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Effects of fertilization on hybrid and native poplar clones

by

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Abstract

Trees of four poplar clones were grown for two years on an agricultural field (54°N, 112°W, elevation 620 m) and fertilized at planting with 27.9 g of nitrogen (N) or 83.4 g of NPKSCuZn (NPK+). Growth response to the fertilizer treatments varied between treatments and clones throughout the experiment. After two years height, basal diameter and leaf area differed significantly with control<N<NPK+.

Leaf N (% dry weight) was significantly affected by fertilizer treatment and clone in both years with an overall trend of leaf N increasing from control to N to NPK+ treatments. Generally leaf P and K (% dry weight) were also significantly affected by fertilizer treatment and clone. Comparison of nutrient levels in the harvested whole trees versus sample tree leaves collected from the actively growing terminal leaders showed significant differences in several key nutrients.

Overall the results indicate that nutrients supplied by the fertilizer treatments were taken up by the target trees, increasing leaf tissue levels resulting in increased leaf area and mass and overall biomass accumulation.

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Finally, thank you God for your wonderful creation, its complexity and splendor we can never fully understand nor truly appreciate. Amen!

For there is hope of a tree, if it be cut down, that it will sprout again, and that the tender branch thereof will not cease.

Job 14:7

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1.0 Introduction

1.1 Background

Poplars (*Populus* spp.), are fast growing trees widely used for timber, pulp and paper, and have high potential as a source of biomass energy (McLennan and Mamias 1992, Perry *et al.* 2001). With increases in demand for fiber, companies have been pursuing tree improvement and plantation programs with the intent to secure fiber supplies for the future. The increased need for fiber has lead to a growing interest in intensively managed plantation programs moving forward with hybrid poplars, which are seen by many growers as the tree of choice (Dickmann *et al.* 2001). In order for hybrid poplar plantation programs to be economically viable, they have to produce very large volumes of fiber in a short time period compared to natural forests (eg. rotation times of 20-30 versus 70 years). Since hybrid poplars are known to have growth rates that are among the highest of temperate deciduous trees (Larcher 1969), nutrient management practices have proven to be crucial in the success of hybrid poplar programs in some regions (Dickmann *et al.* 2001). With the potential to optimize the nutrient conditions of the sites where these trees are planted, one would expect to be able to increase growth rates above unmanaged trees of similar species. Consequently, information on limitations to growth imposed by natural nutrient regimes, and the nutrient requirements of different species and clones is of great importance.

Nitrogen (N) is the most common gas found in air (~79%) (Brady 1974) and is the nutrient that all plants require in the largest quantity (Dickmann and Stuart 1983). However, despite its apparent abundance and importance, N is generally recognized as the most growth-limiting nutrient in the boreal forest (Wollum and Davey 1975) and, to a larger degree, in plant communities world-wide (Brady 1974). This limitation can greatly affect the productivity of forest tree species (Dickson 1989; Oren *et al.* 2001). Susceptibility to N deficiency stress can be reduced by traditional approaches such as fertilization to supplement already available nutrients on the site (Burdett *et al.* 1984). Nitrogen fertilization is increasingly used to improve harvest yields and reduce rotation age in

managed forest tree plantations, particularly in the context of short-rotation woody crops such as hybrid poplars (Tuskan 1998; Sedjo 2001), however, little effort has been made to understand the implications of fertilizing intensively managed hybrid poplar crop trees in the prairie or boreal regions of western Canada. Hybrid poplar nutrient demands are high and nutrient additions often increase growth (Brown and van den Driessche 2002) by taking up and utilizing nutrients from the soil (Liu and Dickmann 1992). Need for fertilization of hybrid poplars is also accentuated by the apparent increased N requirements of hybrids, produced from breeding programs, having higher foliar N concentrations than their parents (Heilman and Fu Guang 1993, Hansen 1994).

1.2 Nitrogen Allocation in Plants

Poplars can acquire N from the soil as either ammonium (NH_4^+) or nitrate (NO_3^-) ions, however, they show a slight preference for NH_4^+ (Min *et al.* 1999, DesRochers *et al.* 2003). Ammonium is the useable form of N in plants, therefore, following being taken up by roots NO_3^- is reduced to NH_4^+, which can then be used to form amino acids (Raven *et al.* 1992). Amino acids then combine with carbohydrates to form organic molecules, such as glutamine, which is used to transport N from the roots to other parts of the plant. Organic N can then be passed on to other molecules and transformed into other amino acids or N-containing molecules required by the plant (Raven *et al.* 1992, Dickmann *et al.* 2001). The majority of nitrogen taken up by plants ends up in the leaves (Onoda *et al.* 2004) where 71 and 77% is found to be allocated to proteins in evergreen versus deciduous species, respectively (Takashima *et al.* 2004).

The vast majority of proteins found in leaves function in photosynthesis with about half of leaf nitrogen being allocated to photosynthetic proteins (Evans 1989; Evans and Seemann 1989; Onoda *et al.* 2004). Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) is the most abundant and important enzyme of photosynthesis (Evans 1989). RuBisCO constitutes ~50% of photosynthetic N, and requires a large portion of the overall N found in leaves (15-30%) (Evans and Seeman 1989). Contribution of leaf N to RuBisCO is a

determining factor of the overall photosynthetic productivity of a plant. RuBisCO is found in chloroplasts and has two major enzymatic roles; one in photosynthesis and the other in photorespiration. When absorbed, CO_2 levels in the leaf tissue are relatively high in comparison to O_2 levels, therefore, RuBisCO functions in photosynthetic carbon dioxide fixation by catalyzing the carboxylation of ribulose 1,5-bisphosphate (RuBP) to form 3-phosphoglycerate (PGA). This reaction is the first step in the Calvin cycle during photosynthesis. When O_2 levels are high as compared to CO_2 levels, RuBisCO fixes oxygen to RuBP to form only one molecule of PGA, which is used to form carbohydrates, and one molecule of phosphoglycolate, which is used in photorespiration (Raven *et al.* 1992).

Proteins found in cell walls can contribute up to 10% of the nitrogenous compounds found in the cell (Reiter 1998). These proteins carry out many important cellular functions. They include defence, growth, development, signalling, intercellular communication and environmental sensing, and as selective exchange interfaces (Showalter 1993).

Other important components of plants that require nitrogen are: (1) defensive compounds (Gleadon and Woodrow 2002), (2) nucleic acids (Chapin and Kedrowski 1983) and (3) free amino acids and lipids (Chapin *et al.* 1986). Defensive compounds such as alkaloids and cyanogenic glycoside can account for 0-5% of leaf nitrogen (Burns *et al.* 2002), nucleic acids require 10-15% of leaf nitrogen and free amino acids and lipids can total up to 5-10% of leaf nitrogen (Chapin *et al.* 1986).

1.3 Nitrogen use efficiency and photosynthetic nitrogen use efficiency

A general definition of nitrogen use efficiency (NUE) is the output of carbon fixed as plant tissue for every unit of nitrogen acquired from the soil (Pastor and Bridgham 1999). Similarly, photosynthetic nitrogen use efficiency (PNUE) is the ratio between the rate of photosynthesis, which is dependent on the proportion of nitrogen allocated to the photosynthetic apparatus, and the total nitrogen in the plant (leaf) (Poorter and Evans 1998). These two parameters are

very closely related as most of the nitrogen taken up by roots is transported to the leaves, where a large portion is allocated to photosynthetic compounds. Photosynthetic compounds, in turn, fix carbon, during the process of photosynthesis, which is used to form plant tissue. Therefore, NUE can be seen simply as a broader definition of PNUE. Makino *et al.* (1998) states that PNUE depends on three factors: (1) level of allocation of leaf nitrogen to RuBisCO, (2) the specific activity of RuBisCO, and (3) stomatal conductance.

It has been shown that species with a higher PNUE allocate more nitrogen to RuBisCO (Hikosaka *et al.* 1998). Lloyd *et al.* (1992) suggested that the amount of RuBisCO per unit of nitrogen in leaves is higher in species with higher PNUE. The reason for this relationship is the large amount of leaf organic nitrogen (up to 75%) present in the chloroplasts, with most of it in chlorophyll (Evans and Seamann 1989).

Photosynthetic nitrogen use efficiency has also been found to be dependent on specific leaf area (SLA), which is the ratio of leaf area to leaf weight, and is measured in $cm^2\ g^{-1}$. Differences in SLA in poplars show strong dependence on leaf nitrogen and phosphorous concentrations suggesting that high N and P fertility can be related to increased SLA and productivity (van den Driessche 2005). Poorter and Evans (1998) back this claim by stating that species with high SLA typically also have a high PNUE. They continue by saying that species with high levels of PNUE generally also have higher tissue water contents per unit dry mass, higher concentrations of nitrogen based on mass and lower concentrations of cell wall compounds per unit mass.

1.4 Nutrient cycling

Boreal forest trees obtain inorganic nutrients from a fast-turnover pool of nutrients retranslocated within the tree, and from a slow-turnover pool of nutrients cycled through the forest floor (Flanagan and Van Cleve 1983). Both evergreen and deciduous trees resorb (translocate) mineral nutrients from their leaves back into stems and roots during senescence, prior to leaf abscission (Luxmore *et al.* 1981; Nambiar and Fife 1991). Early season growth of many species is

supported by remobilization of stored nutrients before substantial root uptake occurs in the early spring, resulting in a close correlation between initial growth and existing nutrient reserves (Dong *et al.* 2001). Additional nutrients are recycled through leaf fall, decomposition and uptake (McLaughlin *et al.* 1987). Greater resorption of nutrients at senescence might be expected to increase long-term productivity of deciduous trees (Harvey and van den Driessche 1999).

In deciduous tree stands, as succession proceeds and leaf area levels stabilize following crown closure, ecosystem-level N cycling shifts increasingly toward internal recycling within the vegetation, rather than uptake from the soil (Van Cleve *et al.* 1983; Walker and Chapin 1986). Upon stabilization, as much as 50% of the required N for the stand can be translocated from the leaves into woody tissue prior to leaf drop, followed by retranslocation back up to the canopy in the next season to the newly expanding leaves (Bernier 1984). High efficiency in the ability to conserve and reuse nitrogen may be positively related to high growth rates in poplars. Consequently, selection of clones with different growth rates may reflect the extent of nutrient resorption (Pregitzer *et al.* 1990). Resorption rates will likewise affect the extent of nutrient demand from the soil and, depending on soil fertility, may influence the amount of fertilization required to alleviate nutrient shortages.

The timing of uptake of N may affect where it ends up being used in the tree. Studies have shown that N applied to hybrid poplars later in the season is more likely to be recycled within the tree than N applied early in the season (Millard 1995). Nitrogen taken up early in the growing season is transported to actively growing regions of the tree to contribute to increasing photosynthetic capacity and leaf expansion. Late season N is stored in forms and structures that allow it to be more mobile and easily accessible to satisfy requirements as they arise within the tree. A study by Dong *et al.* (2004) found that following cessation of active growth, most N taken up served as storage N instead of structural N. Consequently, an increased percentage of N taken up late in the season was remobilized for new growth in the following season.

Physiologically, different parts of the tree are used as storage organs for different nutrients. The root system is a major storage organ in deciduous trees (Millard 1995). In the genus *Populus*, roots are the main storage organ, but branches and stems are also important reservoirs for starch, sugars, lipids and proteins (Nguyen *et al.* 1990). Leaf proteins are degraded in September as senescence progresses, and leaf N is translocated to the large roots (>3mm diameter) where it is stored for the winter (Sauter *et al.* 1989). The stems, and in some clones, the branches, also represent reservoirs of N, principally in the form of protein (Sauter *et al.* 1989). A study of resorption in hybrid poplars completed by Pregitzer *et al.* (1990) found that the majority of whole tree N was stored in large, structural roots following leaf fall in late October or early November. At this time more than 50% of the whole-tree N was found in roots > 1mm in diameter. Depending on the clone they also calculated that at least 70-80% of the whole-tree N present late in the growing season was conserved within the tree as it entered dormancy. In a study looking at four *Populus trichocarpa* (T) x *Populus deltoides* (D) and TxTxD hybrids in the fall, N, P, K and Cu were resorbed, P less efficiently than N (Harvey and van den Driessche 1999). This may explain why some field studies have shown a strong growth response of poplars to P fertilization (Chapin *et al.* 1983).

There is evidence that stresses induced by either nutrient deficiencies or water shortage can affect levels of nutrient recycling and resorption in hybrid poplars (Harvey and van den Driessche 1999). In trees growing on sites with limited N, nutrient stress may cause yellowing of the older foliage and eventually these leaves may die and fall off if the nutrient stress persists. Yellowing is a typical response to N deficiency as earlier transition of leaves to senescence presumably allows N-limited plants to remobilize resources from the older leaves to support growth in developing regions of the tree (Cooke *et al.* 2005). Under drought conditions, loss of leaves due to moisture stress can affect levels of nutrient resorption (Pugnaire and Chapin 1992). A study looking at levels of resorption in hybrid poplars at varying drought intensities found that this was the main factor determining resorption levels (Harvey and van den Driessche 1999).

Under gradual drought with increasing intensity, the majority of N was resorbed to the stems and roots, the majority of P was resorbed back to the roots and K and Cu were not resorbed and remained in the leaves (Harvey and van den Driessche 1999). Under rapidly induced drought stress, the trees were not able to respond effectively and resorption levels for all nutrients were very low.

1.5 Poplar response to fertilizer

With nitrogen being the major nutrient limiting poplar productivity, additions of nitrogen by fertilization may prove to be beneficial (Heilman *et al.* 1996). Studies with hybrid poplars in coastal regions have shown that they are responsive to additions of nitrogen fertilizer in direct placement applications (van den Driessche 1999). Young poplar plantations often respond positively to fertilization with urea (Heilman 1990), and both ammonium nitrate (Coyne and Van Cleve 1977) and urea have been found to increase productivity in wild aspen stands (Yang 1991). Even though poplars can utilize both ammonium and nitrate, there tends to be a slight preference for ammonium (Min *et al.* 1999). Soil pH can affect the preference for ammonium (NH_4) versus nitrates (NO_3) (van den Driessche 1978, DesRochers *et al.* 2003). Soil pH also affects the availability of other nutrients. In acidic soils phosphorous (P) is adsorbed and is less available to plants than at higher pH, whereas manganese (Mn), boron (B), copper (Cu) and Zinc (Zn) are most available between pH 5 and 6.5 (Mengal and Kirkby 1982). The optimal range for poplar growth is pH 5.0-7.5 (van Oosten 2006).

On sites where available N is limited, N fertilization and foliar N are strongly related with foliar N generally increasing with increasing fertilization rate (Hansen *et al.* 1988). In some situations, nitrogen fertilization has also been shown to significantly increase leaf content of all elements, including those not supplied by fertilizer, indicating that nitrogen can be a limiting factor which can control the overall nutrient status of the plant (van den Driessche 1999a). With the potential for N to be the single most limiting nutrient restricting growth, fertilization with nitrogen alone can provide positive increases in growth. Many

studies using hybrid poplars have shown this type of response (Hansen *et al.* 1988, Hansen 1994, van den Driessche 1999, Brown and van den Driessche 2002). However, fertilization may have little effect if the site is relatively dry, or if other required nutrients are limited, or if the nutrients are not added at sufficient rates (Thomas *et al.* 2000). Soils that have high N fertility have shown little responsiveness to N fertilization (Heilman and Guang 1993).

1.6 Nutrient analysis

There are two main methods that can be used to determine nutrient status of plants. Nutrient status, and as a result fertilizer guidelines, can be based on either soil or plant-tissue nutrient content (Heilman 1993). Plant tissue nutrient content can be easily measured using foliar analysis and is often preferred over soil analysis for woody plants (Lavender 1970) and has a long history of use on *Populus* (White and Carter 1970; Blackmon and White 1972; Heilman 1985). The main reason that foliar analysis is preferred over soil analysis is the lack of a reliable diagnostic method that links soil fertility to hybrid poplar nutrient management as used in traditional agricultural crops (van Oosten 2006).

Use of foliar analysis to determine nutrient deficiencies in poplars should be done proactively as nutrient deficiencies often negatively impact yield without showing clear symptoms (van Oosten 2006). Generally, once a plant exhibits visual symptoms the deficiency is quite severe and, by this time, growth potential may already be compromised or lost. As a result, specific deficiencies are best determined through elemental analysis of recently matured foliage prior to development of deficiency symptoms (Thomas *et al.* 2000).

Timing of foliage collection for analysis is very important as concentrations of N fluctuate seasonally. If collection of tissue is done too early in the growing season, it may indicate exaggerated N concentrations as levels of N in younger stem and leaf tissues are higher than older tissues from the same trees (Cooke *et al.* 2005). A study by Dickmann and Gordon (1975) showed N levels in immature leaf tissue could be as high as 6% of leaf dry weight, which confirms that immature leaves can be powerful N sinks for growth later in the season. In

the Canadian prairie region, leaves collected for foliar analysis should be recently matured and collected in the last week in July to the first week in August (van Oosten 2006). At this time, the trees will be growing at their most rapid rates requiring the highest levels of nutrients to sustain growth, and in post-fertilized stands, they will have had adequate time to draw in any nutrients supplied by spring or early summer fertilizer applications.

Studies by Hansen (1994) have shown that foliar tissue N in the uppermost fully expanded leaves generally increase during the summer until mid-August when declining N levels in leaf tissue are common. It has also been shown that upon fertilization there can be significant increases in foliar tissue nutrients without a significant increase in growth indicating that original fertility levels were sufficient for good tree growth (Hansen 1994). This suggests that hybrid poplars can be luxury consumers when surplus N is available.

Within a species, photosynthetic capacity is largely determined by the amount of nitrogen found in leaves as there is a strong, positive correlation between photosynthetic capacity and the nitrogen content per unit leaf area (Takashima *et al.* 2004). Foliar N differs significantly by species and as a result by clone. Clones with *P. nigra* parentage had the highest foliar concentrations of N which were consistently above 3% leaf dry weight, while clones with *P. trichocarpa* or *P. maximowiczii* parentage ranged from 2.6 to 3.2% leaf dry weight (Hansen 1994). In a study looking at the clones Tristis (*P. tristis* x *P. balsamifera*) and Eugenei (*P. deltoides* x *P. nigra*), the concentration of N in mature leaves was consistently at or above 3% leaf dry weight when sampled in August (Pregitzer *et al.* 1990). Van den Driessche (1999) found that clones differed in their response to nutrients, as shown by relationships between maximum growth and leaf N and P concentrations and changes in other nutrients associated with fertilization.

Hansen (1992) has shown that in most cases plantations had foliar tissue N levels above 3% leaf dry weight, indicating that poplars are very effective in N uptake and as a result fertilization was not necessary. In cases when fertilization was required and nitrogen fertilizer was applied, the total quantity of N in the

canopy increased overall (Pregitzer *et al.* 1990). It is recommended that foliar N concentrations be maintained above 3% leaf dry weight to optimize hybrid poplar growth (Hansen *et al.* 1988). In mid-season, upper canopy leaves of 2.0 to 2.7% leaf dry weight may indicate N deficiency (Heilman 1993). Blackmon and White (1972) found that a foliage content level of 2% leaf dry weight was a minimum for growth in several species of poplars. Approximate values for foliar nutrient concentrations, other than N, that are seen as adequate for good growth, based on % leaf dry weight are P 0.33, K 1.51 and Ca 0.63 (Hansen 1994). Values for other important nutrients have not been as easy to determine or agree upon and therefore remain unavailable in the published literature.

1.7 Fertilization methods

Method of application can have an impact on the fertilizer utilization efficiency of the tree. Fertilizer utilization efficiency is the proportion of fertilizer applied that is taken up by a target crop or tree species. Generally, nitrogen fertilizer utilization efficiency is quite low in plantation trees and rarely exceeds 20% (Baker *et al.* 1974). There are several factors that contribute to this low level of utilization. Aside from tree uptake, fertilizer components can be immobilized in the soil, absorbed by vegetation other than the target species, leached below the rooting zone or converted to gaseous forms and lost (Hansen *et al.* 1988).

There are four main methods by which fertilizer can be applied to poplar plantations. They are 1) fertigation, 2) broadcasting followed by incorporation into the soil, 3) banding followed by incorporation into the soil, or 4) direct placement of fertilizer near the base of the tree. Fertigation is the injection of fertilizer into irrigation water which is distributed to the trees by an irrigation system. Fertigation is not commonly used other than in very dry regions due to availability of water and cost of infrastructure. Economic analyses of irrigation (fertigation) have shown it to be unattractive for most regions because of the high costs of purchasing and operating the required equipment (Dickmann and Stuart

1983), although examples do exist such as in the Tri-Cities region of Oregon where growth rates are extremely high, therefore, offsetting costs (pers. obs.).

Broadcast field fertilization at outplanting with readily available nutrients has shown limited capacity to reduce nutrient stresses. Broadcast field fertilization using traditional agronomic fertilizers releases nutrients immediately upon application with generally low rates of fertilizer use efficiency. Furthermore, the rapid nutrient release characteristic of broadcast fertilization leads to high levels of nutrient volatilization and increased growth of competing vegetation relative to target trees (Jacobs *et al.* 2005).

