles. During the second phase water stress was interrupted, then the third phase followed in which water stress was resumed. During the first water stress phase, water was withheld for eight to ten days so that soil matric potential reached ca. -0.5 MPa, according to a previously determined soil retention curve developed with a pressure plate apparatus. A waterlogging treatment also was imposed which lasted the length of the two drought cycles. Once soil matric potential of the drought treatments reached the target value, watering brought the soil back to field capacity. After two consecutive drought cycles, water stress was interrupted and all the plants received water every 2 to 3 days for 10 days. Finally, in the third phase water stress (drought and flooding) was resumed for another cycle before plants were harvested. A well-watered set of control plants was maintained through all three phases.

There was another controlled factor in this study besides soil water status: N availability. Nitrogen treatment consisted of 2 levels, low N (no supplemental N fertilizer), and high N (supplemental N in ammonium-nitrate form equivalent to 200 kg $ha^{-1}$ applied at the beginning of each drought cycle at the rate equal to one-third of the total amount). Thus, this experiment was composed of six

combinations of water and N levels for each of two clones and was designed as a randomized complete block with a three-factor factorial arrangement in three blocks.

On day 1, 5, and 9 after water treatments began, net photosynthesis (Pn), stomatal conductance (g), transpiration rate (E), and substomatal cavity $CO_2$ concentration ($c_i$) were measured on two new fully expanded leaves for each plant with a portable leaf chamber and infrared gas analyzer (Analytical Development Co., Herts, England). Gas exchange measurements were taken at 1400 hour (solar time). Immediately following the measurements, leaves were excised, placed in liquid N, and stored in a freezer. The frozen leaves were later freeze-dried and ground to powder for ABA analysis. At the end of the drought cycles leaf water potential was measured with a pressure chamber (PMS Instruments) on two fully mature leaves for each plant during 1100 to 1300 hours.

Determination of ABA concentration in the leaves was made using a radioimmunoassay (Weiler 1980, Vernieri and Perata unpublished). A 0.01 g bulk leaf sample of each plant was reconstituted in 1 ml of distilled $H_2O$ in 1.5 ml Eppendorf tubes and soaked approximately 15 hours at $2^oC$ under darkness. A mouse monoclonal antibody (Idetek, Inc. products)(DBPA 1) to free (S)-ABA coupled to the carrier protein (KLH) was used for radioimmunoassays. Three replicate samples of 50 µl were collected from each leaf solution. A tracer solution of 10,000 - 12,000 cpm/100 µl

DL-cis,trans-(G-$^3$H) ABA (Amersham Life Science Products) in a PBS buffer was prepared and 100 µl was added to the samples. After adding 50 µl of antibody solution, the samples were vortexed and incubated for 30 min at 4°C. The samples were then treated with 200 µl of saturated $NH_4SO_4$ solution, vortexed, and incubated for another 30 min at 25°C. The samples were then centrifuged at 13,000 g for 6 min, the supernatant discarded, and the pellet resuspended in 400 µl of 50% saturated $NH_4SO_4$ solution. The centrifuge procedure was repeated and the pellet resuspended in 100 µl distilled water. Finally, 1.2 ml of scintillation cocktail (Packard Opti-fluor) was added to the samples and they were vortexed, incubated for 1 hour at room temperature, and counted for 10 min each with a Packard 1500 Tri-carb liquid scintillation analyzer. Samples were compared to standard solutions of 1, 0.2, 0.1, 0.05, 0.01, and 0.001 ml $^3$H-ABA/liter and converted to units of nanograms (ng) per g of dry leaf weight.

## RESULTS

Water stress caused a substantial induction of ABA accumulation during the first water withholding phase consisting of two drought cycles (Table 4.1) regardless of soil N status. Non-stressed leaves showed small daily

variations throughout this phase of 18 days, fluctuating from 6 to 26 ng ABA g $dw^{-1}$. Approximately 5-fold variations occurred in Tristis and 3-fold in Eugenei. Leaves of flooded plants displayed no significant changes in ABA in both clones, although larger variations in ABA levels were found than in non-stressed plants. For example, flooded Tristis leaves showed a 6-fold increase in ABA levels on day 5, but then dropped back on the ninth day. As flooding continued, no further increase of ABA was observed. Flooded Eugenei, on the other hand, displayed a gradual 10-fold increase of ABA during the first 9 days, but then declined to the control levels throughout the rest of the 18 days of flooding.

In contrast to flooding stress, progressive drought dramatically increased ABA levels in the leaves of both clones. Under mild drought, ABA was increased only by 2- to 3-fold in both clones. However, as the soil dried to -0.5 MPa, Tristis showed almost a 90-fold increase of ABA in the leaves, whereas Eugenei had a 360-fold higher ABA concentration.

Upon re-watering, different responses occurred in the clones. In Tristis, ABA concentrations plunged back to the control levels immediately after the rehydration. On the contrary, Eugenei still maintained 20-fold higher ABA levels in the leaves after the temporary relief from drought, despite significant declines of ABA concentrations when compared to the level at extreme drought. Eugenei then

showed a continuous decline of ABA. On the fifth day after re-watering, ABA levels dropped to only two-fold higher than the control. However, as the second drought became more extreme, ABA levels rose again in both clones. In contrast to the first experience of drought, ABA accumulation was reduced in both clones with the severe soil drying. Tristis showed ca 14-fold increase of ABA concentration, whereas Eugenei had a 28-fold increase.

Nitrogen fertilization substantially altered the response of plants in accumulating ABA (Table 4.2); high N induced higher ABA production in both clones. However, Eugenei tended to have a stronger ABA response to high N than did Tristis. In Tristis, the first cycle of water stress caused ABA increases in both N regimes, but with a 2- to 3-fold higher response in high-N plants. The second cycle of water stress induced ABA accumulation only in high-N plants. Responses of Eugenei plants to N were similar to Tristis. A high residual ABA concentration resided in high-N Eugenei plants after temporary relief from drought, whereas there was no significant amount of residual ABA in the low N plants.

Despite relatively unchanged ABA levels in the flooded plants, photosynthesis started to decline following five days of submergence (Figure 4.1A). However, supplemental N seemed effective in Eugenei in offsetting the negative effects of flooding; similar rates of photosynthesis were shown in both flooded and non-stressed plants. Supplemental

N failed to generate significant changes of Pn in Tristis. Mild drought did not affect Pn in both clones. As soil water deficits developed, significant reductions of Pn to nearly negligible rates occurred in both clones. Similar to the response of ABA, re-watering led to the full and quick recovery of Pn in Tristis regardless of N levels. In Eugenei, however, high N plants recovered completely after the relief from drought; but low N plants showed a slower return, which was opposite to the changing pattern of ABA. When another drought developed, both clones tended to become more resistant to declines of Pn, particularly in low-N plants.