The majority of previous fertilizer research done has shown that banding or direct placement are the best methods for fertilizer application and current practice is to band fertilizer along the row to be planted (van den Driessche 1999b) or between rows of existing trees. Banding can be done cost-effectively with mechanical equipment and has been shown to be advantageous in the application of phosphate fertilizers since they are highly insoluble and largely immobile in the soil (van den Driessche 1999b). Banding also allows for more specific placement of fertilizers putting them close to the tree roots and out of the reach of weeds (van den Driessche 1999b).

As compared to banding, direct placement of fertilizers in the root zone of poplar trees can result in increases in both growth response and efficiency of nutrient uptake. Van den Driessche (1999b) showed that application of fertilizer in readily soluble forms of N and P to cuttings shortly after planting caused large increases in all measures of growth. Placement of fertilizer was approximately twice as effective as banding in increasing stem volume. Also, the total amount of fertilizer applied was a quarter of that banded per hectare. Efficiencies for N and P in placement versus banding were about 10-fold between the two methods. Growth might be further increased if nutrients added at planting were supplied in slowly soluble forms (Brown and van den Driessche 2002). Application of controlled-release fertilizer in the outplanting hole could be a useful alternative to help improve fertilizer use efficiency and alleviate competition problems associated with banding and especially broadcast fertilization, thereby

promoting early regeneration success of outplanted seedlings (Jacobs *et al.* 2005). The greatest disadvantage to direct placement of fertilizers is that currently this method requires manual application, greatly increasing the cost of application. However, savings due to decreased amounts of fertilizer being required and increased uptake efficiencies may offset this increased application cost.

1.8 Timing of fertilization

Heilman (1992) suggests that there are two main strategies in timing of fertilization. The first is a conservative approach, and fertilizer is only applied when growth decline or some form of deficiency is detected. This tends to maximize fertilizer use by the crop and economic return on the fertilizer investment. In practice, it implies that fertilizer is not applied at planting, but perhaps two or three years after planting when foliar analysis or perhaps soil testing have shown the necessity (van den Driessche 2003). The second approach strives to maintain optimal nutrient conditions throughout the rotation (Heilman 1992). This may require fertilization at, or before planting, as well as throughout the rotation. In the most intensive form, it is achieved by irrigation with nutrient solution on a daily basis (Ingestad and Ågren 1984, Dickmann *et al.* 2001, Nielsen *et al.* 2001). Where there is substantial investment in site preparation, weed control and use of expensive propagules, as from a breeding program, the proportional cost of fertilizer is less (van den Driessche 2003). Fertilization is, therefore, more economically attractive as the intensity and anticipated return on investment of a cultural system increases.

Nutrient enrichment at outplanting may promote early establishment success and growth of seedlings (van den Driessche 1988). Fertilization of TxD cuttings at planting can result in major growth increases over the first year. Increases in stem volume of 2.4-fold were obtained with 200 kg ha^{-1} each of N and P banded adjacent to the row, and 4.3-fold for 50 kg ha^{-1} each of N and P placed adjacent to the cutting (van den Driessche 1999). However, fertilization with N is often delayed for 1 to 2 years after planting to maximize uptake by crop

trees and to minimize leaching losses or uptake by competing vegetation (Hansen 1994). Nutrient uptake changes rapidly in a developing hybrid poplar plantation. Trees are most likely to be nutrient deficient during the years just before canopy closure (Miller 1983) and, therefore, fertilizers are usually applied during the third growing season prior to canopy closure (Thomas *et al.* 2000). In addition, poplars growing on intermediate to poor site classes have a longer response to fertilization than poplars growing on nutrient rich sites (Thomas *et al.* 2000).

1.9 Recommendations for fertilization

Recommendations on the type and amount of fertilizer that should be used in the fertilization of hybrid poplar stands vary greatly depending on the region, clone, and method of application. Therefore, recommendations found in the literature are either very general or they are not very consistent. Some of the recommendations for fertilizer rates are as follows. The recommended amount of banded N per hectare for TxD hybrids in coastal British Columbia is 150-200 kg/ha of N (van Oosten 2006). Urea (46-0-0) is a good fertilizer to use to supply N and, at rates of 150-200 kg/ha of N, one would require 325-430 kg of urea fertilizer per hectare (van Oosten 2006). In the Pacific Northwest, nitrogen is usually the only nutrient added by means of fertilizer (Heilman *et al.* 1995) with application rates between 112 and 336 kg/ha of N (Hansen 1994). In practice, TxD hybrid plantations in coastal regions are fertilized usually receiving 200 kg/ha of N in the third year of growth (van den Driessche 1999). Monoammonium phosphate is commonly used for hybrid poplar fertilization in stands where P deficiency is determined through tissue analysis (van Oosten 2006). In such situations, 220-340 kg/ha of fertilizer is the recommended rate, which supplies between 50 and 75 kg/ha of P (van Oosten 2006).

1.10 Growth and yield response

Fertilization of hybrid poplars has been extensively studied (Dickmann *et al.* 2001) and the trees have proven to be responsive to additions of both N and

P fertilizers (van den Driessche 1999). Growth response to both N and P applied separately, have shown that fertilization with N and P at planting could prove advantageous (van den Driessche and Brown 1996). On Vancouver Island, fertilization with N in year 3 significantly increased height (19%) and addition of N, P, and K, further increased height (39%) over controls (Zabek 1995). Another study completed on Vancouver Island showed stem volumes increased up to three-fold through one growing season and up to two-fold through four growing seasons when sufficient quantities of N, P, K, S and trace elements were added shortly after planting (van den Driessche 1999). In the hybrid poplar clone NC5271 (*P. nigra*), fertigation with rates of 200 ppm N, with N, P and K supplied in a ratio of 3:1:2, increased growth rates by 35% with a total increase in plant biomass of 91% (Glynn *et al.* 2003).

Combined fertilization and irrigation of forest trees has produced greater stem volume growth than either fertilization or irrigation alone in dry regions. Experiments in the USA Lake States region have shown that irrigation and fertilization of poplar "biomass" plantations had the potential to increase growth (Hansen 1988, Strong and Hansen 1991). A test of placement fertilization and irrigation in a 2 x 2 factorial experiment with aspen at the Weyerhaeuser Tree Improvement Centre at Drayton Valley, Alberta, Canada, showed a strong interaction between both factors (van den Driessche *et al.* 2003). Fertilizer and irrigation treatments applied together increased mean height 19% and stem volume 92% after three growing seasons. Fertilizer without irrigation had no effect on growth and decreased survival 17% compared with control. Based on many of these results one may conclude that response to fertilizer treatments can be maximized if soil moisture is not a limiting factor to tree growth.

1.11 Physiological effects

Poplars have been shown to respond physiologically and morphologically to increases in nutrient availability, as well as to nutrient deficiencies. Theories of optimal allocation predict that plants preferentially allocate resources to processes and structure that maximize acquisition of limiting resources, including

proportional increases in root growth in response to nutrient limitation (Chapin 1991). More specifically, there is much evidence that N availability influences root versus shoot allocation. Plants generally allocate more photosynthates to shoots and less to roots as the availability of N increases (Axelsson and Axelsson 1986, Birk and Vitousek 1986, Ingestadt and Agren 1988). In another fertilizer study using hybrid poplars, fertilized treatments showed a 35% decrease in carbon allocation belowground as compared to control whereas above-ground biomass increased (Sigurdsson *et al.* 2001). The increases in above-ground biomass were mainly due to higher levels of branching and shoot extension.

Increased N availability has also been shown to have morphological effects on hybrid poplar canopy structure. Increases in N levels increase number and size of leaves as well as level of syllepsis (branch emergence from buds formed in the same growing season) in hybrid poplars (Cooke *et al.* 2005). These factors contribute to increased canopy size and, as a result, increased photosynthetic productivity and growth. A study by van den Driessche (1999) showed that nitrogen fertilization increased leaf mass and leaf area by up to 2-fold and increased leaf nitrogen content by as much as 153%. Generally higher leaf area and leaf nitrogen contents are associated with higher rates of maximum photosynthesis (Poorter and Evans 1998), which would likely correspond to higher growth rates. Often, increases in leaf area production in TxD clones occur in response to increased N availability, which is in part due to the indeterminate growth habit of *Populus* spp. In TXD hybrids, with high leaf concentrations of N (>4% leaf dry weight), there were significant increases in sylleptic branch biomass (Cooke *et al.* 2005). Dickson (1989) found that sylleptic branches became photosynthetically independent once they had attained 10-15 leaves; thus sylleptic branches are very effective in increasing overall leaf area and photosynthetic productivity of the tree.

Limiting N accelerates leaf maturation in the developing zone, while luxuriant N prolongs leaf maturation. As mature leaves with limited N age, chlorophyll content decreases and this suggests that limiting N conditions also accelerate the final stage of leaf development –senescence. The earlier

transition to senescence presumably allows N-limiting plants to remobilize resources from leaves to support growth in developing parts of the tree (Cooke *et al.* 2005).

Under conditions of moisture stress, some studies have indicated that high levels of N from either highly fertile soils or the addition of N fertilizers may increase poplar susceptibility to a condition called xylem cavitation. Xylem cavitation occurs when xylem vessels become blocked by air bubbles or embolisms, reducing hydraulic conductivity and thus productivity. Xylem cavitation is most prevalent when there is a shortage of available water (Tyree and Ewers 1991). A possible explanation for the occurrence of xylem cavitation is that N treatments increase vessel size, with the assumption that larger vessel size is related to increased probability of cavitation (Zimmermann 1978). Also, with decreased root to shoot ratios as a result of increased aboveground growth in response to N fertilization, water demands become very high and the proportionately smaller root system cannot deliver enough water to the canopy. In a study looking at four TxD hybrid poplar clones, an increase in N fertilization increased the tendency to xylem cavitation, whereas an increase in P reduced cavitation (Harvey and van den Driessche 1997). Therefore, adequate P nutrition may contribute to resistance to xylem cavitation in hybrid poplars. Phosphorus decreased mean vessel diameter and also reduced the amount of damage to membranes under moisture stress. There was also evidence that increasing K supply reduced leaf loss under drought stress, whereas increasing N supply was associated with increasing leaf loss (Harvey and van den Driessche 1999). These results suggest that N fertilization might reduce drought resistance of hybrid poplars unless P and K supplies are increased to match the increase in N supply.

1.12 Thesis objectives

To remain financially viable and meet growth expectations, nutrient supply has become a critical issue for intensively grown poplars. The review of literature suggests that it may be possible to choose *Populus* species and hybrids that are

more productive than others under moisture and nutrient-limited conditions. In addition, supply of appropriate nutrients through fertilization may enhance productivity under deficiency conditions. Although significant research has been done on the effects of nutrient inputs on hybrid poplar growth in the Pacific North-west and the mid-eastern USA Lake States, growing hybrid poplar plantations in northern and prairie climates is still relatively new. This lack of knowledge has created an intense need for research to be completed in this field, thus leading to the following research opportunity.

The main objective of my thesis project was to compare growth and physiological responses of poplars to different levels of nutrient supply during establishment and the variability in responses between hybrid clones and balsam poplar. More specifically I examined differences in: (1) nutrient levels in above-ground and below-ground tissues, (2) leaf, stem and root biomass accumulation (3) photosynthetic rates and (4) water use efficiency.

2.0 Materials and Methods

2.1 Experimental design and layout

2.1.1 Experimental design

The experimental design included three fertilizer treatments and four poplar clones. As a result, there were 12 different treatment combinations randomized within each block, replicated across 5 blocks. Each treatment within a block consisted of two rows of nine trees of the same clone. There were 1080 trees in the entire trial (18 trees/treatment x 12 treatments/block x 5 blocks). Two trees from each treatment (1 from each row) within each block were randomly designated for destructive sampling (D-trees).

2.1.2 Site history and preparation

The trial site is located on land owned by Alberta-Pacific Forest Industries Inc. (Al-Pac) near Athabasca, Alberta in the central mixed wood subregion (Hosie, 1979) (54°N, 112°W, elevation 620 m). Average rainfall for the Athabasca region is 503.7 mm annually with 381.7 mm as rain and 122 mm as

snow (Environment Canada website www.weatheroffice.gc.ca). Summers are cool with mean daily average temperatures for June-August being 15.2° C and winters being much colder with mean daily average temperatures for December-February being –12.8°C (climatic data from Environment Canada weather station 'Athabasca2' summarized from 1971 –2000). The trial site was originally an agriculture field that was farmed for more than 50 years prior to Al-Pac's acquisition in 1998. For approximately one year prior to the establishment of this project, the trial site was in fallow to allow breakdown and incorporation of residual fertilizer and woody biomass from a prior trial conducted by Al-Pac from May 2000 to May 2002. In October 2003 the site was marked using shanks pulled by a tractor to make a grid pattern on the soil surface. These lines were used to mark out where the trees would be planted the following spring.

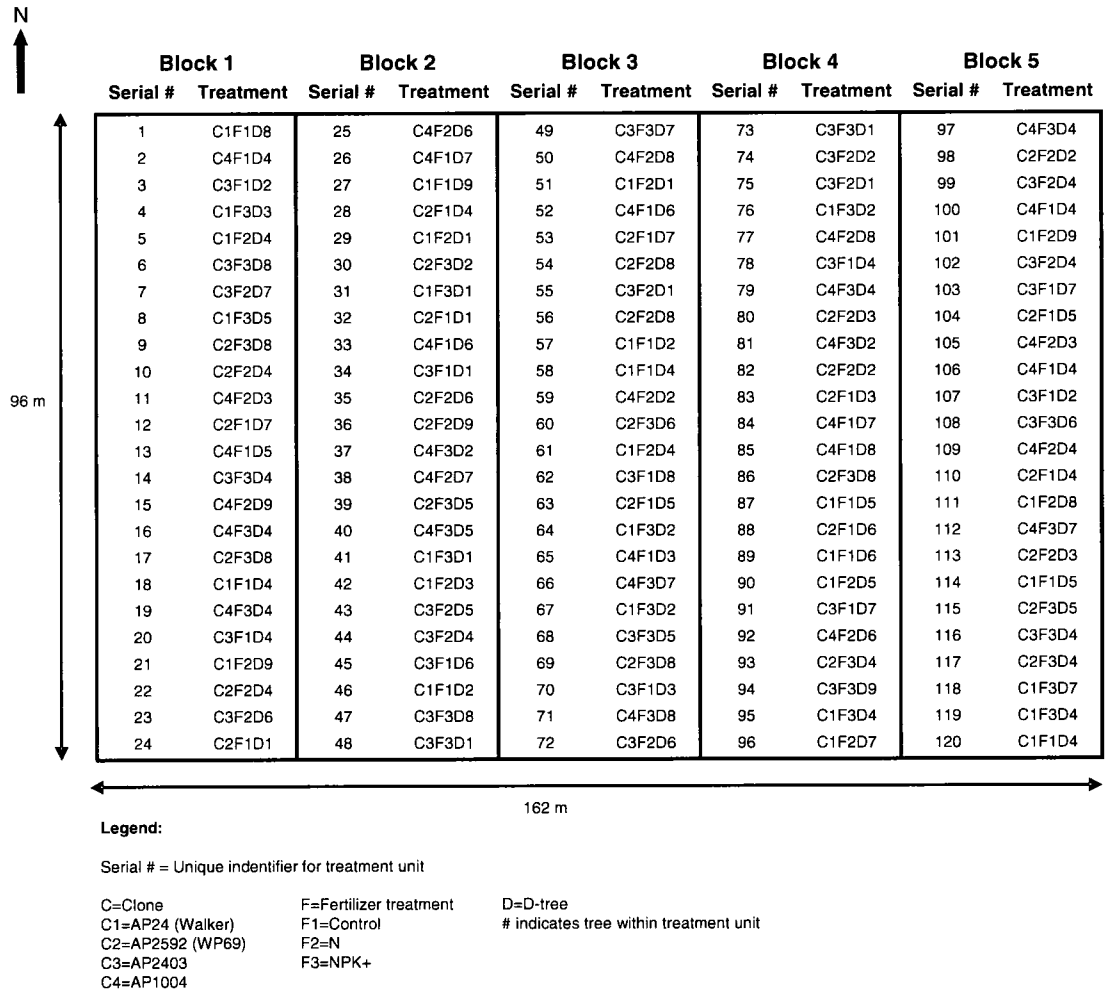
2.1.3 Field trial layout

Spacing for the treatment trees was 3 m x 4 m. The 3 m spacing ran along the treatment rows (east-west) with the treatment rows being 4 m apart (north-south). There was a buffer row of untreated trees planted in between each treatment row and around each individual block. Each treatment row was given a unique serial number (Appendix 1). A stake with the corresponding serial number was placed at the start of each treatment row. D-trees were marked with a wire pigtail for tracking purposes. Blocks were arranged perpendicular to a slight slope running downwards from the west to the east. Arrangement of blocks and the layout of treatment combinations are shown in Figure 2.1.3.1.

2.1.4 Trial maintenance and vegetation control

A pre-plant application of Roundup Transorb® HC (Monsanto, Winnipeg, MB) non-selective herbicide was applied on June 1, 2004 at a rate of 2.5 litres per acre to kill all plant growth on the trial site prior to planting. After planting, on June 23 and 24, 2004, the allies between the rows of trees were rototilled approximately every 2 weeks to keep them as weed free as possible. Weeds around the trees were either sprayed with Roundup Transorb® HC using a

backpack sprayer with a shielded wand to protect the trees or hand-picked. Weed control was required until the growing season ended in early September of both 2004 and 2005.



Block 1		Block 2		Block 3		Block 4		Block 5	
Serial #	Treatment	Serial #	Treatment	Serial #	Treatment	Serial #	Treatment	Serial #	Treatment
1	C1F1D8	25	C4F2D6	49	C3F3D7	73	C3F3D1	97	C4F3D4
2	C4F1D4	26	C4F1D7	50	C4F2D8	74	C3F2D2	98	C2F2D2
3	C3F1D2	27	C1F1D9	51	C1F2D1	75	C3F2D1	99	C3F2D4
4	C1F3D3	28	C2F1D4	52	C4F1D6	76	C1F3D2	100	C4F1D4
5	C1F2D4	29	C1F2D1	53	C2F1D7	77	C4F2D8	101	C1F2D9
6	C3F3D8	30	C2F3D2	54	C2F2D8	78	C3F1D4	102	C3F2D4
7	C3F2D7	31	C1F3D1	55	C3F2D1	79	C4F3D4	103	C3F1D7
8	C1F3D5	32	C2F1D1	56	C2F2D8	80	C2F2D3	104	C2F1D5
9	C2F3D8	33	C4F1D6	57	C1F1D2	81	C4F3D2	105	C4F2D3
10	C2F2D4	34	C3F1D1	58	C1F1D4	82	C2F2D2	106	C4F1D4
11	C4F2D3	35	C2F2D6	59	C4F2D2	83	C2F1D3	107	C3F1D2
12	C2F1D7	36	C2F2D9	60	C2F3D6	84	C4F1D7	108	C3F3D6
13	C4F1D5	37	C4F3D2	61	C1F2D4	85	C4F1D8	109	C4F2D4
14	C3F3D4	38	C4F2D7	62	C3F1D8	86	C2F3D8	110	C2F1D4
15	C4F2D9	39	C2F3D5	63	C2F1D5	87	C1F1D5	111	C1F2D8
16	C4F3D4	40	C4F3D5	64	C1F3D2	88	C2F1D6	112	C4F3D7
17	C2F3D8	41	C1F3D1	65	C4F1D3	89	C1F1D6	113	C2F2D3
18	C1F1D4	42	C1F2D3	66	C4F3D7	90	C1F2D5	114	C1F1D5
19	C4F3D4	43	C3F2D5	67	C1F3D2	91	C3F1D7	115	C2F3D5
20	C3F1D4	44	C3F2D4	68	C3F3D5	92	C4F2D6	116	C3F3D4
21	C1F2D9	45	C3F1D6	69	C2F3D8	93	C2F3D4	117	C2F3D4
22	C2F2D4	46	C1F1D2	70	C3F1D3	94	C3F3D9	118	C1F3D7
23	C3F2D6	47	C3F3D8	71	C4F3D8	95	C1F3D4	119	C1F3D4
24	C2F1D1	48	C3F3D1	72	C3F2D6	96	C1F2D7	120	C1F1D4

Legend:

Serial # = Unique indentifier for treatment unit

C=Clone
C1=AP24 (Walker)
C2=AP2592 (WP69)
C3=AP2403
C4=AP1004

F=Fertilizer treatment
F1=Control
F2=N
F3=NPK+

D=D-tree
# indicates tree within treatment unit

Figure 2.1.3.1. Field layout map of trial blocks and treatment combinations.

2.2 Clones

2.2.1 Description

The clones used were *P. deltoides* x *P.* x *petrowskyana* – 'Walker' hybrid poplar (clone 1), *(P. deltoides* x *P.* x *petrowskyana)* x *(P.* x *petrowskyana)* - 'Okanese' hybrid poplar (clone 2), *(P. deltoides* x *P.* x *petrowskyana)* x *(P.* x *petrowskyana)* – 'WP69' hybrid poplar (clone 3) and *P. balsamifera* - 'AP1004' superior native balsam poplar (clone 4). However, upon more recent consultation with the Prairie Farm Rehabilitation Administration (PFRA), where

several of these clones originated from, and verification by means of DNA fingerprinting, it was found that clones 2 and 3 were the same genotype, which was given different names depending on where it came from. However, due to the late timing of this discovery with respect to this project, for the purposes of this study, they will continue to be considered as different clones and analyzed separately. The clones used have shown rapid growth rates, fair to excellent planting survival, and acceptable winter hardiness, making them of particular interest for future agroforestry and plantation use in northeastern Alberta and Saskatchewan. All buffer trees planted were AP24-Walker hybrid poplar. See Table 2.2.1.2 for a description of the clones used in this experiment.

Table 2.2.1.2. Description of name, type and parentage of poplar clones planted. In the hybrid crosses, the first species is the female, the second is the male.

Clone #	Clone name	Clone type	Parentage
1	Walker	Hybrid poplar	*P. deltoides* x *P.* x *petrowskyana**
2	WP69	Hybrid poplar	*(P. deltoides* x *P.* x *petrowskyana)* x *(P.* x *petrowskyana)*
3	Okanese	Hybrid poplar	*(P. deltoides* x *P.* x *petrowskyana)* x *(P.* x *petrowskyana)*
4	AP1004	Native balsam poplar	*P. balsamifera* x *P. balsamifera*

**P.* x *petrowskyana* is a hybrid cross of *P. laurifolia* x *P. nigra*

2.2.2 Propagation and planting

The poplar clones were propagated using 10 cm dormant cuttings. Cuttings for the three hybrids were collected in March 2004 by the Prairie Farm Rehabilitation Administration Shelterbelt Center, Indian Head, Saskatchewan. The AP1004 native superior balsam poplar cuttings were collected from stoolbeds at Al-Pac in March 2004. Cuttings were stored in plastic bags at –4°C until April 2004 when they were removed from freezer storage on April 15 and soaked for two days in water at room temperature. Cuttings were then planted in 4-15D (164cm^3) cell size styroblocks (Beaver Plastics Ltd., Acheson, AB) in a plastic dome greenhouse facility at Al-Pac. Only natural lighting was provided

during this period. Minimum growing temperatures of 15°C were maintained inside the greenhouse, however maximum temperatures were dependent mainly on outside conditions. Trees were watered on average two times/week and were fertilized with Plant-Prod ® 20-20-20 all purpose fertilizer (Sure-Gro Inc., Brantford, ON) once a week until field planting on June 23 and 24, 2004. After planting, each tree was watered with approximately 2 L of water on June 29 and 4 L of water on July 30 due to extremely dry soil conditions.

2.3 Fertilization

2.3.1 Description

Applied fertilizer treatments were control (no fertilizer), nitrogen fertilizer (N), and nitrogen, phosphorous, potassium, sulphur, zinc and copper combined (NPK+). Fertilizer was pre-measured into individual bags (1 per tree), containing either 27.9 grams of granular N fertilizer (N) or 83.37 g of granular NPKS+Cu+Zn fertilizer mixture (NPK+). The amount and composition of the fertilizers required for the treatments are described in Table 2.3.1.1.

Table 2.3.1.1. Composition of three fertilizer treatments - Control, N, and NPK+. The NPK+ fertilizer treatment is composed of six different fertilizers mixed together in the amounts shown in boldface in the last column and applied at 83.37 g per tree.

Nutrient		**Fertilizer**		
Element	**Amount (g/tree)**	**Type**	**Analysis**	**Amount (g/tree)**
Control				
0	0	0	0	**0**
Treatment N				
N	12 g	$(NH_2)_2CO$	43-0-0	**27.91** g
Treatment NPK+				
N	12 g	$(NH_2)_2CO$	43-0-0	**27.91** g
P	8 g	$Ca(H_2PO_4)_2.\ H_2O$	0-45-0	**40.80** g
K	4 g	K_2SO_4	0-0-50	**9.76** g
S	1.66g	K_2SO_4	0-0-50	9.76 g
S	0.20g	$CuSO_4.5H_2O$	12.6% S	1.59 g
S	0.19g	$ZnSO_4.H_2O$	17.2% S	1.14 g
S	1.94g	S (with bentonite clay carrier)	90% S	**2.16** g
Total S	4.0 g			
Cu	0.40 g	$CuSO_4.5H_2O$	25% Cu	**1.59** g
Zn	0.41 g	$ZnSO_4.H_2O$	36% Zn	**1.14** g
Total weight of NPK+ per tree				83.37 g

2.3.2 Application

The fertilizer treatments were applied on July 9, 2004, 15 days after planting. Fertilizer was placed in a slit made by a spade on the south side of each tree approximately 10-15 cm from the tree base and 5-10 cm below the soil surface. The application strategy behind placement of the fertilizer in this manner was to attempt to maximize availability by placing it in the rooting zone as well as minimizing access to competition or trees in the other treatments. The purpose of having the time period between planting and fertilizing was to allow the trees to become established given the dry conditions at the time of planting, since applying fertilizer under severe drought stress conditions has been known to increase moisture stress and greatly reduce survival (DesRochers *et al.* 2006).

2.4 Sampling and measurements

2.4.1 Soil sampling and moisture level monitoring

Composite soil samples were collected in August 2003 and analyzed to establish a soil characteristic baseline for the site. Three soil samples were taken randomly from each block of the trial to a depth of 45 cm. The samples were split into the A and B horizons. The three samples for the A and B horizons were then combined by horizon to form composite samples. The result was one A and one B sample per block. The soil samples were sent to Norwest Labs, Lethbridge, Alberta and were analyzed for total Kjeldahl N, total C, available P (dilute fluoride acid method), exchangeable K, Ca, Mg, total cation exchange capacity (CEC), and pH (1:2 soil:water).

Soil moisture levels were monitored weekly from planting to the end of August 2004, and from May to the end of August 2005 using a Delta T Profile Probe Type PR1 with a Delta T HH2 Moisture Meter (Delta-T Devices Ltd. Cambridge, UK). Moisture content was measured at four depths (0-10, 10-20, 20-30 & 30-40 cm) and required that permanent access tubes be installed vertically in the soil profile for the duration of the experiment. There were four access tubes installed in each block of the trial and they were located approximately 5 m in from each corner. An average of three measurements were taken each time soil moisture was measured as the probe was rotated 1/3 of a turn within the access tube/measurement to reduce the effects of soil voids or stones.

Readings from the moisture probe were recorded as conductivity (mV), therefore, a general relationship between conductivity and gravimetric soil moisture content was required to convert voltage readings to moisture content (%). To establish this relationship, following recording soil moisture with the moisture probe, soil samples (4) were extracted approximately 2-3 m away from the moisture probe access tubes and divided into layers corresponding to the depths of the moisture probe readings in the spring of 2004. The soil samples were then combined to form a composite sample by layer, weighed wet (field moisture level), dried for 24 hrs at 105^{O}C and then weighed again (van den

Driessche 2003). Gravimetric soil moisture could then be calculated using the following equation:

θ_G (%) =(M_W-M_D)/M_D $g.g^{-1}$ x100

where θ_G *is gravimetric soil moisture in %,*

M_W *is the total mass of the wet sample, and*

M_D *is the total mass of the dry sample*

The moisture probe data and gravimetric soil moisture content were then plotted by depth increment to establish relationships between the two and the following correlation equations for each soil layer were calculated:

Soil moisture 0-10 cm: $y=0.0462x+6.2158$ $R^2=0.49$
Soil moisture 10-20 cm: $y=0.0746x+11.166$ $R^2=0.79$
Soil moisture 20-30 cm: $y=0.0856x+15.687$ $R^2=0.77$
Soil moisture 30-40 cm: $y=0.3713x-150.87$ $R^2=0.83$

These equations were then used to calculate gravimetric soil moisture levels for the duration of the experiment. As reference points for moisture content, (eg. field capacity and wilting point) 25 soil samples were collected, with five/block, and separated into layers 0-10cm, 10-20cm, 20-30 cm and 30-40 cm. The soil samples from each layer were combined to form one composite sample/layer and were analysed for soil water retention at soil water potential intervals from 1/10 to 15 bar at Norwest Labs (Water Retentivity of Soil at Specified Values of Matric Suction, 8-2, Agronomy No 9, Part 1).

2.4.2 Growth measurements

Height and root collar diameter of all treatment trees (excluding D-trees) were measured immediately following planting and at the end of growing season 1 (November 2004) and again at the end of growing season 2 (October 2005). All root collar diameters (mm) were measured using a HJK M4306 digital caliper

(G. Hjukstrom Ltd. Surrey, BC), above the original cutting that the tree was propagated from or as near to the soil surface as possible. All heights (cm) were measured using a measuring stick from the ground surface to the base of the terminal bud.

2.4.3 Tissue sampling/nutrient analysis

On August 24, 2004, 46 days after fertilization, while the trees were still actively growing, leaf tissue samples were collected. Three of the uppermost, fully expanded leaves were collected from each of the eight trees within a treatment row (D-trees were excluded) to form composite samples of 24 leaves. Leaf sampling methodology was based on criteria described by van den Driessche (1974, 1999). Therefore, with two rows of trees/treatment combination in each block (times five blocks) there were 120 samples in total. Leaf areas were measured using a LI-COR 3100 leaf area meter (LI-COR Biosciences, Lincoln, NE) and samples were dried at 80°C for 24 hours and then weighed prior to grinding. Samples were finely ground using a Brinkmann MM2 ball grinder (Brinkmann Instruments Ltd. Rexdale, ON) and then sent to Norwest Labs to be analyzed for: N, P, K, Ca, Mg, S, Fe, Mn, Cu, Zn, B, Mo and Na. To determine levels for P, K, Ca, Mg, Fe, Mn, Cu, Zn, B, Mo and Na samples of tissue were dry ashed in a muffle furnace at 500°C, digested in a 30% HCl solution and then individual nutrient levels were determined by atomic emission spectroscopy (ARL 34000 RTB ICP spectrometer, Thermo Instruments, Mississauga, ON). Nitrogen and S levels were determined by combustion (LECO CNS-2000 Elemental Analyser, model FP 428, Leco Instruments, St-Josephs, MI). Additional sub-samples of tissue from each leaf sample were sent to the Isotope Science Laboratory at the University of Calgary to determine leaf carbon isotope ratios ($\delta^{13}C$). Sampling, handling, and tissue analysis were repeated in August 2005 using the same methodology as in 2004 except leaf area was not measured.

2.4.4 Destructive tree sampling/nutrient analysis

In July 2005, height and root collar diameter were recorded for all of the D-trees in blocks 1 and 2 before they were destructively harvested to look at whole tree biomass, leaf, stem and root volumes and tissue nutrient concentrations. D-trees were dug up manually with as complete a root system as possible and the roots were washed to remove all soil. The trees were then separated into leaves, shoots and roots to form individual samples (3 samples/tree) and dried for 48 hours at 80°C. Following drying, the samples were weighed and ground, and then sent to Norwest Labs to be analyzed for: N, P, K, Ca, Mg, S, Fe, Mn, Cu, Zn, B, Mo and Na. The same methods for nutrient analysis were used as described previously.

2.4.5 Gas exchange

Measurements of net assimilation (NA), stomatal conductance (g_s) and transpiration (E) were taken on August 22 and August 23, 2005 using a CIRAS-1 infrared gas analyzer (PP Systems, Haverhill, MA). Measurements were taken on the first two healthy looking trees in each treatment row for a total of four measurements/treatment/block.

Instantaneous water-use efficiency (WUE_i) was calculated as NA/E using values for NA and E from the infrared gas analyzer (IRGA) output data. Ratios of $\delta^{13}C$ provided by analysis of the 2004 and 2005 leaf tissue, indicating static water-use efficiency, allowed for comparisons to be made between the two measurement methods.

2.5 Statistical analysis

Measurements of growth characteristics, tissue nutrient analysis and gas exchange measurements were treated by analysis of variance for a 3x4 factorial, randomised complete block design using the general linear model procedure for mixed model analysis using SAS (SAS 9.1, SAS Institute Inc, Cary, NC). Initial seedling height and basal diameter were used as covariates in the analysis of height and diameter growth but were not significant ($p>0.05$) and, therefore,

analysis for growth was rerun without covariates. Least square means were compared using the Student-Newman-Keuls Test with a significance level of $p<0.05$. The linear model used for this analysis was as follows:

$$Y_{ijkl} = \mu + B_i + C_j + T_k + CT_{jk} + BT_{ik} + BC_{ij} + BCT_{ijk} + E_{ijkl}$$

Where:

Y_{ijkl} = the measurement of the k^{th} treatment in the i^{th} block of the j^{th} clone on the l^{th} tree;

μ = the overall mean;

B_i = the random effect of the i^{th} block (1-5)

C_j = the fixed effect of the j^{th} clone (1-4)

T_k = the fixed effect of the k^{th} fertilizer treatment (1-3)

CT_{jk} = the fixed effect of the k^{th} fertilizer treatment on the j^{th} clone

BT_{ik} = the random effect of the interaction between the i^{th} block and the k^{th} fertilizer treatment

BC_{ij} = the random effect of the interaction between the i^{th} block and the j^{th} clone

BCT_{ijk} = the random effect of the interaction between the i^{th} block, j^{th} clone and k^{th} fertilizer treatment

E_{ijkl} = the residual error.

Two-tailed, student t-tests for two samples of unequal variance were performed using Microsoft Excel®, (Microsoft Office 2007®, Microsoft Corporation, Redmond, WA) to determine if leaf sample nutrient levels were the same as whole D-tree nutrient levels for each nutrient quantified in the tissue analysis based on percent content by weight. The threshold chosen for statistical significance was 0.05 ($\alpha \leq 0.05$).

Correlation analyses were completed by plotting measured trait values against each other using Microsoft Excel®, (Microsoft Office 2007®, Microsoft Corporation, Redmond, WA) for leaf weight and area, leaf weight and nutrient

composition and content, nutrient composition and tree height and basal diameter, and leaf weight and leaf C isotope ratios.

3.0 Results

3.1 Soil sampling and moisture level monitoring

Pre-plant soil analysis for the A horizon, across the 1.5 ha site, found the mean soil pH was 6.62±0.09, N levels were 0.16±0.02%, P levels were 12.2±1.11mg/kg and K levels were 0.342 meq/100g. Soil organic matter was between 3.49 and 5.42%.

Baseline soil moisture levels for depths 0-10cm, 10-20cm, 20-30cm, and 30-40cm at -33 J/kg (field capacity) were 31.3, 30.3, 22.9 and 32.3%, and at -1500 J/kg (wilting point) were 11.8, 13.7, 11.4 and 18.6%, respectively. Soil moisture monitoring throughout the growing seasons of 2004 (year 1) and 2005 (year 2) showed that soil moisture content of the site for top 40 cm of the soil horizon generally stayed between field capacity and wilting point (Figure 3.1.1 and 3.1.2).

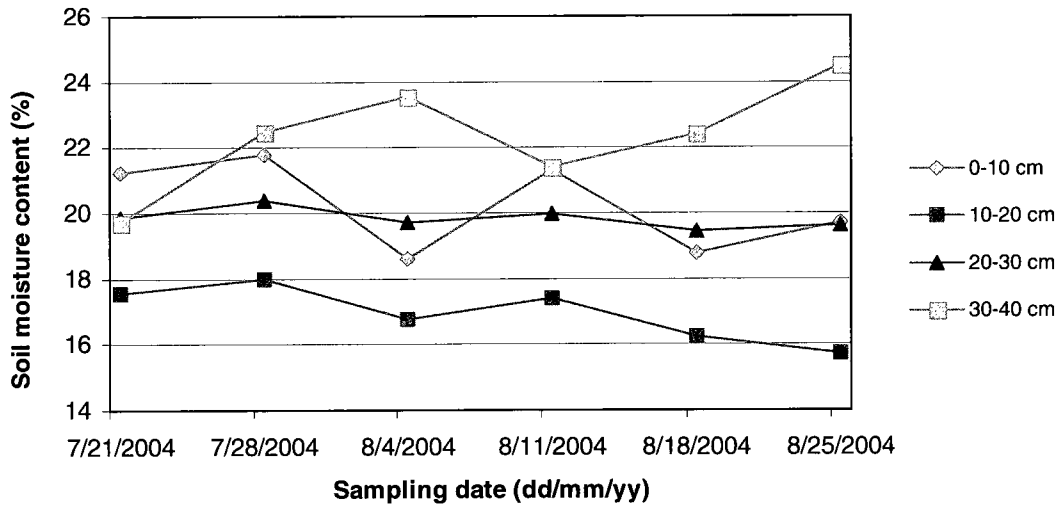


Figure 3.1.1. Soil moisture content (%) from July 21, 2004 to August 25, 2004 for 0-10cm, 10-20cm, 20-30cm and 30-40cm into the soil profile from the soil surface.

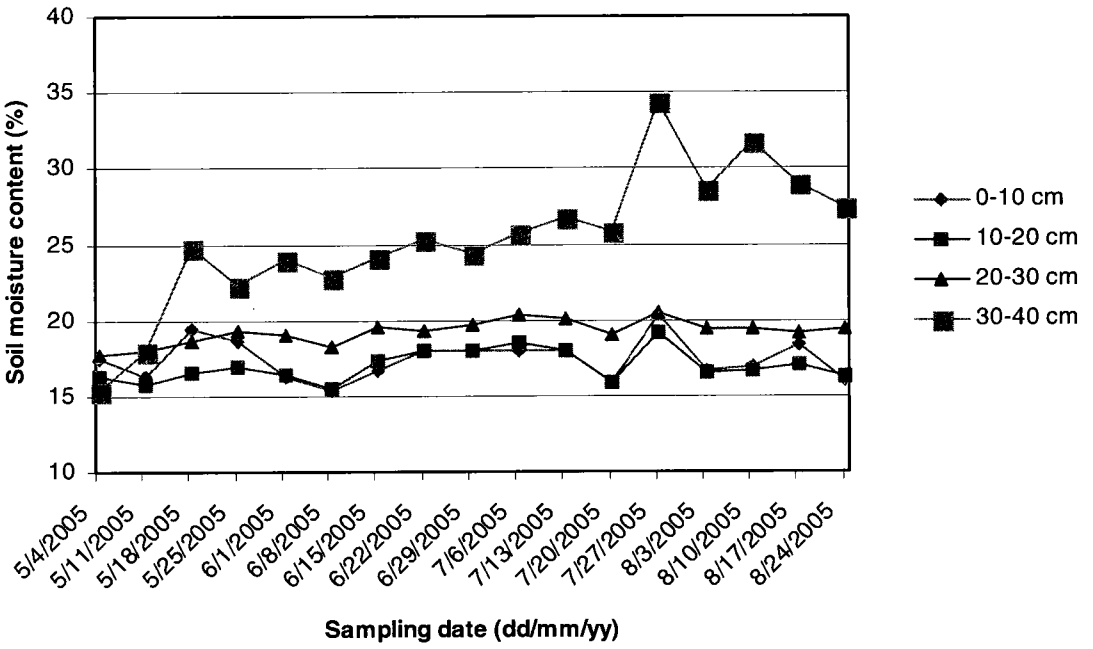


Figure 3.1.2. Soil moisture content (%) from May 4, 2005 to August 24, 2005 for 0-10cm, 10-20cm, 20-30cm and 30-40cm into the soil profile from the soil surface.

3.2 Growth measurements

Both fertilizer treatment and clone significantly influenced height and basal diameter after years one and two of growth ($p<0.05$) (Table 3.2.1). Height response to N fertilization alone was not significantly greater than control in year 1 but was in year 2 with a 9% increase across all four clones. There was a significant response in tree height growth for NPK+ fertilizer in both years with increases of 14% after year 1 and 16% after year 2 compared to the control trees. Height was also significantly greater for NPK+ than N for both years. Analysis of year 2 height data showed a significant interaction between fertilizer and clone ($p=0.01$), due mainly to the increased height growth of clone 4 in response to the N treatment. Increased basal diameter as a result of N fertilization was not significant in year 1 as compared to control, but was in year 2 with an 11% increase across all four clones. There were also significant increases in diameter growth for NPK+ in both years with increases of 25% after

year 1 and 24% after year 2 compared to control. Significant differences were also observed for diameter growth between the NPK+ and N treatments for both years.

Table 3.2.1. Means, standard errors (±SE), and p-values for height (cm) and basal diameter (mm) after years 1 (2004), and 2 (2005), in response to three fertilizer treatments and four poplar clones.