Stomatal conductance was closely coupled to photosynthetic activity (Figure 4.1B). Flooded Tristis was able to maintain rates of g during the first five days of flooding. On day 9, g was reduced to almost 40% of the non-flooded plants. Subsequently as flooding lengthened, high N, but not low N stimulated stomatal reopening. In Eugenei, the effects of N under flooding were apparent. High-N plants were able to maintain high rates of g throughout the flooding period, whereas low-N plants displayed pronounced stomatal closure.

Under progressive drought, g closely followed the changes of leaf ABA levels. Mild drought did not induce stomatal closure, in correspondence to the low ABA concentrations. Under severe drought, pronounced stomatal closure was observed in both clones, with an accompanying

elevation of ABA concentration. The relief from drought completely released constraints on stomatal opening in Tristis, but not in Eugenei, which did not show a full recovery of g or reduction of ABA to pre-stress levels until after 5 days of drought release. With the onset of another drought, low-N plants of both clones demonstrated acclimation to severe drought, whereas high-N plants continued to show substantial reductions of g.

Internal $CO_2$ levels were the reflection of the photosynthetic activity at the chloroplast level. Flooded plants showed relatively constant $c_i$ during flooding, except that low-N plants had a slight rise of $c_i$, an indication of non-stomatal limitations to the decreased Pn (Figure 4.1C). Under drought conditions, however, significant increases of $c_i$, approaching ambient $CO_2$ levels occurred, suggesting strong non-stomatal inhibitions of Pn. As drought repeated, this rise in $c_i$ did not appear. On the other hand, droughted high-N Eugenei showed a dramatic decrease of $c_i$, reflecting stomatal closure.

Three days after interruption of water stress, ABA levels remained different from well-watered plants, reflecting the residual effects of water stress (Table 4.3). Clonal deviations also were apparent, a possible indication of genetic variations in recovery capacity. Three days after interruption of drought stress, the residual ABA effects disappeared in Tristis, whereas Eugenei leaves still retained a significantly high amount of ABA.

When water stress was resumed, a strong ABA response occurred (Table 4.4). Leaf N status stimulated ABA concentrations in leaves and interacted with water availability. Under flooding conditions ABA increases in the leaves were not induced. In contrast, massive ABA accumulations were observed under severe drought. In non-flooded plants, sufficient N supply caused more ABA accumulation than N-deficient counterparts by 3- to 5-fold. Flooded plants with low-N status, on the contrary, displayed higher ABA levels than high-N plants, but not significantly. Despite the similar response patterns of ABA to the previous stress cycles, the resumption of water stress led to a much smaller magnitude of increase of ABA.

The renewal of flooding condition for nine days after stress interruption did not decrease Pn, but limited the promoting effects of N in both clones (Figure 4.2A). The reappearance of droughty conditions caused significant reductions of Pn in Tristis, with high N adding to the negative effects, leading to a further decrease. Eugenei, on the other hand, showed acclimation of Pn to drought, but the positive effects of N were excluded.

In contrast to the first experience of flooding, reintroducing flooding for nine days did not cause substantial stomatal closure in either clone (Figure 4.2B). Soil drying, however, significantly reduced g in both clones. Similar to the Pn response, high N added further stomatal closure in Tristis but not in Eugenei.

Internal $CO_2$ levels remained almost constant in Eugenei, indicating minimum non-stomatal limitations to $CO_2$ assimilation (Figure 4.2C). In Tristis, non-stomatal inhibition of Pn appeared under droughty conditions, evidenced by pronounced elevation of $c_i$.

To examine the effects of ABA accumulation on gas exchange variables during progressive drought, the relationship between the two were plotted (Figure 4.3). Two distinct patterns were observed in the two clones. Tristis appeared more sensitive to increases of ABA concentration; ABA concentrations higher than 10 ng g leaf $dw^{-1}$ caused changes in the gas exchange variables. Photosynthesis started to decline markedly as ABA levels increased beyond 10 ng g $dw^{-1}$; similar trends were shown between g, E and ABA. In contrast, $c_i$ rose as ABA accumulated, probably indicating a direct involvement of ABA in the photosynthetic process. Eugenei seemed tolerant to ABA accumulation up to 10-fold the threshold concentrations in Tristis. When ABA concentrations were below 100 ng g $dw^{-1}$, Pn, g, and E were not affected. As ABA levels increased further, log-linear reductions of Pn, g and E occurred. In contrast to Tristis, $c_i$ was not substantially influenced by ABA accumulation.

During the progressive drying of soil, there was close positive coupling between Pn and g in both clones (Figure 4.4). Pn was linearly increased as g became greater.

# DISCUSSION

Flooding did not cause significant increases of ABA in the leaves of Tristis and Eugenei during two separate phases of flooding. This result contradicts many other findings that flooding caused ABA accumulation in leaves (Wadman-van Schravendijk and van Andel 1985, Zhang and Davies 1987), presumably due to the obstruction of transport of this substance out of the leaves (Setter and Brun 1981, Henson 1984, Jackson and Hall 1987). Despite the small changes in leaf ABA concentration during waterlogging in the present experiment, the depression of Pn and stomatal closure were pronounced following 5 days of submergence. These are common responses to flooding as observed in other studies (Drew 1990, Van Der Moezel et al. 1989, Davies and Flore 1986c, Bradford 1983a). The depressions of photosynthesis and $CO_2$ diffusion not associated with ABA accumulation suggest that flooding stress did not induce synthesis of ABA in roots (Zhang and Davies 1986) or leaves (Jackson and Hall 1987), nor did it obstruct ABA translocation out of leaves in the two poplar clones.

Factors other than ABA (Munns and King 1988) may be involved in the interference of gas exchange processes in flooded poplars. One possibility might be the diminished supply of cytokinins to the leaves. According to Drew (1990), transfer and supply of growth promoting regulators, such as IAA, gibberillins and cytokinins, are all diminished

under flooding. Because cytokinins are primarily produced in the roots (Cornish and Radin 1990), it is highly likely that the synthesis of cytokinin in flooded poplar roots was greatly decreased, causing significant reduction of the amount that was translocated. As one of the antagonists of ABA, cytokinin was reportedly able to reverse the effects of ABA when fed into the transpirational stream (Blackman and Davies 1985). The role of cytokinin is possibly to regulate the sensitivity of stomata to ABA concentrations (Cornish and Radin 1990). If cytokinins were present in the leaves, ABA concentrations had to be 10-fold higher to initiate stomatal response (Radin and Hendrix 1988). Therefore, it is conceivable that even if ABA production was not increased by flooding, the existing concentrations of ABA might be sufficient to induce stomatal closure and depression of photosynthesis because of a diminished supply of cytokinins from the flooded roots, possibly by means of redistribution of ABA between the apoplastic and chloroplastic pools (Radin and Hendrix 1988). Another possibility could be reduced root growth as oxygen was depleted, weakening sink strength and causing diminished transport of assimilates (Bradford and Hsiao 1982).