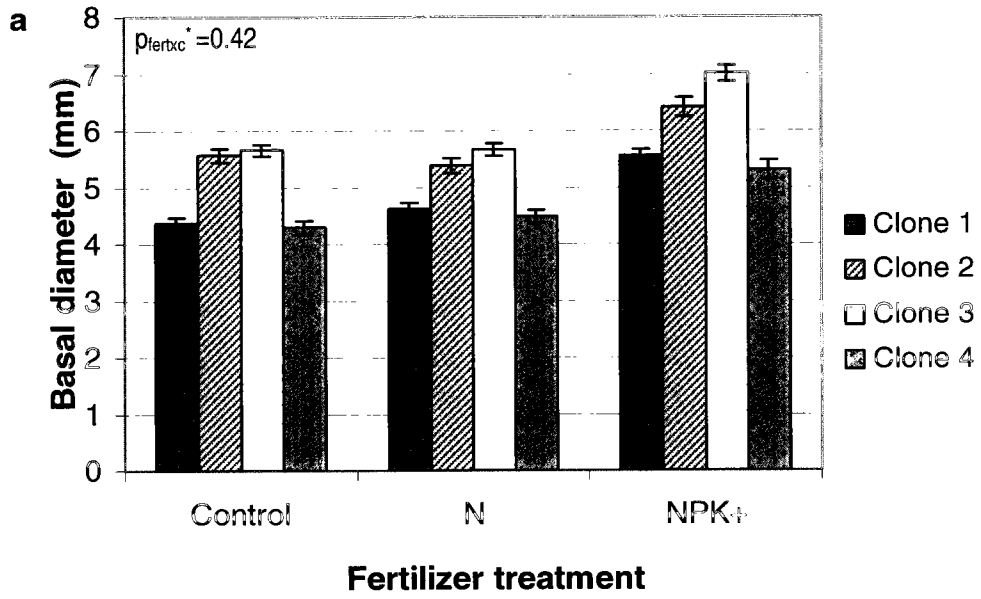
		Height (cm) Year 1		Height (cm) Year 2		Basal diameter (mm) Year 1		Basal diameter (mm) Year 2	
Fertilizer (1=none, 2=N, 3=NPK+)	Clone*	Mean	±SE	Mean	±SE	Mean	±SE	Mean	±SE
1	1-4	45.35b	0.97	96.65c	2.35	4.98b	0.06	13.11c	0.29
2	1-4	45.14b	0.97	105.37b	2.32	5.04b	0.06	14.67b	0.33
3	1-4	51.84a	1.02	112.23a	2.49	6.08a	0.08	16.37a	0.36
$p_{(fertilizer)}$		**<0.001**		**0.001**		**<0.001**		**0.04**	
1-3	1	46.39c	0.78	75.05c	2.2	4.84c	0.07	8.49d	0.2
1-3	2	56.93b	0.89	125.97a	2.53	5.79b	0.09	16.71b	0.33
1-3	3	59.99a	0.78	125.50a	2.36	6.11a	0.08	17.99a	0.29
1-3	4	25.92d	0.54	90.26b	2.34	4.69c	0.08	14.67c	0.37
$p_{(clone)}$		**<0.001**		**<0.001**		**<0.001**		**0.006**	
1	1	41.75	1.29	61.92	2.97	4.37	0.10	7.55	0.23
1	2	56.17	1.42	124.57	3.55	5.57	0.12	15.95	0.48
1	3	58.66	1.14	119.14	3.82	5.66	0.10	15.85	0.42
1	4	24.29	0.80	72.72	3.45	4.30	0.11	11.87	0.51
2	1	45.97	1.35	74.58	4.23	4.62	0.11	8.42	0.38
2	2	53.62	1.58	119.70	4.72	5.38	0.13	16.06	0.59
2	3	56.15	1.48	122.26	3.93	5.66	0.12	17.75	0.51
2	4	25.05	0.89	105.61	3.46	4.48	0.11	15.46	0.61
3	1	51.46	1.19	83.13	3.58	5.54	0.12	9.56	0.39
3	2	60.95	1.53	135.45	4.79	6.41	0.16	18.06	0.64
3	3	65.16	1.20	135.14	4.34	7.00	0.14	20.34	0.49
3	4	28.56	1.07	96.16	4.41	5.31	0.17	16.74	0.68
$p_{(fertilizer\ x\ clone)}$		**0.26**		**0.01**		**0.42**		**0.04**	

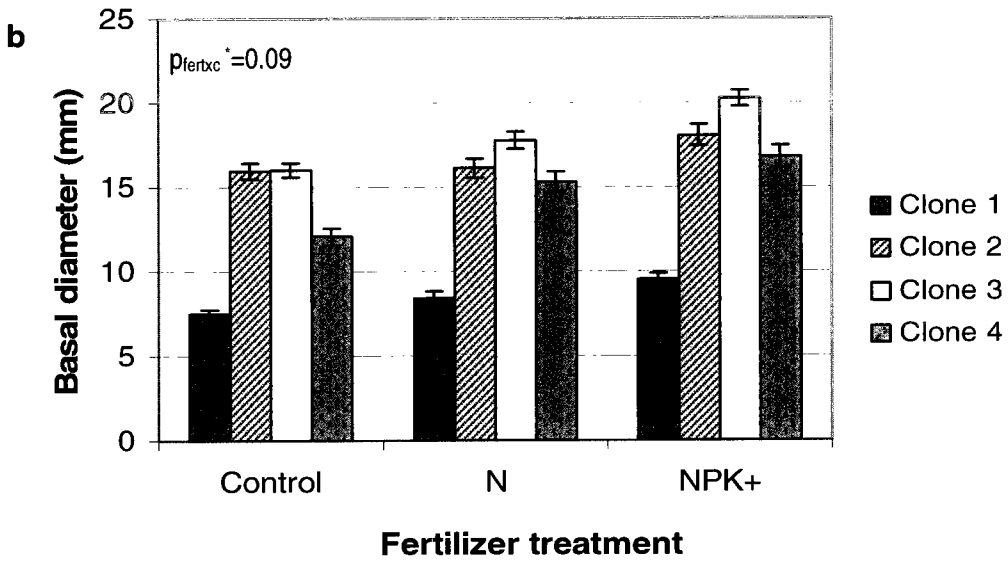
***1=Walker, 2=Okanese, 3=WP69, 4=AP1004**

Note: Different letters following means indicate a significant difference between means for the individual trait and treatment combination. Means for fertilizer treatment are across all four clones while means for clone are across all three fertilizer treatments. Overall p-values for clone and fertilizer are given by trait and year combination.

Growth response was also dependent on clone with clone being significant for height and basal diameter after both years 1 and 2 ($p<0.05$). Overall, clone 3 had the greatest basal diameter and height throughout the experiment having significantly greater height after year 1, and basal diameter after years 1 and 2 than all other clones. Both clones 2 and 3 had significantly greater basal diameter and height than clones 1 and 4 over the duration of the experiment. After year 1, clone 1 had significantly greater height and similar basal diameter to clone 4, but after year 2, clone 4 had significantly outgrown clone 1 for both measured traits.

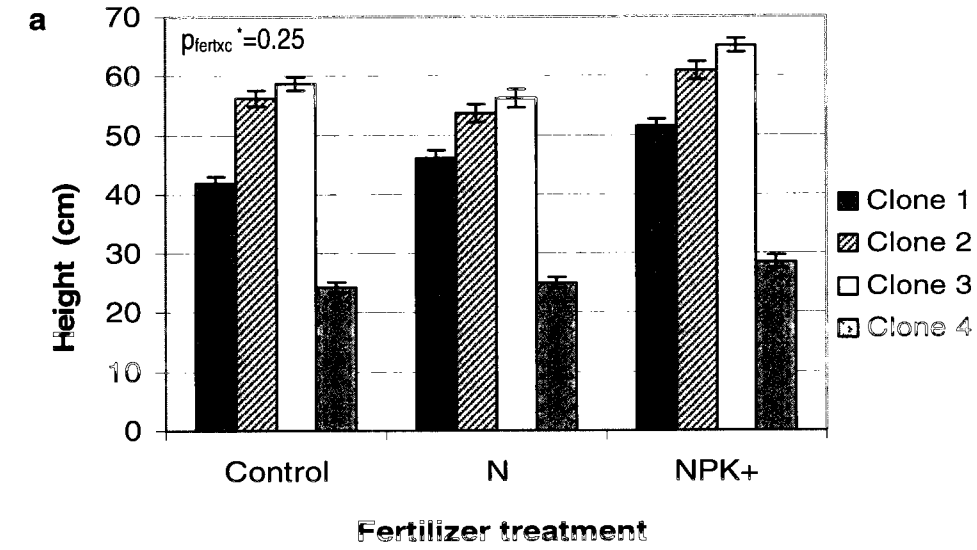
After year 1, clones 1 and 4 showed increasing basal diameter with control<N<NPK+. Clones 2 and 3 showed decreased basal diameter in response to N but increased basal diameter in response to NPK+ as compared to the control (Figure 3.2.1.1). After year 2 all of the clones showed control<N<NPK+ (Figure 3.2.1.2). Responses to fertilizer treatments were very similar for height. After year 1, clones 1 and 4 showed increasing height with control<N<NPK+, but clones 2 and 3 showed decreased height for N and increased height for NPK+ with respect to the control (Figure 3.2.1.3). After year 2, clones 1 and 3 showed a response in height to fertilizer treatment with control<N<NPK+ ($p=0.001$). Clone 2 showed a negative response to N but a positive response to NPK+ and clone 4 showed a positive response to both, with N being greater than NPK+ compared to the control (Figure 3.2.1.4). Survival was 98% after year 1 and 91% after year 2.

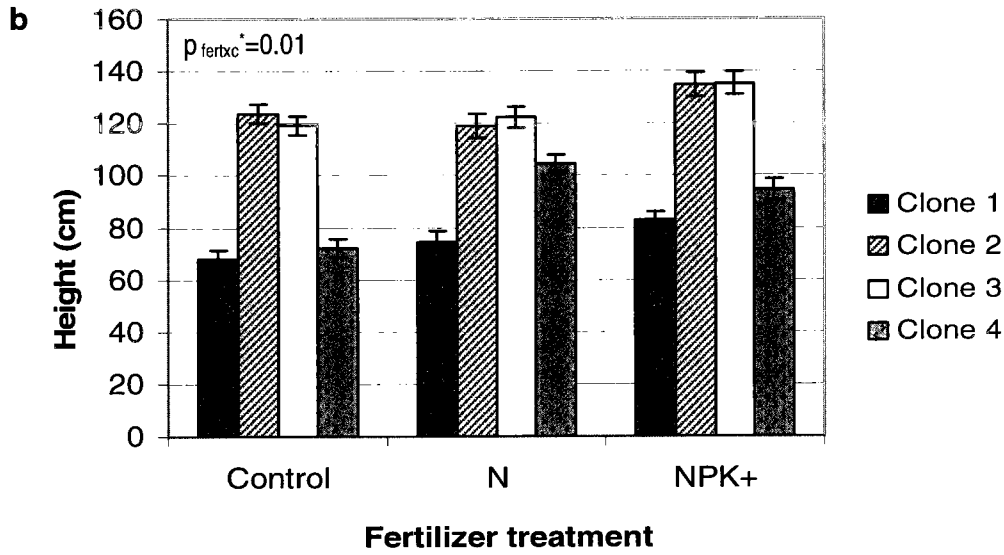




*p_{fertxc} = p-value for fertilizer x clone interaction

Figure 3.2.1.1. Mean basal diameter (mm) (±SE) by clone for each fertilizer treatment after 1 (a) and 2 (b) seasons of growth.





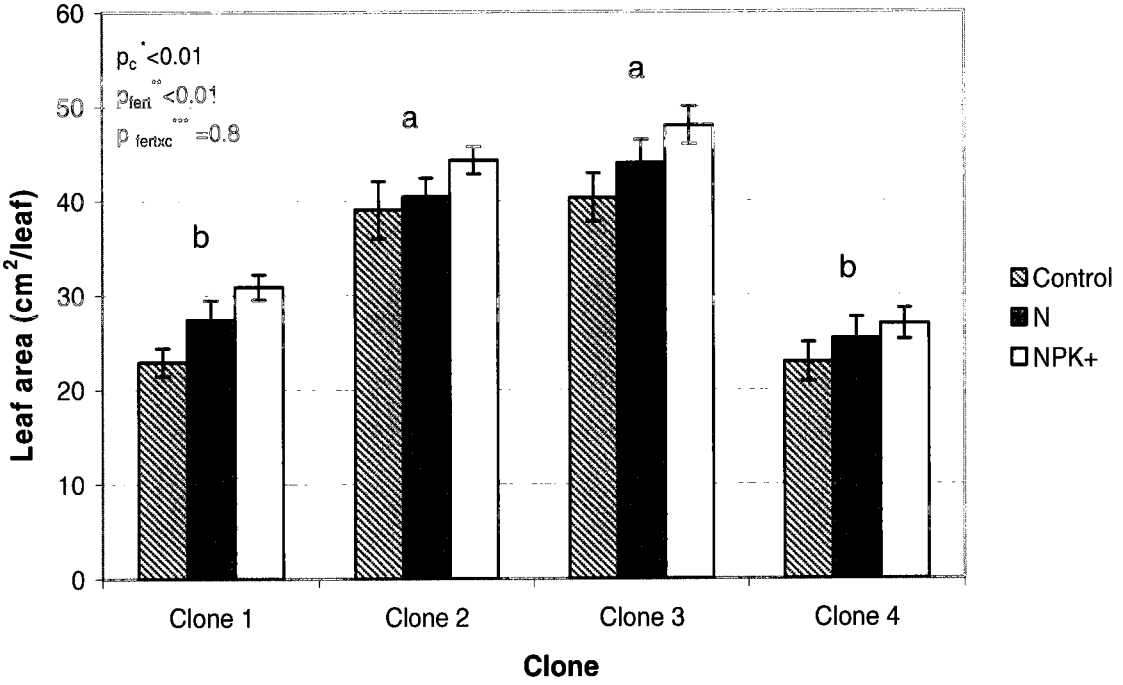
*p_{fertxc} = p-value for fertilizer x clone interaction

Figure 3.2.1.2. Mean height (cm) (±SE) by clone for each fertilizer treatment after 1 (a) and 2 (b) seasons of growth.

3.3 Tissue sampling/nutrient analysis

3.3.1 Year 1 results

Individual leaf area was significantly affected by fertilizer treatment after year one (p=0.003). Addition of N increased leaf area by 9% and NPK+ increased leaf area by 19% above control for all four clones combined. Leaf area was also significantly affected by clone (p<0.001). Clones 2 and 3 had larger leaves than clones 1 and 4. There was a relationship between leaf area and fertilizer treatment with leaf area increasing with control<N<NPK+ for all four clones (Figure 3.3.1.1). Largest leaf areas were obtained by clone 3 treated with the NPK+ fertilizer, which increased leaf area by 19% over control.



*p_c = p-value for clone
**p_{fert} = p-value for fertilizer
***p_{fertxc} = p-value for fertilizer x clone interaction

Figure 3.3.1.1. Mean individual leaf area (cm^2/leaf) (±SE) for each fertilizer treatment by clone. Letters above bars indicate significant differences between clones.

Effect of fertilizer treatment on individual leaf weight was significant after year 1 ($p=0.006$) with significant differences between all three treatments. The NPK+ treatment had the greatest leaf weight being 5% higher than the N treatment and 20% higher than the control leaf sample. Effect of clone on leaf weight was significant ($p<0.001$). Clone 3 had the highest leaf weight followed by clone 2 with clones 1 and 4 lowest (Table 3.3.1.1).

Leaf weight plotted against leaf area for the raw data showed a very strong relationship between these two traits across a wide range in both leaf areas and weights (Figure 3.3.1.2) with a positive correlation ($R^2 = 0.94$).

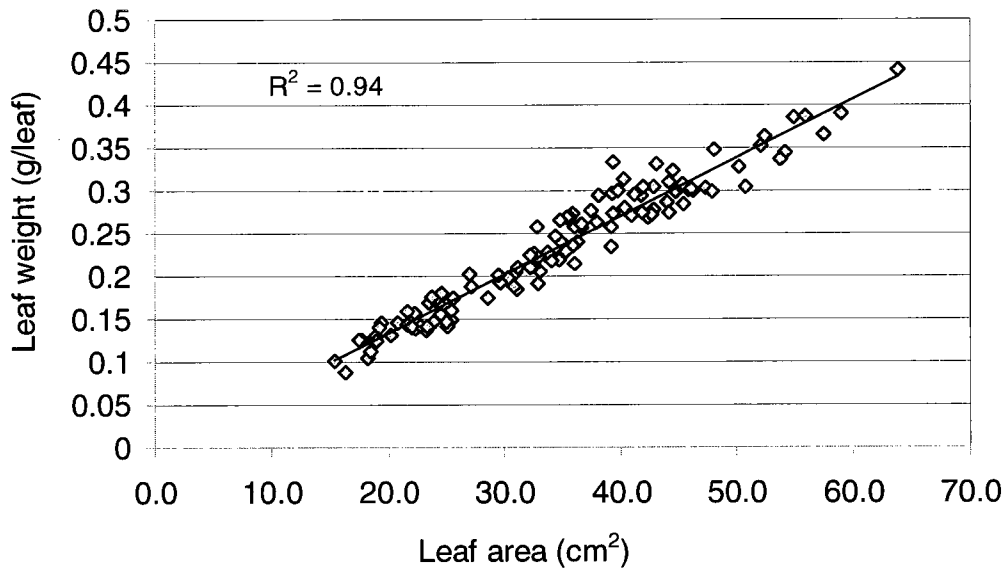


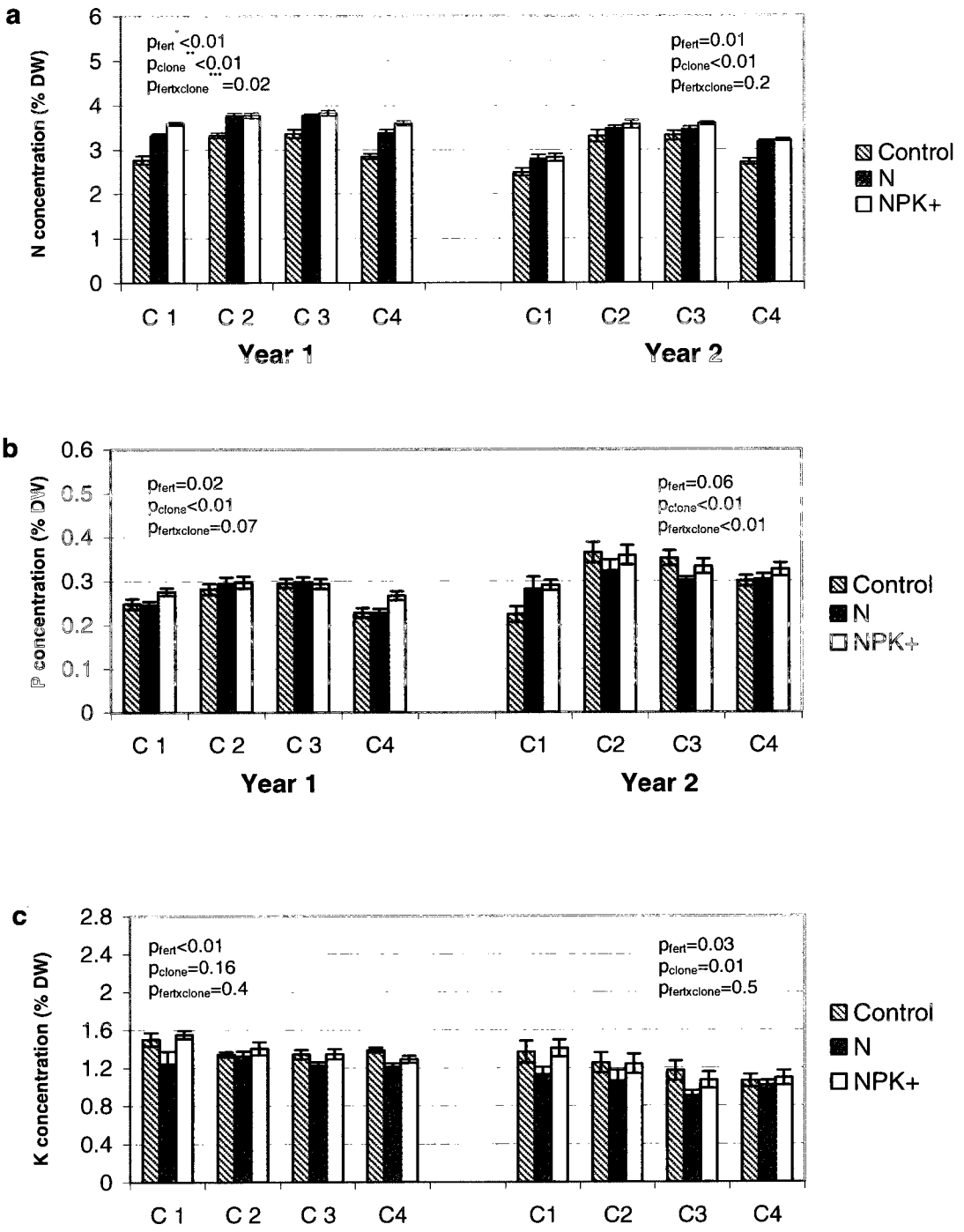
Figure 3.3.1.2. Year 1 leaf weight (g/leaf) versus leaf area (cm^2) for three fertilizer treatments and four clones combined (n=120).

Table 3.3.1.1. Means, standard errors (±SE), and p-values for leaf area (year 1) and leaf weight (years 1 and 2) in response to fertilizer treatment and clone.

		Leaf Area Year 1 (cm^2/leaf)		Leaf Weight Year 1 (g/leaf)		Leaf Weight Year 2 (g/leaf)	
Fertilizer(s) (1=none, 2=N, 3=NPK+)	Clone(s) 1=Walker, 2=Okanese, 3=WP69, 4=AP1004	Mean	SE	Mean	SE	Mean	SE
1	1-4	31.30c	1.75	0.208b	0.012	0.418	0.029
2	1-4	34.29b	1.66	0.237a	0.012	0.540	0.053
3	1-4	37.52a	1.61	0.250a	0.011	0.559	0.041
$p_{fertilizer}$		**0.003**		**0.006**		**0.106**	
1-3	1	27.07b	1.10	0.170c	0.007	0.187b	0.016
1-3	2	41.25a	1.32	0.283b	0.008	0.580a	0.060
1-3	3	44.08a	1.43	0.305a	0.009	0.636a	0.031
1-3	4	25.08b	1.16	0.170c	0.008	0.619a	0.026
p_{clone}		**<0.001**		**<0.001**		**<0.001**	

Note: Different letters following means indicate a significant difference between means for the individual trait being measured. Means for fertilizer treatment are across all four clones while means for clone are across all three fertilizer treatments.

There was a significant interaction between fertilizer treatment and clone for leaf N level ($p=0.02$) as seen by the differential response to the treatments. Leaf N level response, however, increased for all treatments in the same direction, but was not as dramatic for all clone and fertilizer combinations leading to the interaction observed (Figure 3.3.1.3 a). Therefore, treatment effects were analyzed individually. Leaf N (%) was significantly affected by both fertilizer treatment and clone ($p<0.001$). Leaf N was greatest for NPK+ with levels being 3% higher than the N only fertilizer treatment and 19% higher than the control leaf sample. Clones 2 and 3 had higher levels of leaf N than clones 1 and 4.



*p_c = p-value for clone
**p_{fert} = p-value for fertilizer
***p_{fertxc} = p-value for fertilizer x clone interaction

Figure 3.3.1.3. Means (±SE) for nitrogen (a), phosphorous (b), and potassium concentration (c), as percent dry weight (DW), for fertilizer treatment and clone combinations for years 1 and 2.

Leaf P (% dry weight) was significantly influenced by fertilizer treatment ($p=0.02$) with NPK+ being greater than control and N alone. Clone also influenced leaf P level ($p=0.001$) with clones 2 and 3 having the highest level, clone 1 being next and clone 4 having the lowest P level. Individual clones showed variable response in leaf P to the fertilizer treatments with trends for clones 1 and 4 being similar and clones 2 and 3 being similar (Figure 3.3.1.3b). Leaf K was significantly affected by fertilizer treatment, with the N treatment resulting in lower leaf K than control and NPK+ across all four clones (Figure 3.3.1.3c). Fertilizer treatment also significantly affected levels of S, B, Cu, Zn, Ca and Mn (all $p<0.05$) (Appendix B). Leaf levels of S, B, Zn, Ca, Fe, Mg and Mn were significantly different in different clones (Appendix B). Resolution of the nutrient testing methods that were used could not accurately determine levels of Mo and Na for all treatment combinations, therefore no statistical analysis could be completed for these nutrients.

3.3.2 Year 2 results

Individual leaf weight was significantly affected by clone in year 2 ($p<0.001$) with clones 2, 3 and 4 being greater than clone 1 (Table 3.3.1.1). Leaf weight increased from year 1 to year 2 overall (Figure 3.3.2.1). Clone 4 showed the greatest increase with leaf weight increasing approximately 3.6 times from year 1 to 2, clones 2 and 3 were similar at approximately 2 times greater and clone 1 was relatively unchanged. Leaf nitrogen level was significantly affected by fertilizer ($p=0.007$) and clone (Figure 3.3.1.3a) ($p<0.001$). N and NPK+ treatments were statistically the same but higher than control by 9% and 11%, respectively. With respect to clone, clones 2 and 3 were the same with the highest leaf N level, clone 4 was next and clone 1 had the lowest leaf N level averaged across the fertilizer treatments. Overall trend for leaf N level in all clones showed an increase from control to N to NPK+ treatments (Figure 3.3.1.3a). Leaf phosphorous levels could not be analyzed for individual clone or treatment effects as there was a significant interaction between clone and

fertilizer treatment (p=0.001). However, trends for clones 1 and 4 showed increasing P levels from control to N to NPK+, whereas clones 2 and 3 had the highest leaf P in the control, and lowest leaf P in the N treatment (Figure 3.3.1.3b). Leaf K levels were significantly affected by both fertilizer treatment (p=0.03) and clone (p=0.01). Control and NPK+ had significantly higher levels of K than the N treatment. This trend was the same as that found in the first season and was consistent across all four clones (Figure 3.3.1.3c). Clone 1 had the highest leaf K content with clone 2 being next and clones 3 and 4 having the lowest K levels. Leaf S and Zn were also significantly affected by fertilizer treatment (Appendix B). Clone significantly affected S, B, Cu, Zn, Ca, Fe, Mg and Mn (Appendix B). Resolution of the nutrient testing methods used could not accurately determine levels of Mo and Na for all treatment combinations, therefore no statistical analysis could be completed for these nutrients.

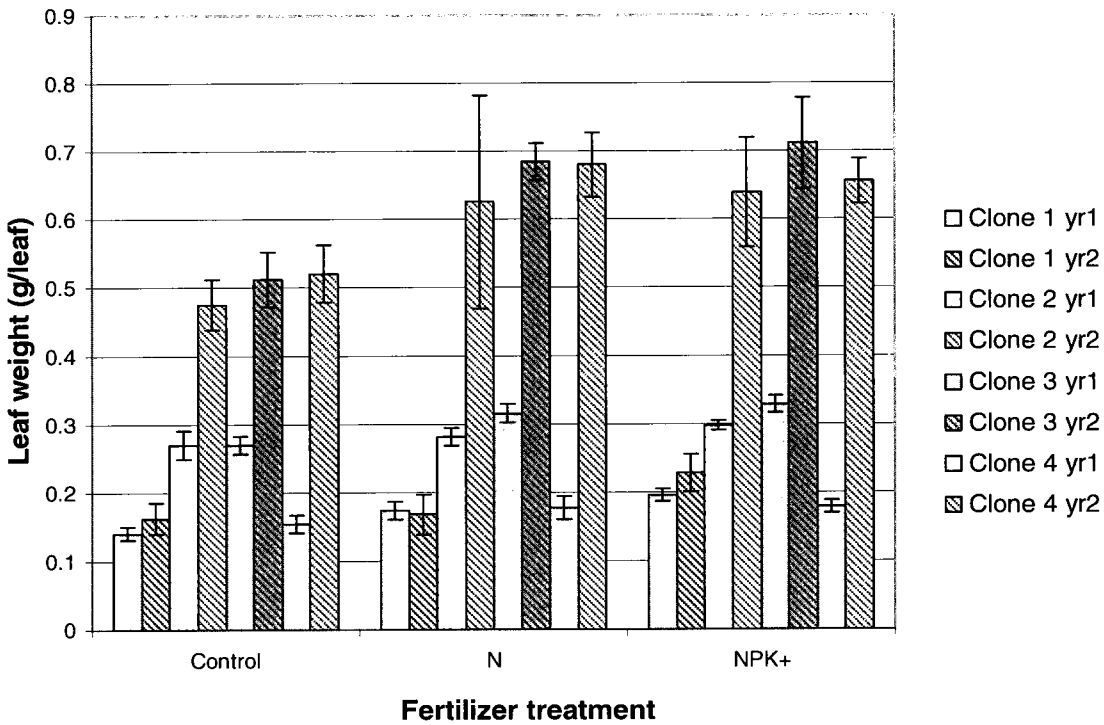


Figure 3.3.2.1. Comparison of mean leaf weight (g/leaf) (±SE), by clone and year, to fertilizer treatment.

Comparison of mean year 2 treatment values of leaf N, P, and K concentrations with year 2 leaf weight values showed that there was a relationship between nutrient content in percent dry weight and total amount of nutrient in grams with respect to mean leaf sample weight. For N, increasing leaf weight resulted in increased overall leaf N content (positive slope) as a percent dry weight (R^2=0.67). Total leaf N (g), calculated by taking N%/100 multiplied by dry weight also showed an increase in total leaf N in response to increases in leaf weight (R^2=0.97) (Figure 3.3.2.2). The relationship between P concentration (R^2=0.39) and total P (R^2=0.98) was similar with increases in P concentration and total P as leaf sample weight increased (Figure 3.3.2.3). Potassium content, however, did not follow the same trend as N and P for concentration. Potassium concentration decreased as leaf area increased resulting in a negative slope (R^2=0.49) while total K in grams for the total leaf sample increased (R^2=0.95) (Figure 3.3.2.4). Overall fertilizer treatments N and NPK+ had higher leaf sample weights and nutrient levels than control (F1). Relationships in the data between nutrient levels and leaf sample weight were not as strong in year 1 as year 2.

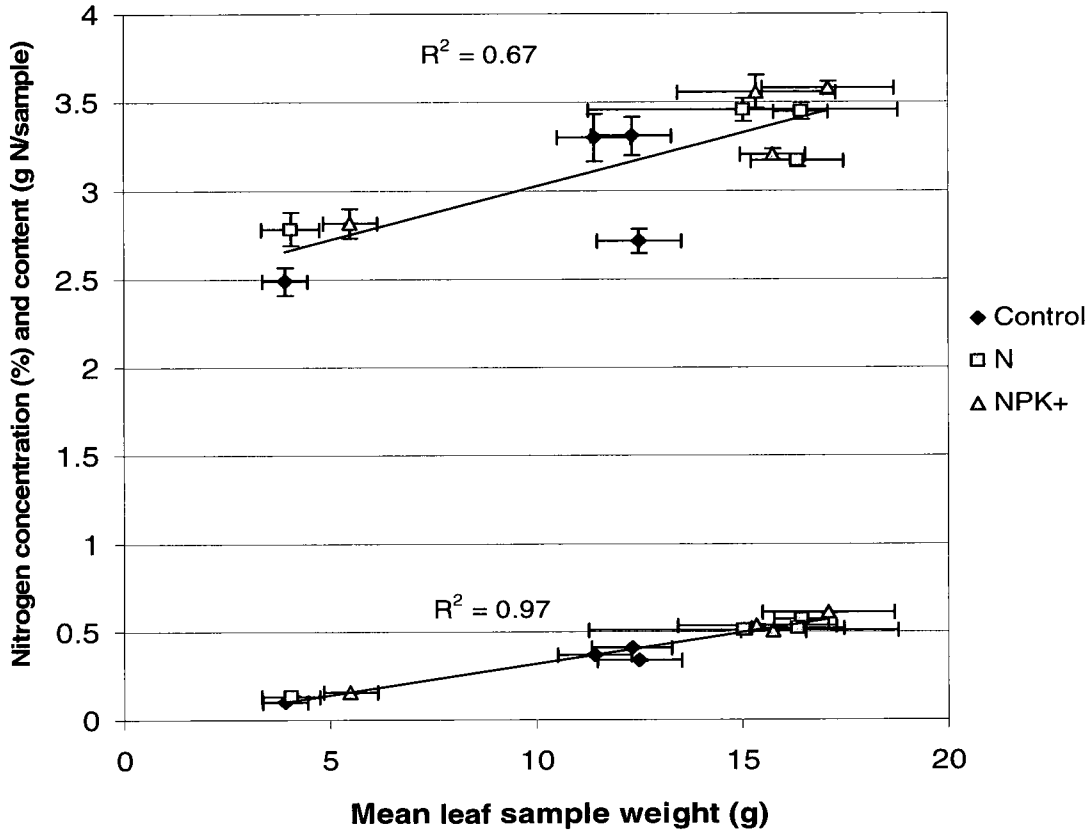


Figure 3.3.2.2. Nitrogen concentration in percent (±SE) (top data series) and content in grams N/sample (±SE) (bottom data series) versus mean leaf sample weight in grams (±SE) for year 2.

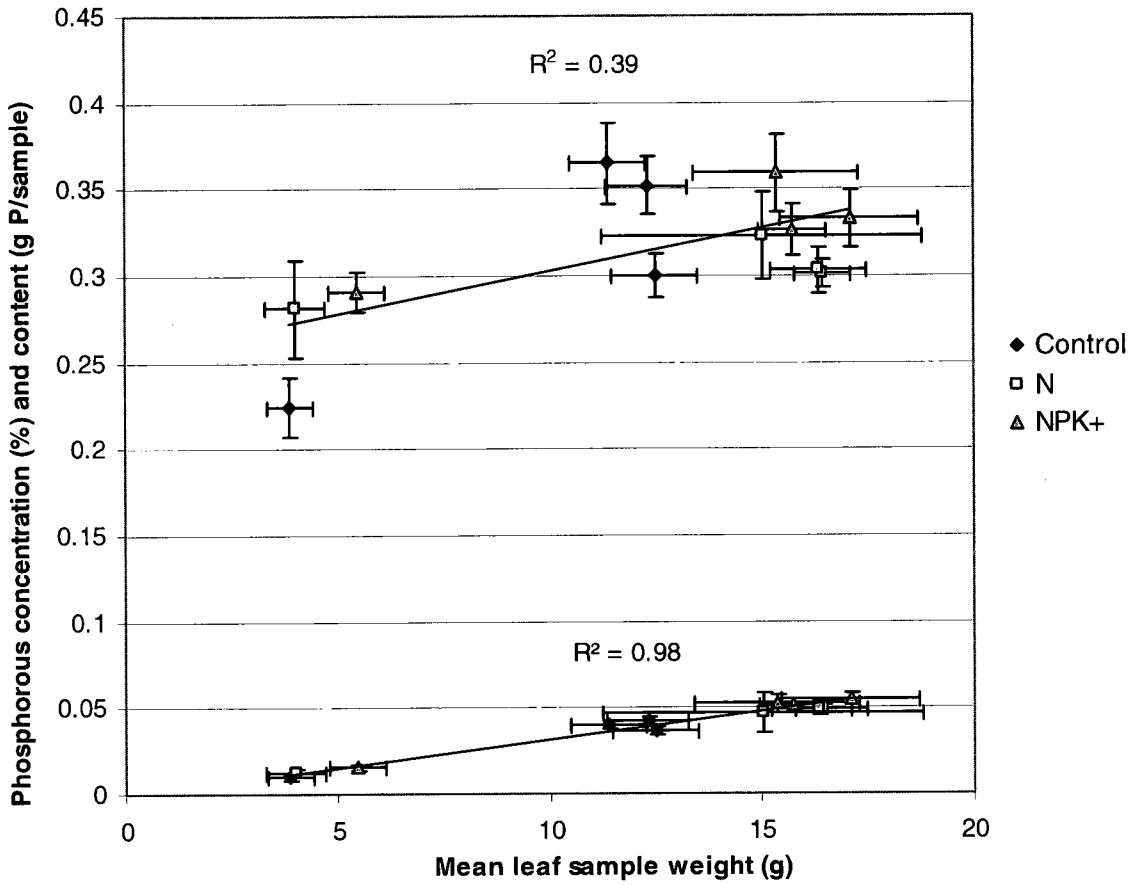


Figure 3.3.2.3. Phosphorous concentration in percent (±SE) (top data series) and content in grams P/sample (±SE) (bottom data series) versus mean leaf sample weight in grams (±SE) for year 2.

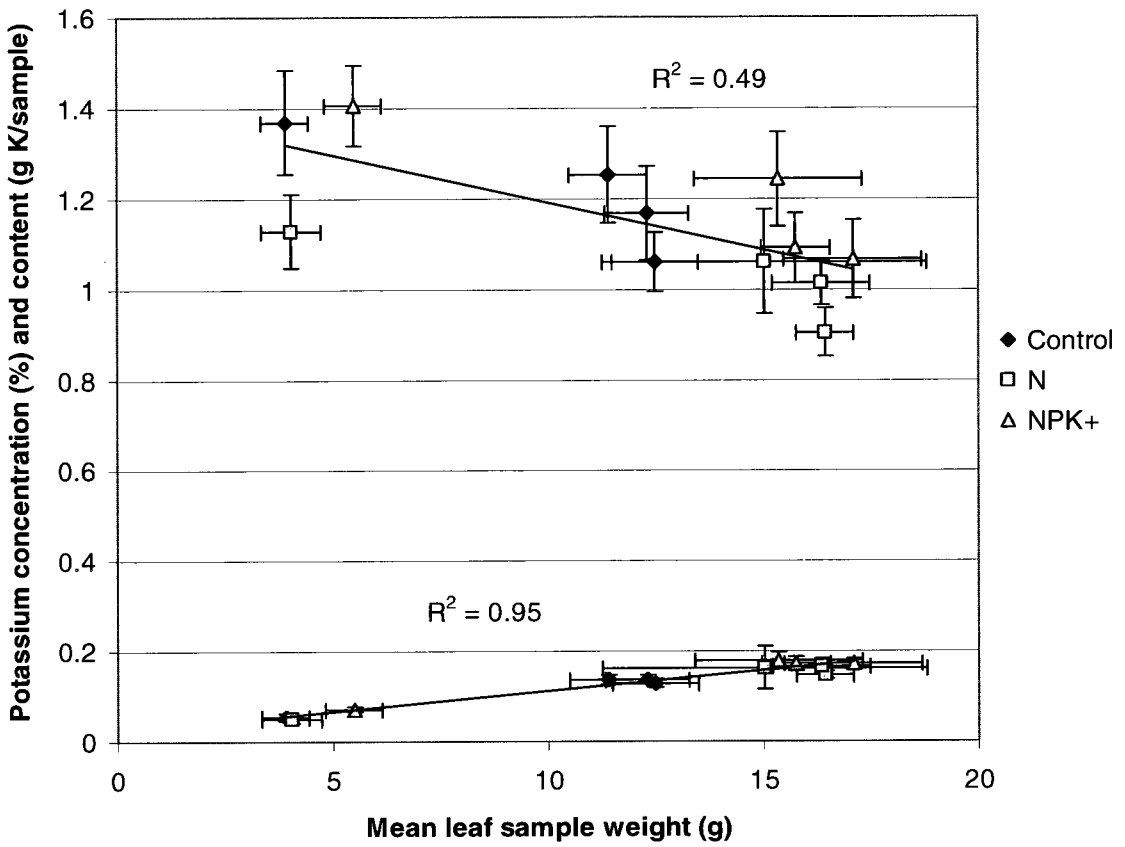
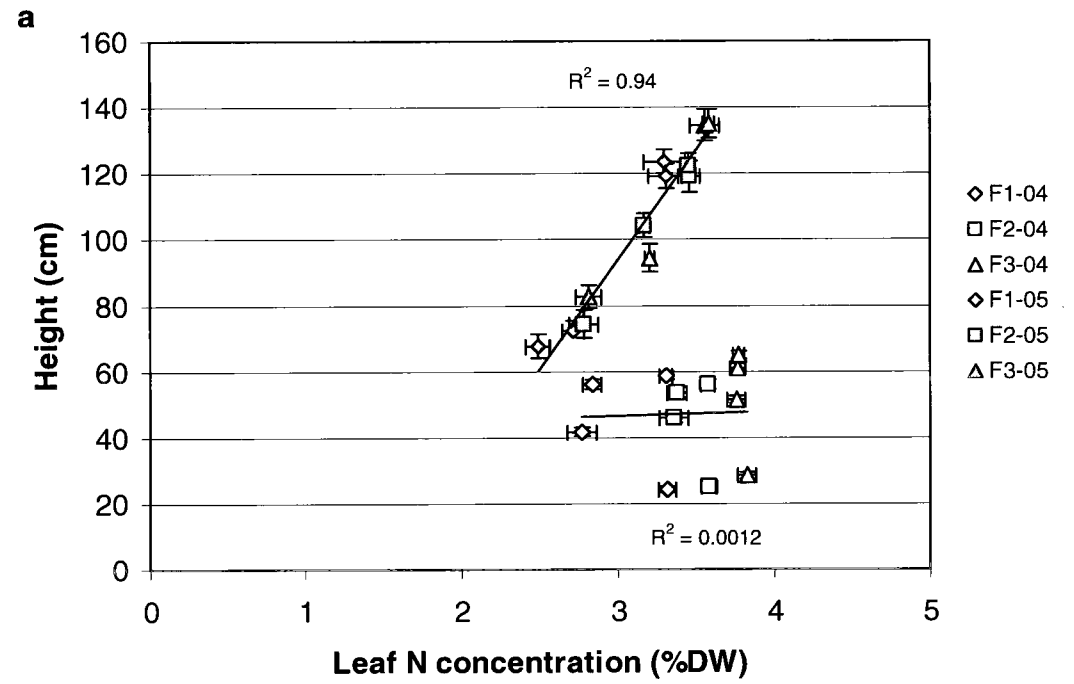


Figure 3.3.2.4. Potassium concentration in percent (±SE) (top data series) and content in grams/leaf (±SE) (bottom data series) versus mean leaf sample weight in grams (±SE) for year 2.

Leaf N levels were compared to growth parameters for the year 1 and 2 data. Year 1 results showed little relationship between N level and both height (R^2=0.001) and basal diameter (R^2=0.23). However, year 2 results showed very strong relationships between N content and height (R^2=0.94) and N content and basal diameter (R^2=0.90) (Figures 3.3.2.5a & b).