The observation that flooded Eugenei with high N in the soil solution was able to maintain comparable Pn and g to well-drained controls in this study led to the speculation that soil N status may alter the sensitivity of stomata through the regulation of cytokinin production. Eugenei has

been shown more responsive to high soil fertility than Tristis (Chapter 3). Low N supply inhibited cytokinin production (Drew 1990). Eugenei might be able to efficiently utilize an ample N supply to stimulate cytokinin synthesis in the flooded roots and transfer it to the leaves. Cytokinins can act as an antagonist to desensitize stomata by preventing the alteration of cytokinin to ABA ratios, thus maintaining a normal physiology. Low N supply, however, did not enhance ABA production in the two clones investigated, in disagreement with the suggestion by Drew (1990). It could also be possible that low N may alter the partitioning of ABA among the active and inactive pools such that the release of ABA into the apoplastic region was enhanced (Radin and Hendrix 1988), and as a consequence, stomatal and photosynthetic changes were induced.

In contrast to flooding, progressive drought substantially induced ABA accumulation in the leaves, in agreement with many other studies that showed that soil water deficits stimulated ABA production (Zhang and Davies 1989 a&b, Neales et al. 1989, Harris et al. 1988). The depression of Pn and g by drought was closely associated with increasing ABA concentrations in the leaves, supporting strongly the contention that ABA accumulation under water deficits accounted for the physiological changes. However, it is not clear why leaves responded to water deficits with such high concentration of ABA (90-fold higher in Tristis and 360-fold higher in Eugenei than well-watered controls),

since a small amount of ABA released to the apoplastic region at the guard cell plasmalemma may be sufficient to induce stomatal closure (Creelman 1989). The high ABA buildup in poplars may exert other impacts in addition to causing reduced stomatal aperture, such as leaf senescence and abscission to reduce leaf area (Cornish and Radin 1990), one of the strategies to avoid desiccation in face of prolonged soil water deficiency (Zhang and Davies 1989 a&b). Abscisic acid may also directly interfere with the photosynthetic process (Chaves 1991), presumably by causing a lower affinity of the carboxylation enzyme to its substrate (Chaves 1991). The elevation of $c_i$ as stomata tended to close during the first soil drying cycle probably suggested that a non-stomatal inhibition of photosynthesis was also associated with the high amounts of ABA induced by water deficits. Such elevation of $c_i$ diminished during the second drought cycle, conceivably an indication of photosynthetic acclimation. Instead, the restricted $CO_2$ diffusion due to stomatal closure that drives down $c_i$ below saturation for Pn (Cornish and Radin 1990) became a dominant factor responsible for the depression of Pn in Eugenei, but not in Tristis.

The massive buildup of ABA in the leaves occurred only at the first extreme drought, probably indicating that water deficits imposed a shock to those plants that had never experienced a serious drought. With the onset of another drought, plants did show a certain degree of acclimation,

such that ABA accumulation was substantially reduced, while Pn, and g were not greatly affected. In particular, low N tended to prevent the accumulation of ABA during the second cycle of drought, whereas high-N plants responded to soil drying by accumulating a large quantity of ABA in the leaves. This finding seems opposite to the observation that low N supply caused leaves to initiate ABA accumulation at a higher soil water potential than high N, while concentration of ABA needed to obtain the same magnitude of stomatal closure in high N plants was 10 to 20 times greater (Radin et al. 1982, Radin 1984). This discrepancy might be the consequences of the different size of plants that received different N regimes, which could cause varied rates of water depletion. As a result, high-N plants might experience greater water deficits than their low-N counterparts (Morgan 1984, Walters and Reich 1989). However, it is uncertain how much this difference in plant size attributed to this discrepancy and whether other factors might be involved.

The two clones displayed apparent variations in their ability to recover from drought stress. Tristis gained full and quick recovery once re-watered, whereas Eugenei showed a slow relief from drought, which was associated with higher residual ABA. Progressive drought resulted in an additive synthesis of ABA. Once the drying process was terminated, the rate of ABA synthesis would decline and the accumulated ABA turned over, converting it to phaseic acid (PA) until pre-stress levels were approached (Cornish and Radin 1990).

It is conceivable, therefore, that Eugenei may possess a system that prevents the quick turnover of ABA to PA. However, exact mechanism(s) of Eugenei vs. Tristis's ABA physiology remains unknown.

Photosynthesis, g, and E showed continuous response to increased ABA in both clones. However, the sensitivity of Pn, g, and E to increased ABA concentration differed in the clones. Tristis showed immediate declines of Pn, g, and E as ABA levels increased, indicating a high sensitivity. On the other hand, Eugenei was tolerant of an increase of ABA levels up to 5- to 10-fold that in Tristis. This insensitive response to ABA accumulation by Eugenei may explain the loose stomatal control over water loss in this clone, leading to the higher leaf water deficits, observed by Mazzoleni and Dickmann (1988). Moreover, not only was Eugenei relatively insensitive to ABA increases, but it continued to accumulate ABA to a much higher concentration than in Tristis. These two features may explain the earlier senescence and abscission of Eugenei leaves under prolonged drought. On the other hand, the sensitivity of stomata to ABA accumulation in Tristis could enable plants of this clone to sense and regulate water consumption, thus avoiding desiccation in a prolonged droughty condition. However, the conservative strategy of water loss in Tristis is at the expense of reduced $CO_2$ fixation, leading to smaller biomass production than in Eugenei (Pregitzer et al. 1990).

Increase of $c_i$ was also associated with ABA accumulation in Tristis, whereas $c_i$ remained unaltered in Eugenei. As $c_i$ can reflect photosynthetic activity at the chloroplast level, it can be speculated that non-stomatal limitations to Pn contribute predominantly to the depression of Pn during drought in Tristis, whereas stomatal limitations were a major constraint to Pn in Eugenei.

It is concluded from this study that flooding-induced modifications in gas exchange are not mediated by the accumulation of ABA in leaves, but rather through an imbalance in growth regulators or/and partitioning of ABA among active and inactive pools. Soil-water deficiency induced responses in gas exchange are mediated by ABA accumulation in leaves. High N stimulates ABA synthesis and accumulation in leaves, which accounts for the sensitive physiological responses in the face of drought, contradicting the finding that low-N plants are more sensitive to the decline of soil moisture in initiating ABA production (Radin et al. 1982, Radin et al. 1985, Cornish and Radin 1990).