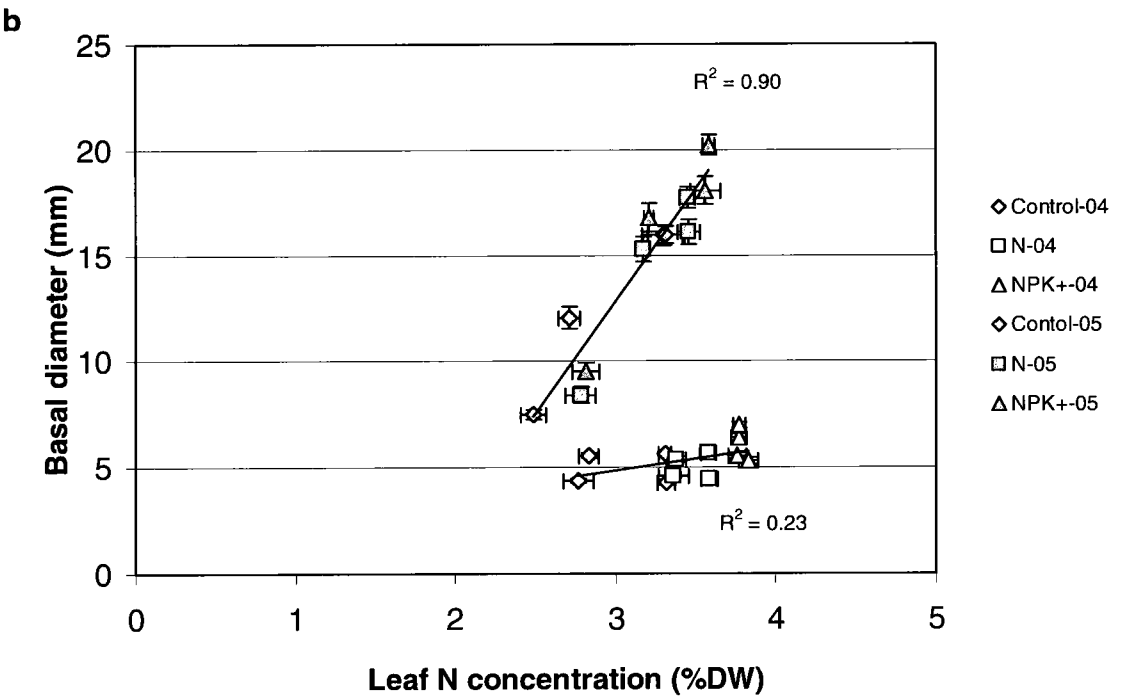


Figure 3.3.2.5. Mean height (cm) (±SE) versus leaf N concentration (%DW) (±SE) (a) and mean basal diameter (mm) (±SE) versus leaf N concentration (%DW) (±SE) (b) for fertilizer treatment and year across all four clones.

3.4 Destructive tree sampling/nutrient analysis

Differences in leaf, stem and root dry weights were significantly different between clones. Clone 3 had the highest and clone 1 had the lowest dry weights of the four clones (Figure 3.4.1). Stem weight accounted for the highest proportion of tree total mass, with leaves next and roots contributing the least. Root-to-shoot ratios calculated from the dry weights of the root and stem tissue from the D-trees showed no significant effect of fertilizer treatment ($p=0.16$) even though NPK+ showed a 22% and 21% lower root-to-shoot ratio than control and N, respectively. Root-to-shoot ratio was significantly affected by clone ($p=0.017$) with clone 1 being significantly lower than clones 3 and 4 and clone 2 not being significantly different than any of the other clones.

There were no significant differences for leaf, stem or root nutrient concentrations in response to fertilizer treatment. Leaf P, S, Ca, Cu, Fe, and Mg levels were significantly affected by clone (Appendix B). Levels of K, S, B, Zn, Ca, Fe, and Mg were significantly affected ($p<0.05$) by clone for stem tissue (Appendix B). There were no significant differences in root nutrient levels between fertilizer treatments or clones. Resolution of the nutrient testing methods used could not accurately determine levels of Mo and Na for all treatment combinations, therefore no statistical analysis could be completed for these nutrients.

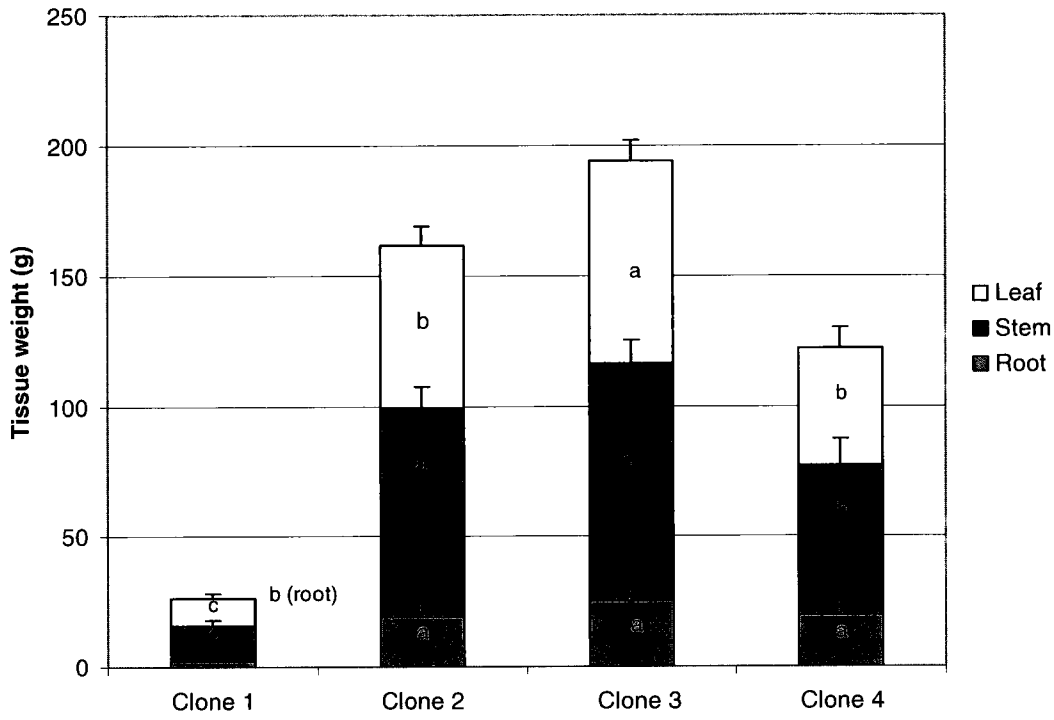


Figure 3.4.1. Clonal differences in mean (±SE) leaf, stem and root tissue dry weights (g) for D-trees. Letters in bars indicate significant differences ($p \leq 0.05$) between clones for leaf, stem and root portions of the trees sampled.

Comparison of nutrient levels in the whole tree analysis (D-trees) to the analysis of the samples of leaves collected from the actively growing terminal leaders (see Materials and Methods 2.4.3 and 2.4.4 for collection criteria) showed differences in several key nutrients. Results from paired t-tests showed significant differences ($p \leq 0.05$) between sample and whole tree nutrient values for N, P, Ca and Fe for all three fertilizer treatments, Bo for treatments 1 and 3, K and Zn for treatment 2 and Mn for treatment 1 (Figures 3.4.2 a and b).

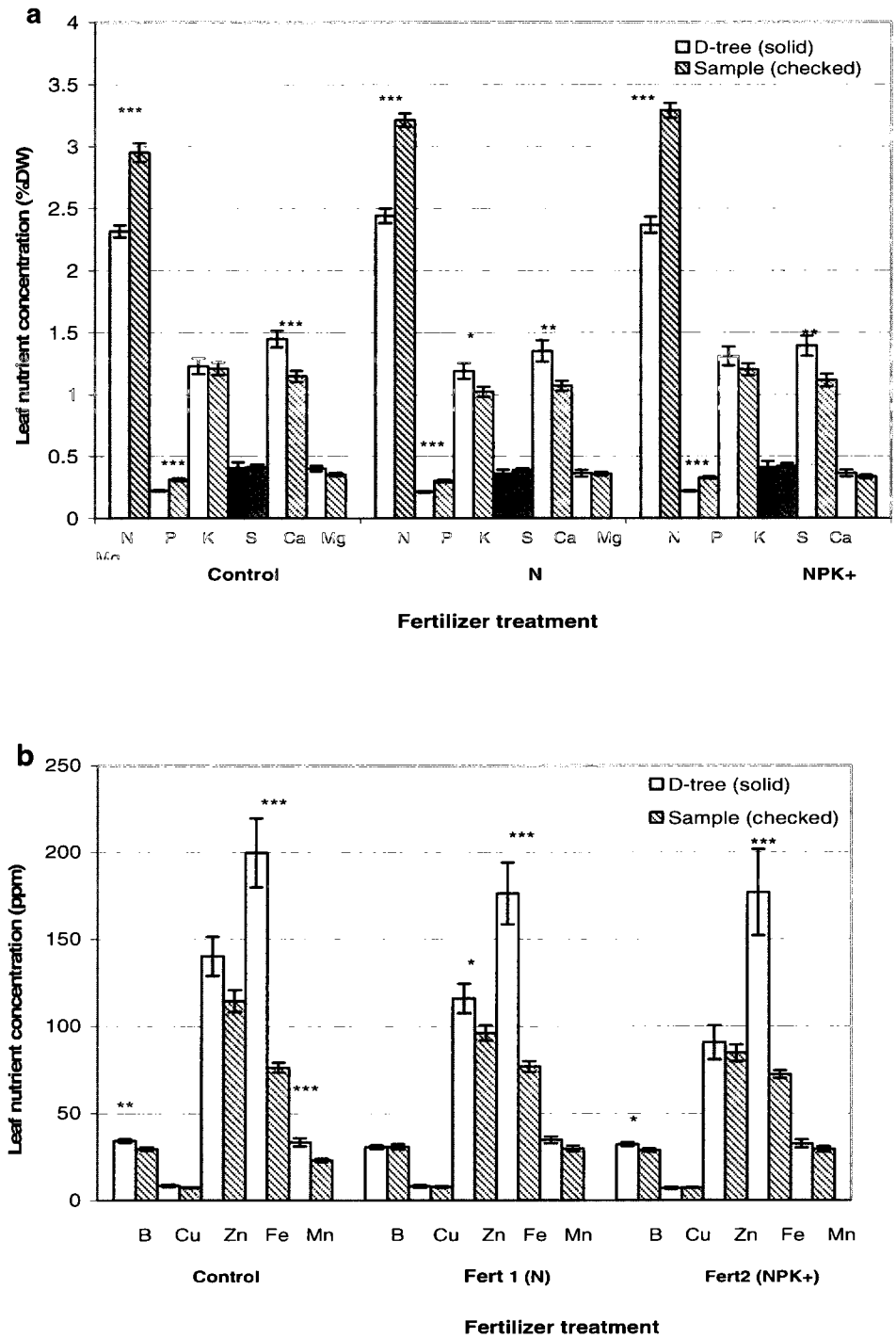


Figure 3.4.2. Mean leaf N, P, K, S, Ca and Mg concentrations (%DW) (±SE) (a) and mean B, Cu, Zn, Fe, and Mn concentrations (ppm) (±SE) (b) for whole D-tree (solid bars) versus sample tree tissue analysis (checked bars) for all three fertilizer treatments. Whole D-tree analysis included all leaves harvested from individual D-trees following extraction, whereas sample tissue analysis comprised of a composite collection of leaves collected from the terminal leaders of all trees/treatment/ block by clone. Levels of significance for t-tests comparing paired whole tree and sample values are denoted as *** = $p<0.001$, ** = $p<0.01$ and * = $p<0.05$.

3.5 Gas exchange

There were no significant differences in transpiration rate (E), net assimilation (NA), or stomatal conductance (g_s) with respect to fertilizer type. However, there were differences in E ($p=0.026$) (Figure 3.5.1 a) and NA ($p<0.001$) (Figure 3.5.1 c) between the clones. Clone 4 had higher rates of E and NA than the other clones with clones 2 and 3 being 2nd highest and clone 1 being the lowest (Figure 3.5.1). There were no clonal differences for g_s (Figure 3.5.1 b). There were no significant differences in WUE for fertilizer treatment but there were for clone ($p<0.001$). Clone 4 had the highest WUE but it was not significantly different from clones 2 and 3. Clone 1 had the lowest WUE which was significantly different from the other three clones (Figure 3.5.1 d).

There were no significant differences in leaf carbon isotope ratios ($\delta^{13}C$). in year 1, but there were for both fertilizer ($p=0.001$) and clone ($p<0.001$) in year 2. Fertilizers 2 and 3 showed higher $\delta^{13}C$ (less negative values) than fertilizer 1. Clones 2 and 3 showed the highest $\delta^{13}C$, with clone 1 next, and clone 4 being the most negative. Ranking for response to fertilizer by clone was C3<C2<C1<C4 for all three fertilizer treatments (Figure 3.5.2). When $\delta^{13}C$ was plotted against leaf sample weight it was evident that there was a consistent relationship with $\delta^{13}C$ increasing with increased leaf sample weight for all four clones (Figure 3.5.2). Clone 1 appeared to have a more rapid change in $\delta^{13}C$ with a smaller change in leaf weight than the other clones.

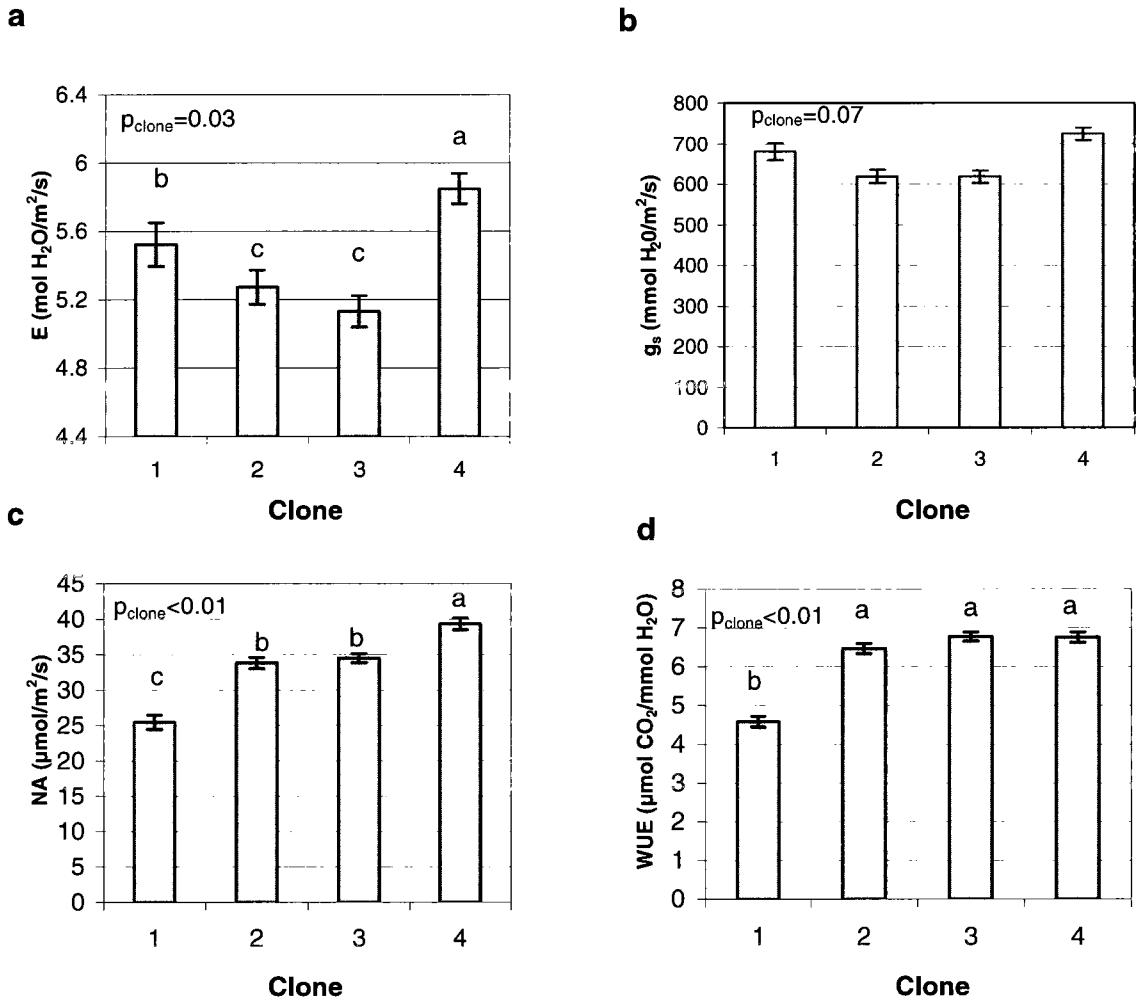


Figure 3.5.1. Mean (±SE) for transpiration rate (E) (a), stomatal conductance (g_s) (b), net assimilation (NA) (c), and water use efficiency (WUE) (d) measured for the four clones across the three fertilizer treatments. Letters above bars indicate significant differences between clones.

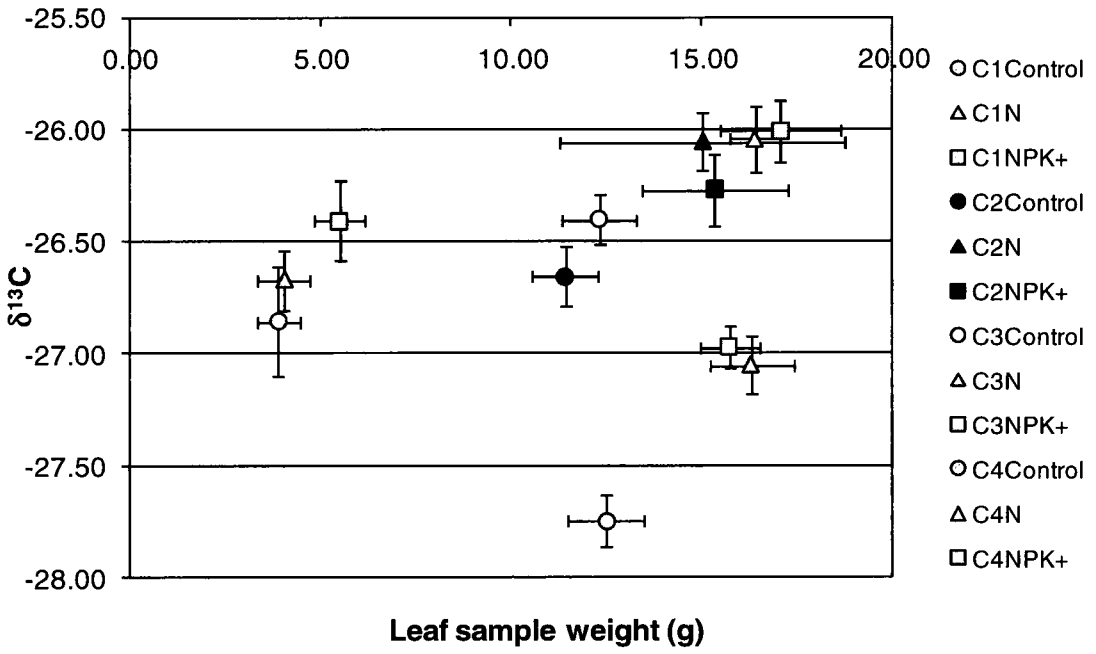


Figure 3.5.2. Leaf carbon isotope ratios ($\delta^{13}C$) (±SE) plotted against leaf sample weight (g) (±SE) for each clone and fertilizer treatment combination.

4.0 Discussion

Available resources provided by the site are critical in the overall productivity of hybrid poplar stands. Sites that provide optimal performance for hybrid poplars are those with moist, well aerated soils that are rich in nutrients (Baker and Broadfoot 1979). Soils should also be sufficiently deep (>1m to the water table), have a medium texture (sand/loam), and have a soil pH in the 5.0-7.5 range (Baker and Broadfoot 1979). Suitable annual precipitation is in the 390-430 mm range (Vanin and Birgon 1995). Acceptable soil pH is critical as pH outside the acceptable range can affect the ability of poplars to take up nutrients (Vanin and Burgon 1995). The site where my research was conducted largely meets these criteria. Mean soil pH across the five blocks of the trial was 6.62±0.1, which is well within the optimal range, therefore, nutrient uptake should not have been adversely affected. The soil was over 1 meter deep, with clay

loam in the A horizon and heavier clay in the B horizon. However, the high levels of clay on this site may be potentially limiting to growth due to imperfect drainage and reduced aeration. Soil organic matter was 4.1±0.4 % which is within the acceptable range for soil/nutrient relations. Although soil nutrient analysis was done on the site prior to installation of the trial, there is still insufficient information available in the literature to determine what soil nutrient levels are adequate to maintain or optimize tree growth for the prairie/boreal region. Overall, however, soil conditions on the site, other than potential nutrient limitations, were likely not limiting to tree growth.