Table 4.1 ABA accumulations (ng g $dw^{-1}$ ± se) induced by water deficits during the first water-withholding phase.

| Cycle | Day | Flood | | Control | | Drought | |
|---|---|---|---|---|---|---|---|
| | | Tristis | Eugenei | Tristis | Eugenei | Tristis | Eugenei |
| 1 | 1 | 9.94±1.70 | 11.20±3.58 | 8.26±0.92 | 8.10±1.01 | 9.88±1.88 | 19.03±4.16 |
| | 5 | 61.60±32.4 | 39.63±11.0 | 5.62±1.70 | 16.01±4.55 | 21.76±6.06 | 50.86±15.2 |
| | 9 | 17.96±8.97 | 112.56±29.7 | 26.48±6.71 | 22.76±3.98 | 2289.02±616.84 | 8266.8±1353.7 |
| 2 | 1 | 13.66±3.21 | 15.72±3.48 | 8.91±1.09 | 20.85±4.18 | 17.56±2.54 | 410.73±307.3 |
| | 5 | 29.64±7.81 | 5.13±1.00 | 11.78±3.52 | 20.67±6.18 | 10.25±1.83 | 49.53±13.47 |
| | 9 | 5.16±1.85 | 12.33±5.81 | 6.01±1.90 | 12.79±8.07 | 81.27±29.3 | 360.90±99.33 |

Table 4.2 ABA accumulations (ng g $dw^{-1}$ ± se) induced by N fertilization during the first water withholding phase.

| Cycle | Day | Tristis | | Eugenei | |
|---|---|---|---|---|---|
| | | Low N | High N | Low N | High N |
| 1 | 1 | 9.23±1.24 | 9.49±1.29 | 8.17±0.80 | 17.75±3.63 |
| | 5 | 16.19±4.53 | 43.14±22.2 | 16.54±6.30 | 55.33±10.3 |
| | 9 | 371.94±188.6 | 1183.70±29.7 | 2177.73±758 | 3423.70±1388.6 |
| 2 | 1 | 11.86±2.28 | 14.89±1.94 | 12.73±1.18 | 285.47±206.4 |
| | 5 | 14.56±4.09 | 19.89±4.96 | 8.65±2.05 | 41.57±9.94 |
| | 9 | 4.35±1.62 | 57.28±20.93 | 28.86±8.43 | 228.49±78.93 |

Table 4.3 Clonal difference in ABA levels (ng g $dw^{-1}$ ± se) after three days of interruption from water stress.

| Clones | Flood | Control | Drought |
|---|---|---|---|
| Tristis | 19.15 ± 4.27 | 13.88 ± 3.14 | 26.47 ± 4.18 |
| Eugenei | 32.47 ± 7.33 | 38.26 ± 5.77 | 150.17 ± 33.89 |

Table 4.4 ABA (ng g $dw^{-1}$ ± se) response 9 days following resumption of water stress in clones Trsitis (T) and eugenei (E).

| N level | Flood | | Control | | Drought | |
|---|---|---|---|---|---|---|
| | T | E | T | E | T | E |
| Low | 18.0±6.61 | 27.6±4.60 | 5.7±0.64 | 4.1±0.46 | 172.4±75.12 | 416.1±131.70 |
| High | 12.5±4.66 | 10.8±1.58 | 29.7±4.74 | 23.0±2.04 | 983.7±96.87 | 1262.9±158.17 |

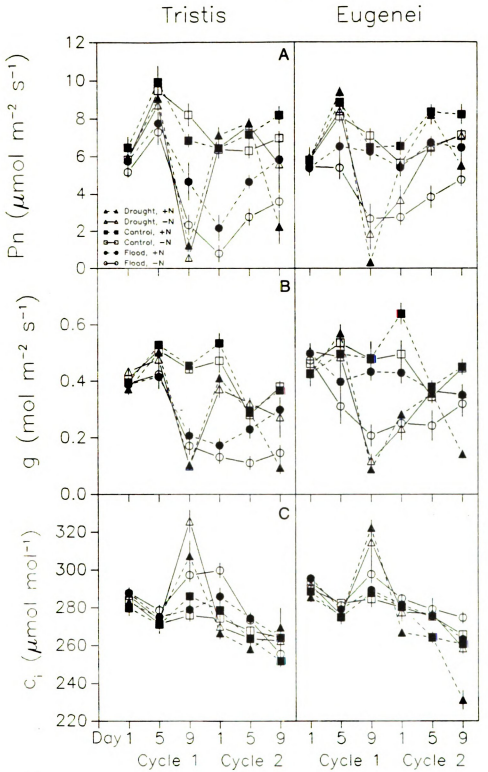


**Figure 4.1** **Effects of water and N regimes on (A) photosynthesis, (B) stomatal conductance, and (C) internal $CO_2$ concentration during the first two cycles of water stress. Vertical lines represent standard error of means.**

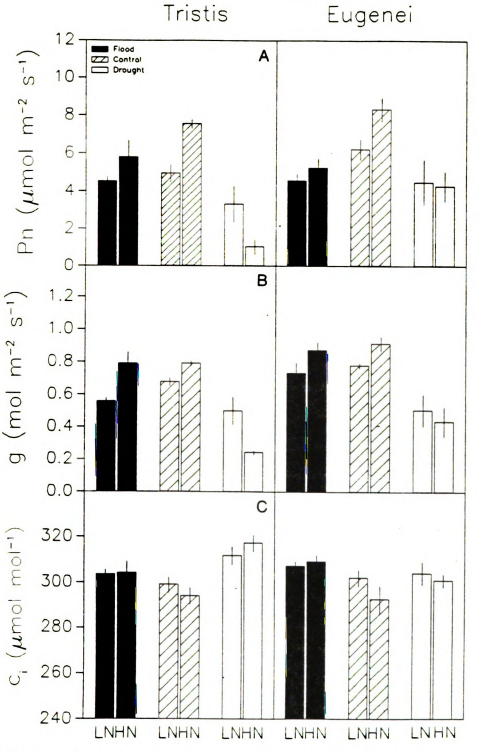


**Figure 4.2** **Photosynthesis (A), stomatal conductance (B), and internal $CO_2$ concentration (C) as affected by water and N regimes 9 days after resumption of a third flooding/drought cycle. LN: no supplemental N; HN: 1.5 g N added. Vertical lines on top of each bar represent standard error of the means.**