It has been previously reported for the prairie region that fertilization has not significantly increased hybrid poplar growth in either height or diameter and has even been found to cause growth reductions and decrease survival (Booth 2008, van den Driessche *et al.* 2003, DesRochers *et al.* 2006). In this experiment, however, NPK+ fertilizer additions significantly increased both height and diameter growth after years 1 and 2, and N alone increased both height and diameter growth after 2 years for all clones tested. Since both the N and NPK+ fertilizer treatments added the same amount of N in the same form, additional growth in the NPK+ fertilizer treatment can likely be attributed to the availability of the additional nutrients from the NPK+ fertilizer.

A factor contributing to the positive growth response to the application of fertilization in this experiment, was the relatively high level of available soil moisture as compared to the site conditions during the experiments by van den Driessche *et al.* (2003) and DesRochers *et al.* (2006). The van den Driessche *et al.* (2003) study was investigating response of trembling aspen (*Populus tremuloides*) to fertilizer and irrigation treatments and was conducted at Drayton Valley, AB. It showed that sufficient moisture was critical to increasing growth rates by adding fertilizer. Their results showed that non-irrigated fertilized trees had no increase in growth, and suffered a decrease in survival of 17% as compared to control trees, while irrigation and fertilizer treatments combined did not affect survival but produced trees with an increase in height of 19% and stem volume of 92% after three growing seasons. During both years of my experiment

the site received sufficient moisture with above average natural rainfall in the growing months from May to September for both seasons. Local precipitation for 2004 was 571.2 mm and in 2005 it was 336.5 mm, with 308.2 mm and 255.7 mm as rainfall each year, respectively, during the growing season from May 1-August 31 (climatic data from Environment Canada weather station 'Athabasca2'). As illustrated in Figures 3.1.1 and 3.1.2 soil moisture levels were above wilting point throughout most of each growing season. The high available moisture levels would have reduced or eliminated soil moisture as a constraint to growth in this experiment.

Adequate levels of available soil moisture during my experiment would have also increased the ability of plant roots to take up water soluble soil nutrients (Tisdale *et al.* 1993), as seen by leaf, stem and root tissue nutrient levels. Tissue analysis showed that levels of several nutrients increased compared to control with the addition of the fertilizer treatments indicating that the nutrients provided by the fertilizer were taken up by the trees.

Additions of N by fertilization significantly increased: 1) N concentration in leaf tissue, 2) leaf area and weight and ultimately 3) height and basal diameter. Leaf N levels were significantly affected by fertilizer treatment in years 1 and 2 with the NPK+ treatment significantly increasing leaf N level above that of the N only treatment and control for both years. Leaf N levels were strongly correlated with height and basal diameter for year 2 and there was a relationship with basal diameter for year 1. These results suggest that increased leaf N was associated with increased growth, which was evident for both fertilizer treatments across the four clones. The significant increases in growth due to the addition of N fertilizer in this study shows that soil N was likely limiting growth on the site. This is evident by the increased growth in response to the addition of N in the N only fertilizer treatment. Leaf tissue N also indicated a potential deficiency in year 2 with leaf N levels in control trees being below 3%, which is considered to be a boundary between low and adequate leaf N level in hybrid poplars (Pregitzer *et al.* 1990, Hansen 1994).

Sample leaf N was also significantly higher than whole D-tree leaf N. Sample leaf tissue was taken from the uppermost fully expanded leaves on the terminal leader, where maximum photosynthetic rates are typically observed, which may account for the higher levels of leaf N observed in the upper canopy. Whole tree leaf N included all of the leaves from the trees, and had a lower mean level of N as the older/lower leaves likely had lower N content and would tend to decrease the overall mean N content in the foliage sample (Hansen 1994). Even though mean levels of whole tree leaf N were between 2.0 and 2.5%, actual levels of N in the older/lower leaf portion of the sample would have likely been below these levels and the high N levels in the younger leaves would have partially buffered the lower N levels in the older leaves. The overall decreased N levels found in the whole tree sample suggests that a portion of the N may have been retranslocated from the lower leaves to the upper canopy, since nitrogen is highly mobile in plants (Tisdale *et al.* 1993).

A large portion of the increased N uptake would have been used in the production of amino acids. Nitrogen makes up a large component of amino acids found in proteins, especially those found in the photosynthetic apparatus, such as RuBisCO (Raven *et al.* 1992). With increased N uptake and utilization, it may be assumed that there was an increase in production of RuBisCO, although this was not measured. Increased levels of RuBisCO would therefore be relative to the overall increased size of the leaves, resulting in higher levels of photosynthesis, leading to increased production of carbohydrates enabling and sustaining the elevated growth rates that were observed.

Levels of leaf P were only significantly affected by the NPK+ treatment in year 1. Levels of leaf P stayed relatively constant across the year 2 and whole D-tree tissue analysis. The rate of P uptake increased in response to increased growth rate as indicated by the trees in the N and NPK+ fertilizer treatments being able to maintain constant levels of leaf P. Phosphorous provided by the NPK+ fertilizer may have contributed to this uptake to offset demands due to the increased growth observed between the N and NPK+ treatments. The additional P may account for a large portion of the increased growth as some fertilizer

experiments have shown that P treatments can lead to larger increases in growth than N alone (van den Driessche and Brown 1996), with responses reduced under dry soil conditions (van den Driessche 1996). Soil pH on the site should have been optimal for P availability as maximum available P is found in the pH range 5.5 to 6.5 (Tisdale *et al.* 1993). Placement of P fertilizer near the base of the trees, as compared to broadcasting followed by incorporation, or banding, would also have reduced the rate at which P would have been bound to soil particles. Having P fertilizers more concentrated, as is the case with placement applications, reduces exposure to the soil particle surface and adjacent soil surface binding sites, creating a temporary buffer, limiting the rate of P adsorption to the soil particles. Placement of concentrated water soluble P fertilizers such as the superphosphates and ammonium phosphates, as compared to other methods of application, generally increases availability and utilization by plants (Tisdale *et al.* 1993). However, with the high moisture levels present, and over the duration of the experiment, available P supplied by the fertilizer may have eventually leached out of the rooting zone, increasing exposure and the probability of being bound to the soil particles. As a result the overall availability of P would have become increasingly dependent on the existing soil water-P balance and rate of plant uptake (Tisdale *et al.* 1993). Following exposure to the soil particles, high levels of clay in the A and B soil horizons could partially limit P availability on this site. Soil P and P applied in fertilizers can be rapidly bound to soil particles, especially clays, where there is high reactive mineral surface area. Therefore, combined unfavorable clay type soils and high moisture conditions, with respect to P availability, increases the importance of targeted placement of the fertilizer into the rooting zone of newly planted trees.

Once in the plant, P is highly mobile. Significant differences in whole D-tree versus sample leaf P may indicate that P was mobilized from the older leaf tissues and transferred to the actively growing terminal portions of the tree. Leaf P also increased with leaf size indicating that P levels were likely adequate to maintain the elevated growth rates found in response to the fertilizer treatments.

P levels commonly accepted as adequate for poplar growth are from 0.25-0.5% (van den Burg 1985, van den Driessche 1998), which would put the results from this analysis at or just below adequate depending on the samples observed.

Year 1 and 2 tissue analysis data showed significantly lower levels of K for the N only treatment than both control and NPK+ treatments. This trend was consistent for all individual clone and fertilizer treatment combinations indicating that the demand for K in response to increased growth rates, seen in the N only fertilizer treatment, was proportionally higher than K uptake by roots and mobilization from other tissues within the plant. As a result, limitations to the rate of K uptake caused a dilution effect of the available plant K resulting in overall lower leaf K concentrations. Evidence of this is seen in the relationship between leaf weight and leaf K concentration (Figure 3.3.2.2c). Increased leaf weight, indicating increased growth, showed decreased leaf K concentrations across all clone and fertilizer treatment combinations. With increases in leaf weight, total leaf K, measured in grams per leaf, did increase but not at a sufficient rate required to maintain a steady concentration of leaf K. The greater the leaf weight/size the higher the level of dilution that was observed (Figure 3.3.2.2c). With the already low leaf K levels seen across all of the clone and treatment combinations, dilution of leaf K in response to the N treatment may have either induced or magnified a low level K deficiency, as levels of leaf K considered to be adequate to maintain normal growth are stated to be from 1.5-2.2% (van den Burg 1985). Potassium levels in the year 1 analysis ranged from 1.25-1.40%, year 2 analysis ranged from 1.02-1.21% and whole D-tree analysis ranged from 1.19-1.31%; all indicating that K was likely low to deficient in all leaves. Addition of K in the NPK+ fertilizer compensated for the increased demand due to increased growth rate, and caused leaf K to return to similar levels as seen in the control.

Year 2 leaf sample levels (where the trend for K to be lowest due to N fertilization was most evident) had lower levels of leaf K than whole tree levels, based on the whole D-tree analysis. This is contradictory to the idea that the trees may have been K-deficient. If the trees were in fact in a deficient state it

would be expected that K should be mobilized internally from the older leaves (Cochrane and Cochrane, 2009), which are indirectly represented by the whole D-tree analysis, to the newer leaves, represented by the sample analysis. As a result, leaf sample K should have increased and whole D-tree K should have decreased, as K is considered to have very high mobility in plants (Hodge 2004).

Because K^+ is involved in water relations (Raven *et al.* 1992), especially in osmotic regulation, decreased levels of K in leaves could likely impact the overall productivity and water relations of the leaves, particularly under drought conditions. Decreases in leaf K cause stomatal conductance to decrease as a result of decreased turgor in stomatal guard cells (Tisdale *et al.* 1993). Trends for mean rates of transpiration and stomatal conductance showed decreased levels in trees that were fertilized as compared to control trees, but were not significantly different. Reduced stomatal conductance would have in turn limited both flow of CO_2 into, and water vapor out of, the leaves. When flow of CO_2 is unrestricted between ambient air and the inside of leaf tissues discrimination favoring fixation of $^{12}CO_2$ by RuBisCO is high (Melander and Saunders, 1979). Reduced flow of CO_2 through stomates would have therefore limited the ability of the plants to preferentially fix $^{12}CO_2$, during photosynthesis, over $^{13}CO_2$. Levels of $\delta^{13}C$ in the year 2 leaf samples were significantly higher (less negative) for the N and NPK+ treatments than the control trees, thus further supporting the idea that the fertilizer treatments decreased E and g_s, and may have increased WUE. Trends for mean WUE values were higher, although not significant, for the N and NPK+ treatments over control values providing additional evidence that the fertilized trees had greater WUE due to limited g_s and E. Also, the relationship between $\delta^{13}C$ and leaf weight, showed increases in $\delta^{13}C$ with increases in leaf weight, and was consistent for all four clones as shown in Figure 3.5.2. The increased WUE likely allowed for optimal utilization of resources, mainly water, leading to increased leaf growth. Although increased $\delta^{13}C$ and WUE and decreased E and g_s are often signs of water stress, they can also indicate increased physiological efficiencies as is likely the case here since there were no

significant differences in levels of NA. If the trees were under water stress, differences in NA should have been observed between the treatments.

Sulfur levels followed a similar trend to K levels with the N treatment having lower leaf S than the control and NPK+ treatments, which were fairly similar. It is possible that the tree roots could not take up enough additional S to be able to maintain constant levels of leaf S with the increased growth as a result of the N fertilizer application. Similarly to K, addition of S in the NPK+ fertilizer appeared to compensate for the increased demand, and as a result S levels were brought back up to levels that were similar to control. Leaf S levels should be between 0.29 and 0.45% to maintain adequate growth (van den Burg 1985), indicating that even though the N treatment may have caused a dilution of leaf S, it was not likely enough to initiate internal mobility of S from the older tissue to the newer tissue, which would be an indicator of a potential deficiency. Sulfur has low to moderate mobility in plants and this is shown by the whole D-tree and sample leaf S levels being very similar, also indicating that allocation of S was consistent throughout the growing season throughout the different plant tissues.

Leaf Cu illustrated an unexpected trend which had levels of leaf Cu lowest for the NPK+ treatment, which contained supplemental Cu. Differences were most prevalent in year 1 as they were highly significant for treatment across the four clones. Differences were not significant for year 2 or for the whole D-tree analysis but the trend held for all of the treatment and clone combinations in year 1, 2 and the whole D-tree analysis showing NPK+ having the lowest levels of leaf and stem Cu. In contrast, D-tree root tissue had the highest levels of Cu in the NPK+ treatment. It is most likely that Cu demand, due to the increased growth rate over the N and control treatments, as a result of the NPK+ fertilizer treatment, was proportionally greater than the rate of uptake and transfer of Cu from the soil and into the plant tissues. The result was a dilution of Cu throughout the stem and leaf tissues of the trees. Also, increased levels of N in the plant stem and leaves can reduce Cu mobility, since high N in plants impedes translocation of Cu from older leaves to regions of new growth (Tisdale *et al.* 1993). This idea fits with data from the year 1 and 2 tissue analysis for the

relationship between leaf N and Cu. In the year 1 tissue analysis data, leaf N levels were higher than year 2 and not surprisingly leaf Cu levels were lower than year 2. Also, root tissue data from the whole D-trees showed higher Cu levels in the roots for the NPK+ treatment than the other treatments while stem tissue analysis showed the reverse, indicating that there may have been an inhibiting factor in transfer of Cu from the roots into the stems of the tree. Increased N in the stem tissues, provided by the fertilizer treatments, may have been the reason. Cu levels dropped into levels of deficiency in year 1 for the NPK+ treatment with mean Cu being 3.23 ppm, below the range of 5-20 ppm which is considered to be the normal range in plants (Tisdale *et al.* 1993)

Mean iron (Fe) levels in the whole D-trees were two-fold higher compared with the upper canopy leaf sample tissue. The deficiency range of Fe in poplar leaf tissue is <70 ppm (van den Burg 1985), which is lower than the range seen here suggesting any potential deficiency in the sample tissues was unlikely. Iron is generally known to be immobile in plants thus limiting movement from older tissues to actively growing regions of the plant (Tisdale *et al.* 1993). Immobility of Fe would explain why the whole D-tree samples had much higher Fe levels than the samples from the terminal leaders. In addition the older average age of the leaves of the whole tree sample would have had more time to accumulate Fe than the younger tissues.

Increased growth rates were related to higher specific leaf area and specific leaf weights for the trees in the fertilized treatments where significant increases of up to 33.8% in leaf weight were observed between the year 2 NPK+ treatment and control trees. Overall specific leaf weight increased dramatically from year 1 to 2 for clones 2, 3 and 4, likely leading to the greater increases in growth in year 2. N-levels also increased proportionately to sample leaf weight indicating that addition of N fertilizer was likely responsible for the increases in leaf size that were observed.

The relationship between leaf area and weight was very strong (r^2=0.94) and could therefore provide a tool that could be used to calculate approximate leaf areas or weights if only one of the two variables was known. It would be

expected that individual relationships would vary depending on species, clone or region, however, it was seen here that the relationship held true for all four clones used in this experiment (Figure 3.3.1.2).

Levels of NA did not increase with the addition of N or NPK+ suggesting that leaf N concentration may have been at a luxury consumption level following fertilization. If not at luxury N levels, increased leaf N should have led to increased NA as the majority of N found in leaves is used in the production of photosynthetic components generally leading to increased photosynthetic capacity (Evans and Seeman 1989). Thus, increases in growth were most likely due to increased photosynthetic capacity as a result of increased total leaf area and not increased photosynthesis per unit leaf area. Increased leaf areas would have greatly increased the overall photosynthetic output of the plants resulting in increased vigor and growth, as the trees were not large enough to shade each other, due to their wide spacing, limiting competition for light.

There were significant differences in growth between clones as clones 2 and 3 were taller and had greater basal diameter than clones 1 and 4 in both years of the experiment. The higher growth rates of clones 2 and 3 was likely a result of higher leaf productivity, which may indicate these clones' superior ability to take up greater amounts of nutrients and water. This was reflected in the significantly higher nutrient levels, especially N, in the leaf tissue in both years of the experiment. The allocation of higher levels of N to leaves and photosynthetic components is indicative of higher photosynthetic nitrogen use efficiency (PNUE) Hikosaka *et al.* (1998). Clones 2 and 3 likely had high PNUE as they had larger leaves with higher N composition (Poorter and Evans 1998), and as a result increased productivity, which is determined by photosynthetic output and ultimately captured as growth.

Clones 2 and 3 also had significantly higher levels of P than the other clones in year 1 and this trend continued through year 2 indicating that these clones may have also had an advantage in taking up P. Phosphorous availability and uptake is critical in the early stages of growth following planting enabling plants to grow sufficient root mass to support the tree in its new environment by

exploitation of available nutrients and water. More rapid establishment would have contributed to the clones' 2 and 3's larger size at the end of the experiment.

There were also large differences in response to S accumulation in leaves indicating that there were clonal differences in their ability to uptake and store S. Clone 1 had consistently about 1/3 more leaf S than clones 2 and 3 in all of the leaf stem and root tissue analysis completed for both years of this project, indicating that it may maintain a luxury state of leaf S. Although leaf S levels in clone 4 were near the borderline between low and adequate, it is likely that they were sufficient to maintain growth for this clone. Adequate levels of S for poplar leaf tissue should be between 0.29 and 0.45% dry weight (van den Burg 1985).

Clones 2 and 3 also had significantly lower rates of E and noticeably, however not significantly, lower levels of g_s than the other clones indicating that they may have more efficient mechanisms that controlled water loss from their leaves. Although not statistically significant, the overall trend for WUE for clones 2 and 3 showed higher values than clone 4 and WUE in clones 2 and 3 were significantly higher than in clone 1. Net assimilation rates were similar for clones 2 and 3, and although they were not as high as in clone 4, combined with the overall increased leaf area exhibited by these clones, overall productivity was greater, likely leading to the observed higher growth rates.

Plants of clone 1 had significantly lower height and basal diameter than the other clones after year 2. Leaf weights and N levels were also lower than the other clones by the end of year 2, likely affecting NA, which was 25% lower than clone 2, which was the next lowest clone. Significantly lower levels of Mg and Cu were also indicative of the low photosynthetic productivity of clone 1 as Mg is an important part of the chlorophyll molecule and Cu is required for proper enzyme function in photosynthesis (Raven *et al.* 1992). WUE was also significantly lower for clone 1 than the other clones, but it is likely a function of the very low NA as rates of E for clone 1 were similar to those in other clones (WUE=NA/E).

Although root:shoot ratios for clone 1 were not significantly different from the other clones, clone 1 had extremely low levels of root production showing eight times less root dry weight than clone 2, which was the next lowest. It is

difficult to conclude from this experiment why root production was so low, since it is not possible to determine if it was purely genetic or environmental or an interaction effect. It is plausible, however, that the perceived inability of clone 1 to establish roots was limiting to its growth.

Clone 4 had the highest measured NA rates and E rates for all fertilizer and clone combinations. Generally it would be expected that if a clone had high transpiration rates it would be expected to have low WUE. However, in this case, even though clone 4 had higher transpiration rates than the other clones, its high NA more than made up for this and, as a result, its WUE was also high. Interestingly, to combat the high levels of water that this clone was losing through transpiration, it had the highest root:shoot ratio, although the root:shoot ratios were not significantly different than those in clones 2 and 3. Increased root:shoot ratio allowed trees of clone 4 to better exploit available soil water and thus meet the supply required to maintain the observed high levels of transpiration and photosynthesis.

5.0 Conclusions

Under the conditions provided by the soil and climate at the Al-Pac site, fertilization effectively increased growth rates of both hybrids and a native poplar clone when applied shortly after planting. The NPK+ treatment was more effective at supplying a balanced supply of nutrients as indicated by a greater growth response than N fertilization alone. Fertilizer, especially N, was readily taken up by the trees as indicated by increases in concentration in plant tissues. Clonal responses were also evident, as leaf nutrient levels varied in different parts of the trees at different stages of the experiment. Overall increases in growth were most likely due to increased production of leaves and increased leaf area as shown by the relationships between leaf N, leaf weight and area, and height and basal diameter growth.

The overall positive response of growth to fertilization was not as great as that found in studies done in other regions, such as the Pacific Northwest where fertilization was found to increase growth rates more than two fold (McLaughlin *et*

al. 1987, Kondziolka and Streit 1988, van den Driessche 1999.), but may be enough to warrant fertilization in situations where increased growth can be linked to increased overall survival following planting. Even small or moderate short-term growth increases due to fertilization may be of benefit to newly- planted trees, especially in regions with short growing seasons, by allowing newly-planted trees to: 1) become established and out-compete competition, 2) become large or vigorous enough to survive herbivore damage, and 3) be able to withstand environmental stresses. Lastly, of course, economics have to be taken into consideration to determine if the gains seen in this experiment warrant expensive fertilizer applications.

6.0 References

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Appendix A.

Randomization of treatments showing replicate, treatment row serial number, treatment number, irrigation, fertilizer and clone treatments, and also the position of the D-tree (tree for destructive sampling) in each row.