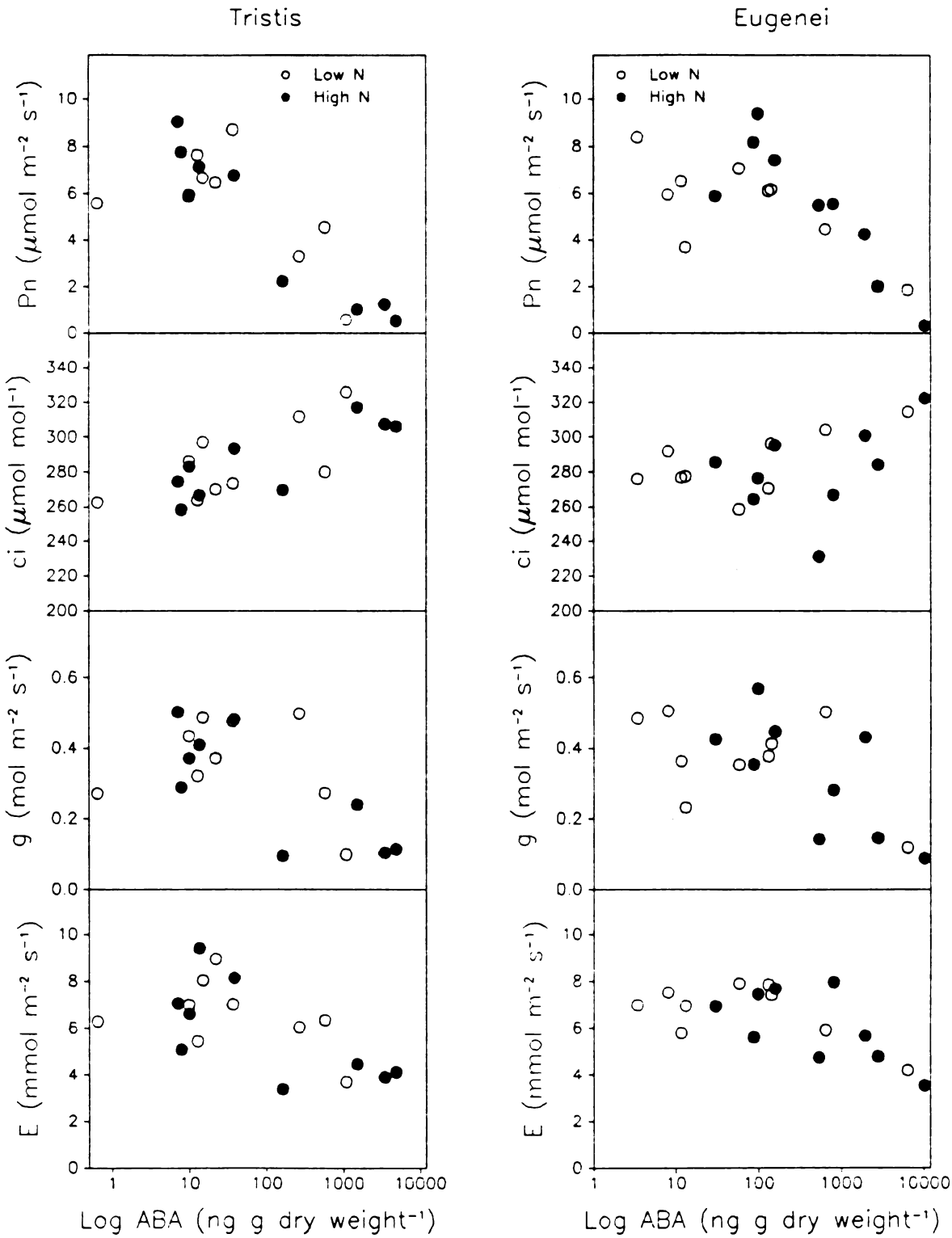


Figure 4.3. Relationships between ABA and gas exchange variables in Tristis and Eugenei. Each data point represents one plant leaf sample.

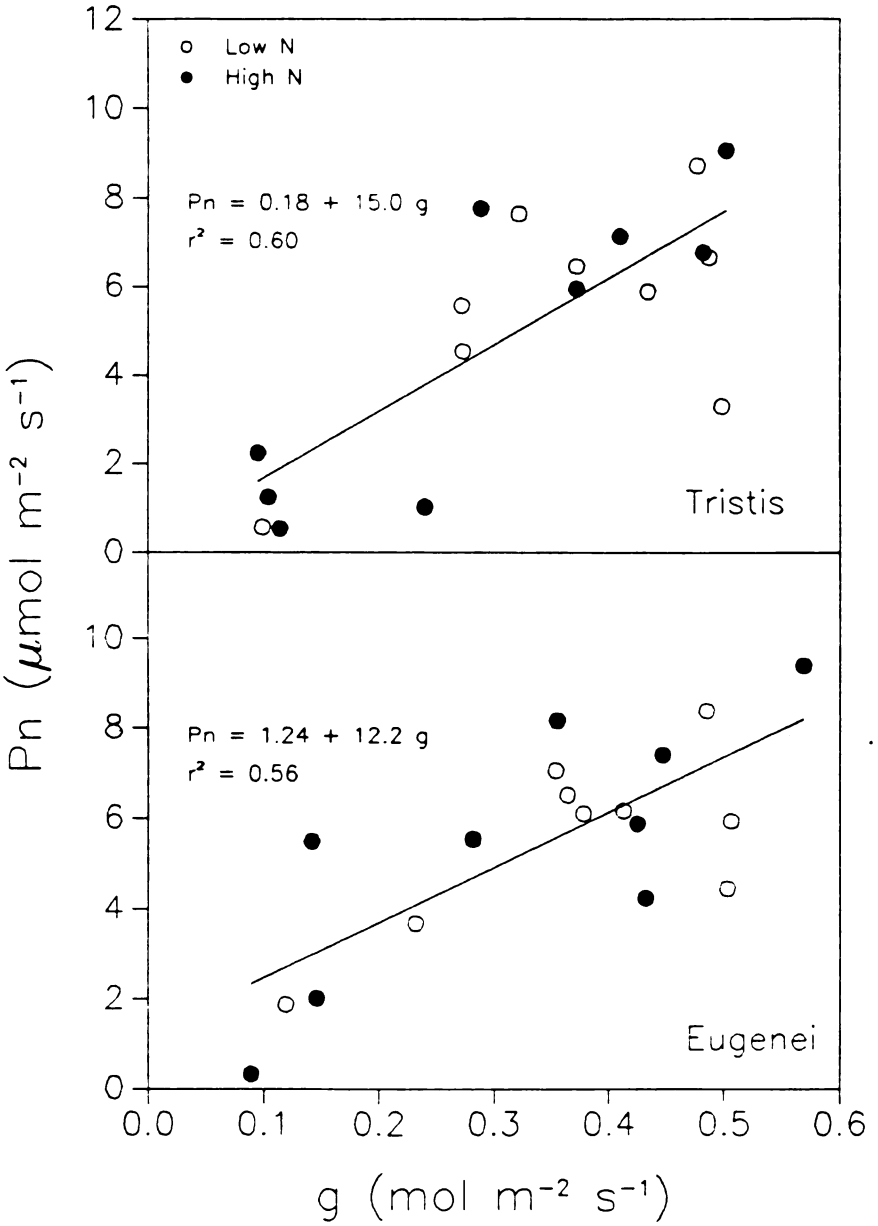


Figure 4.4 Relationship of Pn and g during progressive drought in Tristis and Eugenei.

# CHAPTER V

# CONCLUSIONS

Both Tristis and Eugenei are flooding resistant clones. During a short-term flooding (days), photosynthetic activity was maintained in spite of partial stomatal closure. As flooding lengthened (weeks), photosynthetic capacity was significantly decreased. However, the declines in photosynthesis were reversed with the emergence of adventitious rooting from the submerged stems, a characteristic of flooding resistance. High-N treated plants under flooding showed reversal of the negative effects induced by soil oxygen deficiency, whereas N-deficient plants displayed no sign of recovery throughout the flooding period. The declines of stomatal conductance and photosynthesis under flooding were not associated with an increase of ABA concentration in the leaves, although ABA concentration varied 2- to 3-fold.

Under minimum soil water deficits, additions of N generated positive effects on photosynthesis, in association with high leaf N and chlorophyll contents. On the contrary, as soil progressively dried, high-N plants were severely stressed, showing a drastic decrease of photosynthesis. High N supply imposed a stronger stimulation of ABA

synthesis than the low-N regime during soil drying. Experience of a second drought immediately after re-watering substantially improved the capacity of plants to sustain photosynthesis, whereas a period of drought interruption appeared to increase the sensitivity of high-N plants to soil water deficiency as another drought commenced.

Mild drought did not reduce photosynthesis and stomatal conductance, in correspondence to low leaf ABA concentrations. As soil further dried, photosynthesis was dramatically reduced and stomatal closure occurred in both clones, in association with a massive buildup of ABA in the leaves. This is strong evidence that ABA is responsible for stomatal closure during progressive drought and also implicates direct involvement of ABA in the depression of photosynthesis.

Droughted Tristis plants gained full and quick recovery upon relief from drought, whereas Eugenei showed a slower return. These responses could be explained by the quick drop of leaf ABA to pre-stressed levels in Tristis, whereas significant amounts of ABA were retained in Eugenei.

Compared to Tristis, Eugenei was insensitive to initial increases of ABA. Gas exchange was affected only as ABA accumulated over 100 ng g $dw^{-1}$. Tristis, on the other hand, was sensitive to increases of ABA concentration, starting to show declines of photosynthesis and stomatal conductance when leaf ABA concentration was 10-fold lower than that required in Eugenei leaves. With stomata insensitive to ABA

increase and a higher amount of ABA present, Eugenei will be disadvantaged in the face of prolonged drought, displaying leaf senescence and abscission earlier. Tristis, on the other hand, could resist a longer period of drought through its tight stomatal control over water loss.

Tristis sustained optimal growth over a broader range of soil moisture levels than Eugenei, but it was vulnerable to high N availability as soil dried. Eugenei, on the other hand, grew well under a wider availability of N, but it had a more strict requirement for soil moisture than Tristis.

In comparison to low-N plants, sufficient N supply led to more above-ground biomass accumulation, thus a higher shoot/root ratio. Flooding also resulted in a high shoot/root ratio, but this was primarily because of a substantial reduction of root growth.

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# APPENDIX A

## Appendix A

### The Manipulation of soil moisture levels in pots

The various moisture levels in this study are controlled with a dry-down-recharge cycle technique. That is, once the moisture levels in the pots drop to a certain category, re-watering will be called for to bring the moisture back to field capacity. Obviously, a milder drought stress takes shorter time to go through one cycle, whereas more severe stress takes longer.

The determination of the timing of re-watering is simply monitored by weighing the pots. Since the amount of dry soil in each pot is known, and since the weight of each pot at field capacity is known, too, before planting, the weight of each plant can be estimated by subtracting total weight of each pot before planting from the current weight of each pot. The amount of water in each pot at certain soil moisture content(SMC) can be calculated with the formula: Amount of Water = RWC * weight of dry soil. The total weight of each pot at current can then be estimated by summing the amount of water, weight of pot, weight of dry soil, and weight of the plant. Since various SMC can be referred to corresponding water potential through a moisture

retention curve, the drought stress imposed can be readily quantified.

In this experiment, soil matric potential of -0.02, -0.05, -0.1, or -0.5 MPa means SMC of 14.2, 8.2, 7.9, or 3.8%, respectively, according to the moisture retention curve developed. The desired water potential of each pot can therefore be provided by monitoring the change of total weight of each pot. For example, if a pot weighed 23.23 kg at field capacity before planting, pot weighs 0.37 kg, dry soil weighed 18.25 kg, and if the current pot weighs 24.5 kg with a plant at field capacity, the approximate weight of the plant is 24.5 - 23.23 = 1.27 kg. As the plant transpires, the pot weighs less. At soil water potential of -0.02 MPa (SMC is 14.2%), the total weight of the pot can be estimated using the formula: total weight = 0.142 * 18.25 + 18.25 + 0.37 + 1.27 = 22.48 kg. At -0.05 MPa (SMC is 8.2%), total weight = 0.082 * 18.25 + 18.25 + 0.37 + 1.27 = 21.39 kg. At -0.1 MPa (SMC is 7.9%), total weight = 21.33 kg. At -0.5 MPa (SMC is 3.8%), total weight = 20.58. The total weight at a certain soil water potential can then serve as the time point when re-watering will be called for.

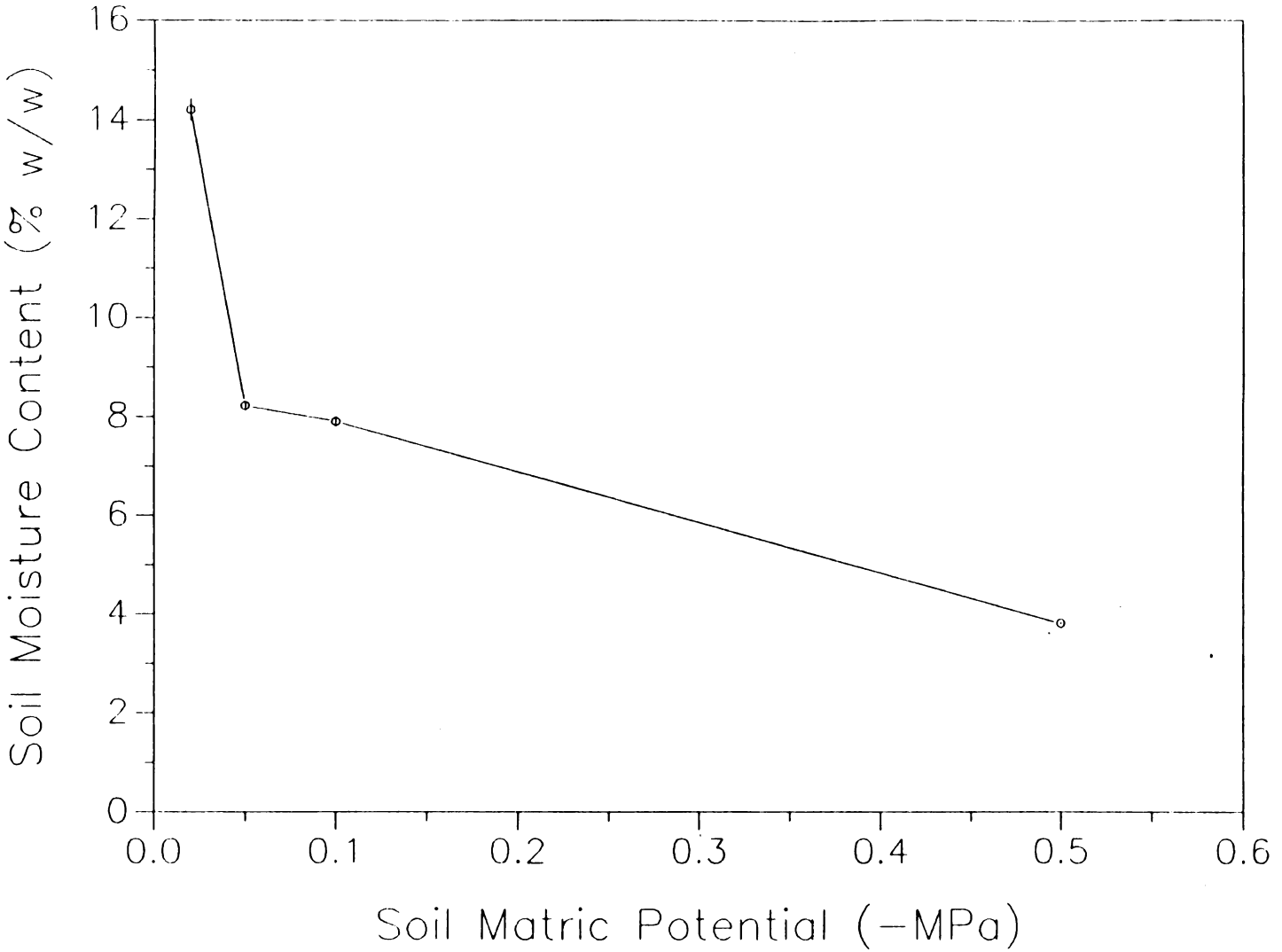


Figure A.1 Soil moisture retention curve. Each data point is the means of 7 to 14 observations.

# APPENDIX B

Table B.1. Means ± standard error of gas exchange variables following three repeated droughts during 1989 experiment.

| Soil matric potential (-MPa) | Soil N level | Pn (μmol/m2/s) | g (mol/m2/s) | E (mmol/m2/s) | ci (μmol/mol) | WUE (μmol/mmol) |
|---|---|---|---|---|---|---|
| | | ----------Tristis---------- | | | | |
| 0 | -N | 2.63±1.10 | 0.13±0.02 | 2.92±0.41 | 307± 7.9 | 0.83±0.17 |
| 0 | +1.5g | 4.29±1.59 | 0.25±0.03 | 5.13±0.33 | 302±12.3 | 0.83±0.26 |
| 0 | +3g | 3.54±1.03 | 0.21±0.02 | 4.08±0.40 | 303±10.4 | 0.83±0.17 |
| 0.02 | -N | 3.95±1.09 | 0.18±0.02 | 4.18±0.34 | 293±10.9 | 0.91±0.20 |
| 0.02 | +1.5g | 4.88±1.33 | 0.22±0.02 | 4.56±0.49 | 293± 9.3 | 0.99±0.19 |
| 0.02 | +3g | 4.77±1.47 | 0.22±0.02 | 4.93±0.31 | 291±12.2 | 0.97±0.26 |
| 0.05 | -N | 4.02±0.85 | 0.18±0.01 | 4.35±0.19 | 297±10.0 | 0.84±0.15 |
| 0.05 | +1.5g | 5.03±0.76 | 0.17±0.05 | 4.16±0.41 | 279±18.4 | 1.20±0.26 |
| 0.05 | +3g | 4.62±1.19 | 0.16±0.02 | 3.90±0.41 | 277±16.1 | 1.21±0.24 |
| 0.1 | -N | 2.94±0.78 | 0.16±0.03 | 4.10±0.57 | 301±11.9 | 0.67±0.19 |
| 0.1 | +1.5g | 2.97±1.02 | 0.08±0.04 | 2.40±0.53 | 269±11.3 | 1.23±0.20 |
| 0.1 | +3g | 2.95±0.95 | 0.10±0.00 | 2.91±0.19 | 277±15.1 | 1.01±0.27 |
| 0.5 | -N | 3.73±0.97 | 0.17±0.02 | 3.98±0.41 | 295±12.0 | 0.91±0.22 |
| 0.5 | +1.5g | 2.41±0.61 | 0.08±0.01 | 2.36±0.32 | 292± 9.5 | 0.91±0.16 |
| 0.5 | +3g | 3.17±0.81 | 0.09±0.00 | 2.55±0.12 | 273±11.5 | 1.20±0.24 |
| | | ----------Eugenei---------- | | | | |
| 0 | -N | 3.83±1.04 | 0.20±0.01 | 4.61±0.24 | 300±18.7 | 0.77±0.28 |
| 0 | +1.5g | 5.12±1.28 | 0.27±0.03 | 5.20±0.32 | 298±13.9 | 0.92±0.25 |
| 0 | +3g | 3.55±1.37 | 0.21±0.03 | 4.98±0.26 | 301±16.7 | 0.67±0.27 |
| 0.02 | -N | 3.50±0.86 | 0.22±0.01 | 4.80±0.32 | 303± 9.6 | 0.70±0.16 |
| 0.02 | +1.5g | 4.14±1.61 | 0.25±0.02 | 5.38±0.26 | 304±14.7 | 0.71±0.30 |
| 0.02 | +3g | 4.36±1.30 | 0.24±0.00 | 4.85±0.20 | 301±13.0 | 0.83±0.26 |
| 0.05 | -N | 3.63±1.26 | 0.24±0.02 | 5.23±0.37 | 307±11.1 | 0.69±0.21 |
| 0.05 | +1.5g | 3.88±0.92 | 0.18±0.02 | 3.76±0.28 | 281±14.6 | 1.11±0.21 |
| 0.05 | +3g | 4.53±0.93 | 0.15±0.01 | 3.55±0.27 | 283±16.0 | 1.20±0.24 |
| 0.1 | -N | 2.65±0.81 | 0.18±0.01 | 3.96±0.30 | 311± 9.9 | 0.65±0.15 |
| 0.1 | +1.5g | 3.56±0.55 | 0.18±0.01 | 3.96±0.20 | 297±11.2 | 0.88±0.19 |
| 0.1 | +3g | 3.01±0.66 | 0.09±0.01 | 2.83±0.23 | 283±14.8 | 0.97±0.22 |
| 0.5 | -N | 1.82±1.12 | 0.20±0.04 | 4.38±0.53 | 321±12.1 | 0.39±0.19 |
| 0.5 | +1.5g | 2.36±0.79 | 0.08±0.01 | 2.38±0.28 | 289±11.4 | 0.94±0.24 |
| 0.5 | +3g | 2.64±0.81 | 0.09±0.01 | 2.68±0.18 | 289±14.6 | 0.93±0.26 |

Table B.2 Means ± standard deviation of leaf and stem biomass after 48 days of water stress in 1989 experiment.

| Clone | Soil matric potential(-MPa) | Soil N level | Sample size | Leaf (g) | Stem (g) |
|---|---|---|---|---|---|
| Tristis | 0 | -N | 3 | 16.2 ± 2.2 | 14.3 ± 7.3 |
| | 0 | +1.5g | 3 | 20.8 ± 4.4 | 16.3 ± 9.1 |
| | 0 | +3g | 3 | 18.7 ± 3.1 | 12.5 ± 6.6 |
| | 0.02 | -N | 3 | 13.3 ± 1.7 | 17.1 ± 3.0 |
| | 0.02 | +1.5g | 3 | 20.4 ± 3.0 | 28.2 ± 2.8 |
| | 0.02 | +3g | 3 | 12.7 ± 6.5 | 16.5 ± 8.4 |
| | 0.05 | -N | 3 | 9.7 ± 0.4 | 12.0 ± 0.8 |
| | 0.05 | +1.5g | 3 | 11.7 ± 6.1 | 14.8 ± 8.4 |
| | 0.05 | +3g | 3 | 14.1 ± 1.4 | 19.4 ± 3.0 |
| | 0.1 | -N | 3 | 16.1 ± 1.4 | 22.4 ± 1.7 |
| | 0.1 | +1.5g | 3 | 12.2 ± 6.1 | 21.8 ± 1.7 |
| | 0.1 | +3g | 3 | 17.2 ± 1.1 | 20.1 ± 0.7 |
| | 0.5 | -N | 3 | 11.1 ± 1.8 | 11.9 ± 0.4 |
| | 0.5 | +1.5g | 3 | 14.8 ± 0.7 | 17.6 ± 0.2 |
| | 0.5 | +3g | 3 | 13.2 ± 0.8 | 15.5 ± 0.6 |
| Eugenei | 0 | -N | 3 | 17.0 ± 0.1 | 22.1 ± 1.0 |
| | 0 | +1.5g | 3 | 25.7 ± 1.5 | 29.4 ± 2.2 |
| | 0 | +3g | 3 | 22.8 ± 1.6 | 25.5 ± 3.4 |
| | 0.02 | -N | 3 | 13.3 ± 0.7 | 13.9 ± 0.9 |
| | 0.02 | +1.5g | 3 | 25.5 ± 1.7 | 18.8 ± 9.6 |
| | 0.02 | +3g | 3 | 24.7 ± 1.1 | 16.8 ± 8.4 |
| | 0.05 | -N | 3 | 15.1 ± 1.4 | 9.8 ± 4.9 |
| | 0.05 | +1.5g | 3 | 24.3 ± 2.5 | 22.8 ± 1.1 |
| | 0.05 | +3g | 3 | 13.1 ± 6.8 | 14.6 ± 7.5 |
| | 0.1 | -N | 3 | 8.5 ± 4.2 | 14.9 ± 1.9 |
| | 0.1 | +1.5g | 3 | 20.0 ± 2.3 | 17.3 ± 0.9 |
| | 0.1 | +3g | 3 | 19.3 ± 2.6 | 19.9 ± 1.0 |
| | 0.5 | -N | 3 | 11.4 ± 0.6 | 11.7 ± 0.7 |
| | 0.5 | +1.5g | 3 | 11.9 ± 6.4 | 15.6 ± 1.0 |
| | 0.5 | +3g | 3 | 15.9 ± 0.8 | 15.8 ± 0.5 |

Table B.3 Means of morphological and growth variables at the end of 1990 experiment.

| Clone | Water level | N | No. of leaves | Leaf Area (cm2) | Root/shoot ratio | SLW (g/m2 dw) | Chl (mg/g dw) | Leaf N (g/m2) | Height (cm) | Leaf biomass (g) | Stem biomass (g) | Root biomass (g) | Whole plant biomass (g) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Tristis | Flooded | -N | 31±1.2 | 65.8±4.1 | 0.23±0.06 | 75.4±5.0 | 4.0±0.3 | 1.09±0.006 | 120±3.6 | 8.0±1.6 | 14.4±1.6 | 4.9±0.9 | 27.3±2.5 |
| | | +N | 34±0.9 | 91.2±7.9 | 0.18±0.02 | 61.5±2.6 | 6.6±1.1 | 1.12±0.002 | 123±7.6 | 9.3±3.0 | 13.4±2.1 | 3.9±0.4 | 26.7±5.4 |
| | Watered | -N | 29±2.0 | 66.4±6.7 | 1.30±0.15 | 62.9±1.3 | 3.1±0.5 | 1.03±0.002 | 121±5.5 | 8.8±1.0 | 12.2±0.8 | 26.8±2.5 | 47.8±3.0 |
| | | +N | 47±1.5 | 138.0±8.6 | 0.50±0.11 | 51.0±0.7 | 7.9±0.9 | 1.61±0.001 | 158±3.9 | 25.9±0.5 | 30.8±2.1 | 28.5±6.6 | 85.1±7.7 |
| | Drought | -N | 33±3.2 | 87.7±9.3 | 0.72±0.20 | 55.5±1.4 | 6.1±0.8 | 1.14±0.003 | 123±5.9 | 9.2±1.9 | 14.2±0.7 | 16.1±3.9 | 39.6±5.9 |
| | | +N | 40±0.3 | 111.6±4.1 | 0.47±0.09 | 49.0±1.0 | 8.4±1.0 | 1.47±0.001 | 137±1.5 | 16.6±1.4 | 20.1±0.8 | 17.2±3.7 | 54.0±4.9 |
| Eugenei | Flooded | -N | 28±1.2 | 73.7±3.1 | 0.30±0.08 | 61.8±3.5 | 3.7±0.7 | 0.94±0.004 | 109±3.9 | 7.6±1.3 | 10.1±1.2 | 5.3±1.4 | 23.0±3.3 |
| | | +N | 37±1.2 | 116.4±5.8 | 0.25±0.06 | 57.0±1.4 | 7.0±0.7 | 1.10±0.001 | 132±2.3 | 16.1±0.4 | 17.4±1.4 | 8.2±1.9 | 41.7±2.7 |
| | Watered | -N | 28±0.7 | 72.1±5.1 | 1.18±0.30 | 53.5±1.1 | 4.7±1.0 | 1.10±0.002 | 111±3.6 | 7.4±0.1 | 9.1±0.6 | 19.4±4.6 | 35.9±4.4 |
| | | +N | 44±1.9 | 151.6±3.4 | 0.46±0.05 | 47.9±0.7 | 8.6±0.7 | 1.43±0.001 | 151±3.1 | 25.8±0.2 | 23.4±1.7 | 22.9±3.0 | 72.1±4.5 |
| | Drought | -N | 30±1.9 | 71.9±6.4 | 0.76±0.20 | 55.8±1.0 | 5.8±0.7 | 1.20±0.001 | 108±3.8 | 8.9±1.4 | 9.5±1.1 | 13.2±1.6 | 31.7±0.8 |
| | | +N | 36±1.5 | 109.4±5.5 | 0.39±0.07 | 51.3±1.4 | 8.2±0.5 | 1.53±0.001 | 119±5.2 | 15.5±0.8 | 14.2±1.3 | 11.8±2.5 | 41.5±3.9 |