Block	Rep	Serial #	Treatment #	Fertilizer (1=none, 2=N, 3=NPK+)	Clone #	Clone Name	D-Tree
1	1	1	1	1	1	Walker	**8**
1	2	2	4	1	4	AP 1004	**4**
1	2	3	3	1	3	OKANESE	**2**
1	2	4	9	3	1	Walker	**3**
1	1	5	5	2	1	Walker	**4**
1	1	6	11	3	3	OKANESE	**8**
1	2	7	7	2	3	OKANESE	**7**
1	1	8	9	3	1	Walker	**5**
1	1	9	10	3	2	WP 69	**8**
1	1	10	6	2	2	WP 69	**4**
1	2	11	8	2	4	AP 1004	**3**
1	1	12	2	1	2	WP 69	**7**
1	1	13	4	1	4	AP 1004	**5**
1	2	14	11	3	3	OKANESE	**4**
1	1	15	8	2	4	AP 1004	**9**
1	1	16	12	3	4	AP 1004	**4**
1	2	17	10	3	2	WP 69	**8**
1	2	18	1	1	1	Walker	**4**
1	2	19	12	3	4	AP 1004	**4**
1	1	20	3	1	3	OKANESE	**4**
1	2	21	5	2	1	Walker	**9**
1	2	22	6	2	2	WP 69	**4**
1	1	23	7	2	3	OKANESE	**6**
1	2	24	2	1	2	WP 69	**1**
2	1	25	8	2	4	AP 1004	**6**
2	2	26	4	1	4	AP 1004	**7**
2	1	27	1	1	1	Walker	**9**
2	2	28	2	1	2	WP 69	**4**
2	1	29	5	2	1	Walker	**1**
2	1	30	10	3	2	WP 69	**2**
2	2	31	9	3	1	Walker	**1**
2	1	32	2	1	2	WP 69	**1**
2	1	33	4	1	4	AP 1004	**6**
2	2	34	3	1	3	OKANESE	**1**
2	1	35	6	2	2	WP 69	**6**
2	2	36	6	2	2	WP 69	**9**
2	2	37	12	3	4	AP 1004	**2**
2	2	38	8	2	4	AP 1004	**7**
2	2	39	10	3	2	WP 69	**5**
2	1	40	12	3	4	AP 1004	**5**
2	1	41	9	3	1	Walker	**1**
2	2	42	5	2	1	Walker	**3**
2	2	43	7	2	3	OKANESE	**5**

2	1	44	7	2	3	OKANESE	4
2	1	45	3	1	3	OKANESE	6
2	2	46	1	1	1	Walker	2
2	1	47	11	3	3	OKANESE	8
2	2	48	11	3	3	OKANESE	1
3	2	49	11	3	3	OKANESE	7
3	1	50	8	2	4	AP 1004	8
3	1	51	5	2	1	Walker	1
3	1	52	4	1	4	AP 1004	6
3	1	53	2	1	2	WP 69	7
3	2	54	6	2	2	WP 69	8
3	1	55	7	2	3	OKANESE	1
3	1	56	6	2	2	WP 69	8
3	2	57	1	1	1	Walker	2
3	1	58	1	1	1	Walker	4
3	2	59	8	2	4	AP 1004	2
3	1	60	10	3	2	WP 69	6
3	2	61	5	2	1	Walker	4
3	2	62	3	1	3	OKANESE	8
3	2	63	2	1	2	WP 69	5
3	2	64	9	3	1	Walker	2
3	2	65	4	1	4	AP 1004	3
3	1	66	12	3	4	AP 1004	7
3	1	67	9	3	1	Walker	2
3	1	68	11	3	3	OKANESE	5
3	2	69	10	3	2	WP 69	8
3	1	70	3	1	3	OKANESE	3
3	2	71	12	3	4	AP 1004	8
3	2	72	7	2	3	OKANESE	6
4	2	73	11	3	3	OKANESE	1
4	2	74	7	2	3	OKANESE	2
4	1	75	7	2	3	OKANESE	1
4	2	76	9	3	1	Walker	2
4	1	77	8	2	4	AP 1004	8
4	1	78	3	1	3	OKANESE	4
4	2	79	12	3	4	AP 1004	4
4	2	80	6	2	2	WP 69	3
4	1	81	12	3	4	AP 1004	2
4	1	82	6	2	2	WP 69	2
4	1	83	2	1	2	WP 69	3
4	1	84	4	1	4	AP 1004	7
4	2	85	4	1	4	AP 1004	8
4	2	86	10	3	2	WP 69	8
4	1	87	1	1	1	Walker	5
4	2	88	2	1	2	WP 69	6
4	2	89	1	1	1	Walker	6
4	1	90	5	2	1	Walker	5
4	2	91	3	1	3	OKANESE	7
4	2	92	8	2	4	AP 1004	6
4	1	93	10	3	2	WP 69	4
4	1	94	11	3	3	OKANESE	9
4	1	95	9	3	1	Walker	4
4	2	96	5	2	1	Walker	7
5	2	97	12	3	4	AP 1004	4
5	2	98	6	2	2	WP 69	2

5	2	99	7	2	3	OKANESE	**4**
5	2	100	4	1	4	AP 1004	**4**
5	2	101	5	2	1	Walker	**9**
5	1	102	7	2	3	OKANESE	**4**
5	1	103	3	1	3	OKANESE	**7**
5	1	104	2	1	2	WP 69	**5**
5	1	105	8	2	4	AP 1004	**3**
5	1	106	4	1	4	AP 1004	**4**
5	2	107	3	1	3	OKANESE	**2**
5	2	108	11	3	3	OKANESE	**6**
5	2	109	8	2	4	AP 1004	**4**
5	2	110	2	1	2	WP 69	**4**
5	1	111	5	2	1	Walker	**8**
5	1	112	12	3	4	AP 1004	**7**
5	1	113	6	2	2	WP 69	**3**
5	1	114	1	1	1	Walker	**5**
5	2	115	10	3	2	WP 69	**5**
5	1	116	11	3	3	OKANESE	**4**
5	1	117	10	3	2	WP 69	**4**
5	2	118	9	3	1	Walker	**7**
5	1	119	9	3	1	Walker	**4**
5	2	120	1	1	1	Walker	**4**

Appendix B
Summary of Data

Growth Measurements

		Height F2004* (cm)		Height F2005 (cm)		Caliper F2004 (mm)		Caliper F2005 (mm)	
Fertilizer (1=none, 2=N, 3=NPK+)	Clone	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
1		45.35b	0.97	96.65c	2.35	4.98b	0.06	13.11c	0.29
2		45.14b	0.97	105.37b	2.32	5.04b	0.06	14.67b	0.33
3		51.84a	1.02	112.23a	2.49	6.08a	0.08	16.37a	0.36
$p_{(fertilizer)}$		**0.0002**		**0.0428**		**<0.0001**		**0.00**	
	1	46.39c	0.78	75.05c	2.20	4.84c	0.07	8.49d	0.20
	2	56.93b	0.89	125.97a	2.53	5.79b	0.09	16.71b	0.33
	3	59.99a	0.78	125.50a	2.36	6.11a	0.08	17.99a	0.29
	4	25.92d	0.54	90.26b	2.34	4.69c	0.08	14.67c	0.37
$p_{(clone)}$		**<0.0001**		**0.0024**		**<0.0001**		**<0.0001**	
1	1	41.75	1.29	67.77	3.65	4.37	0.10	7.50	0.23
1	2	56.17	1.42	123.68	3.62	5.57	0.12	15.96	0.47
1	3	58.66	1.14	119.14	3.82	5.66	0.10	15.99	0.41
1	4	24.29	0.80	72.21	3.44	4.30	0.11	12.06	0.52
2	1	45.97	1.35	74.46	4.19	4.62	0.11	8.41	0.37
2	2	53.62	1.58	119.07	4.65	5.38	0.13	16.10	0.58
2	3	56.15	1.48	122.26	3.93	5.66	0.12	17.75	0.50
2	4	25.05	0.89	104.28	3.55	4.48	0.11	15.29	0.59
3	1	51.46	1.19	82.71	3.42	5.54	0.12	9.53	0.38
3	2	60.95	1.53	134.64	4.71	6.41	0.16	18.06	0.64
3	3	65.16	1.20	135.14	4.34	7.00	0.14	20.23	0.48
3	4	28.56	1.07	94.44	4.19	5.31	0.17	16.78	0.67
$p_{(fertxclone)}$		**0.25**		**0.01**		**0.42**		**0.09**	

*F=Fall

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		Leaf Area (cm^2)		Leaf Weight (g)		Nitrogen (%)		Phosphorus (%)		Potassium (%)		Total Sulfur (%)		Boron (ppm)		Copper (ppm)	
Fertilizer (1=none, 2=N, 3=NPK+)	Clone	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
1		31.30c	1.75	0.21b	0.012	3.07c	0.057	0.26b	0.007	1.39b	0.025	0.44b	0.019	24.16a	0.56	5.35a	0.28
2		34.29b	1.66	0.24a	0.012	3.55b	0.042	0.27b	0.007	1.25a	0.037	0.41c	0.017	21.50b	0.45	5.38a	0.26
3		37.52a	1.61	0.25a	0.011	3.67a	0.031	0.28a	0.006	1.40b	0.029	0.47a	0.022	19.39c	0.55	3.23b	0.20
$P_{(fertilizer)}$		**0.003**		**0.01**		**<0.0001**		**0.02**		**0.003**		**0.01**		**0.0002**		**0.0001**	
	1	27.07b	1.10	0.17c	0.007	3.22b	0.072	0.26b	0.006	1.43a	0.056	0.62a	0.017	18.17c	0.59	3.13b	0.23
	2	41.25a	1.32	0.28b	0.008	3.61a	0.050	0.29a	0.008	1.36ab	0.029	0.41b	0.007	23.13a	0.49	5.05a	0.26
	3	44.08a	1.43	0.30a	0.009	3.65a	0.054	0.30a	0.006	1.30b	0.028	0.41b	0.005	23.60a	0.56	5.53a	0.36
	4	25.08b	1.16	0.17c	0.008	3.26b	0.067	0.24c	0.007	1.30b	0.023	0.32c	0.011	21.82b	0.69	4.89a	0.32
$P_{(clone)}$		**<0.0001**		**<0.0001**		**<0.0001**		**0.001**		**0.16**		**<0.0001**		**<0.0001**		**0.0002**	
1	1	22.94	1.47	0.14	0.010	2.77	0.092	0.25	0.012	1.50	0.072	0.57	0.038	20.96	0.87	3.59	0.35
1	2	39.02	3.04	0.27	0.021	3.32	0.056	0.28	0.013	1.34	0.027	0.43	0.015	24.86	1.02	5.72	0.38
1	3	40.31	2.56	0.27	0.013	3.36	0.094	0.30	0.010	1.34	0.049	0.42	0.011	26.40	0.81	6.69	0.46
1	4	22.93	2.05	0.15	0.013	2.84	0.060	0.23	0.011	1.39	0.024	0.33	0.031	24.42	1.12	5.39	0.56
2	1	27.40	2.07	0.17	0.013	3.31	0.041	0.25	0.008	1.24	0.133	0.58	0.011	18.36	0.50	3.51	0.37
2	2	40.44	1.96	0.28	0.013	3.76	0.059	0.29	0.015	1.33	0.049	0.38	0.009	22.51	0.62	5.87	0.25
2	3	43.96	2.46	0.32	0.014	3.77	0.037	0.30	0.011	1.23	0.035	0.39	0.006	23.17	0.76	6.43	0.41
2	4	25.38	2.27	0.18	0.017	3.38	0.061	0.23	0.010	1.21	0.039	0.29	0.005	21.96	0.89	5.70	0.48
3	1	30.86	1.31	0.20	0.009	3.58	0.045	0.28	0.009	1.55	0.043	0.70	0.015	15.19	0.67	2.30	0.39
3	2	44.28	1.47	0.30	0.007	3.77	0.049	0.30	0.014	1.40	0.069	0.42	0.008	22.03	0.60	3.57	0.27
3	3	47.98	2.00	0.33	0.012	3.83	0.060	0.29	0.011	1.35	0.054	0.42	0.007	21.23	0.50	3.46	0.40
3	4	26.94	1.65	0.18	0.009	3.58	0.052	0.27	0.010	1.29	0.036	0.34	0.005	19.09	1.00	3.58	0.39
$P_{(fertxclone)}$		**0.81**		**0.57**		**0.02**		**0.07**		**0.48**		**<0.0001**		**0.52**		**0.11**	

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		Zinc (ppm)		Calcium (%)		Iron (ppm)		Magnesium (%)		Manganese (ppm)		Molybdenum (ppm)		Sodium (%)		C13	
Fertilizer (1=none, 2=N, 3=NPK+)	Clone	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
1		86.37b	3.21	1.00	0.021	146.41	14.31	0.34a	0.009	22.10c	1.00	0.60	0.07	0.01	0.0004	-26.55	0.14
2		103.75a	3.88	1.00	0.022	141.15	13.11	0.31b	0.008	26.13b	0.93	0.51	0.01	0.01	0.0004	-26.73	0.15
3		68.81c	3.10	0.95	0.024	126.27	9.41	0.28c	0.007	29.25a	0.94	0.51	0.01	0.01	0.0000	-26.51	0.15
$P_{(fertilizer)}$		**0.001**		**0.02**		**0.05**		**0.0003**		**0.001**		**0.17**		**0.24**		**0.61**	
	1	63.01b	3.37	1.03a	0.023	107.46b	7.15	0.26b	0.005	24.28	1.38	0.55	0.03	0.010333b	0.0003	-26.62	0.17
	2	94.28a	4.03	1.04a	0.023	99.91b	3.63	0.35a	0.007	25.49	0.95	0.59	0.08	0.01b	0.0000	-26.58	0.19
	3	98.33a	4.31	1.02a	0.017	93.59b	2.37	0.35a	0.008	26.08	1.39	0.50	0.00	0.01b	0.0000	-26.62	0.18
	4	89.61a	4.28	0.83b	0.016	250.81a	13.60	0.27b	0.006	27.45	1.09	0.50	0.00	0.011667a	0.0007	-26.55	0.13
$P_{(clone)}$		**<0.0001**		**0.0002**		**<0.0001**		**<0.0001**		**0.28**		**0.48**		**0.12**		**0.96**	
1	1	62.6	4.87	0.99	0.027	115.98	18.30	0.29	0.007	17.88	1.36	0.62	0.08	0.01	0.0010	-26.42	0.35
1	2	95.0	4.64	1.08	0.044	100.94	9.07	0.39	0.008	22.51	1.17	0.75	0.25	0.01	0.0000	-26.73	0.27
1	3	96.5	2.74	1.05	0.028	98.36	5.04	0.38	0.008	24.08	2.56	0.50	0.00	0.01	0.0000	-26.57	0.26
1	4	91.4	6.38	0.86	0.027	270.34	28.47	0.29	0.010	23.93	2.16	0.51	0.01	0.01	0.0013	-26.49	0.26
2	1	76.7	4.82	1.07	0.034	106.63	9.52	0.26	0.006	23.83	1.55	0.52	0.02	0.01	0.0000	-26.92	0.25
2	2	114.2	4.88	1.03	0.048	98.89	5.53	0.34	0.006	25.92	1.83	0.51	0.01	0.01	0.0000	-26.59	0.41
2	3	121.0	6.34	1.04	0.030	90.69	2.70	0.35	0.014	27.09	2.69	0.50	0.00	0.01	0.0000	-26.71	0.28
2	4	103.2	6.89	0.86	0.032	268.40	20.89	0.28	0.010	27.69	0.99	0.50	0.00	0.01	0.0015	-26.68	0.26
3	1	49.7	4.70	1.04	0.054	99.76	7.31	0.24	0.007	31.14	2.05	0.52	0.02	0.01	0.0000	-26.53	0.29
3	2	73.7	4.58	1.02	0.030	99.89	3.78	0.33	0.013	28.05	1.49	0.50	0.00	0.01	0.0000	-26.43	0.31
3	3	77.5	5.13	0.97	0.029	91.71	4.21	0.31	0.009	27.07	2.08	0.50	0.00	0.01	0.0000	-26.57	0.42
3	4	74.3	6.47	0.78	0.018	213.70	17.63	0.26	0.008	30.73	1.80	0.50	0.00	0.01	0.0000	-26.49	0.19
$P_{(fertxclone)}$		**0.4**		**0.30**		**0.28**		**0.43**		**0.06**		**0.73**		**0.27**		**0.98**	

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		Leaf Weight (g)		Nitrogen (%)		Phosphorus (%)		Potassium (%)		Total Sulfur (%)		Boron (ppm)		Copper (ppm)		Zinc (ppm)	
Fertilizer (1=none, 2=N, 3=NPK+)	Clone	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
1		10.02b	0.71	2.95b	0.075	0.31	0.012	1.21a	0.05	0.42a	0.021	29.54	1.04	7.45	0.25	114.46a	6.32
2		12.95a	1.28	3.21a	0.054	0.30	0.010	1.02b	0.04	0.39b	0.015	30.81	1.45	7.83	0.50	96.14b	4.31
3		13.41a	0.99	3.29a	0.059	0.33	0.009	1.20a	0.05	0.42a	0.021	28.87	0.94	7.37	0.34	84.60c	4.83
$P_{(fertilizer)}$		**0.11**		**0.01**		**0.06**		**0.03**		**0.01**		**0.35**		**0.40**		**0.004**	
	1	4.48b	0.38	2.69c	0.055	0.27c	0.012	1.31a	0.06	0.60a	0.013	25.68b	1.06	6.18b	0.75	80.63b	5.74
	2	13.92a	1.43	3.44a	0.060	0.35a	0.014	1.19b	0.06	0.37b	0.005	29.88a	1.16	8.07a	0.23	88.23b	3.86
	3	15.27a	0.75	3.45a	0.045	0.33ab	0.009	1.05c	0.05	0.37b	0.005	33.05a	1.61	8.34a	0.24	87.71b	4.37
	4	14.85a	0.63	3.03b	0.049	0.31b	0.008	1.06c	0.04	0.30c	0.006	29.87a	1.09	7.45a	0.27	134.87a	6.06
$P_{(clone)}$		**<0.0001**		**<0.0001**		**0.001**		**0.01**		**<0.0001**		**0.01**		**0.002**		**<0.0001**	
1	1	3.90	0.55	2.49	0.079	0.22	0.018	1.37	0.12	0.63	0.016	26.07	2.41	5.56	0.47	96.17	12.57
1	2	11.40	0.89	3.30	0.135	0.37	0.023	1.25	0.11	0.38	0.009	29.85	2.37	8.31	0.25	98.38	8.68
1	3	12.30	0.96	3.31	0.108	0.35	0.017	1.17	0.10	0.37	0.011	31.09	1.92	8.67	0.41	102.38	9.68
1	4	12.49	1.01	2.71	0.067	0.30	0.012	1.06	0.07	0.29	0.005	30.80	1.48	7.09	0.18	159.10	7.88
2	1	4.04	0.70	2.78	0.093	0.28	0.028	1.13	0.08	0.53	0.018	26.68	2.31	8.35	2.38	84.21	8.06
2	2	15.02	3.76	3.46	0.067	0.32	0.025	1.06	0.12	0.36	0.004	30.17	1.93	7.74	0.26	86.33	3.93
2	3	16.42	0.65	3.45	0.047	0.30	0.008	0.91	0.05	0.37	0.006	35.44	4.28	8.16	0.42	84.67	5.60
2	4	16.33	1.13	3.17	0.034	0.30	0.013	1.02	0.05	0.30	0.010	30.12	1.87	7.16	0.20	126.96	7.71
3	1	5.49	0.66	2.81	0.082	0.29	0.011	1.41	0.09	0.64	0.021	24.53	0.74	5.00	0.23	63.78	5.77
3	2	15.34	1.93	3.56	0.093	0.36	0.022	1.24	0.10	0.37	0.011	29.62	1.89	8.17	0.59	79.99	5.82
3	3	17.08	1.60	3.58	0.037	0.33	0.017	1.07	0.09	0.37	0.007	32.63	1.37	8.20	0.42	76.09	4.47
3	4	15.74	0.80	3.21	0.030	0.33	0.015	1.09	0.08	0.32	0.011	28.69	2.33	8.11	0.76	118.55	11.46
$P_{(fertxclone)}$		**0.64**		**0.21**		**0.001**		**0.58**		**<0.0001**		**0.90**		**0.19**		**0.68**	

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		Calcium (%)		Iron (ppm)		Magnesium (%)		Manganese (ppm)		Molybdenum (ppm)		Sodium (%)		C13	
Fertilizer (1=none, 2=N, 3=NPK+)	Clone	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
1		1.15	0.044	76.09	2.85	0.35	0.014	23.04b	1.02	0.71	0.024	0.014	0.0009	-26.92b	0.11
2		1.07	0.039	76.78	3.11	0.36	0.014	29.76a	1.66	0.72	0.018	0.014	0.0010	-26.43a	0.10
3		1.12	0.051	72.28	2.29	0.33	0.014	29.33a	1.59	0.73	0.019	0.016	0.0013	-26.41a	0.09
$P_{(fertilizer)}$		**0.15**		**0.41**		**0.33**		**0.06**		**0.57**		**0.232**		**0.001**	
	1	1.20a	0.052	73.97b	4.37	0.31c	0.011	30.12a	2.28	0.74	0.022	0.015	0.0014	-26.62b	0.12
	2	1.20a	0.048	71.06b	2.37	0.38b	0.017	26.04ab	1.21	0.71	0.021	0.014	0.0012	-26.32a	0.09
	3	1.19a	0.038	68.19b	1.85	0.41a	0.014	23.77b	1.06	0.71	0.030	0.015	0.0011	-26.15a	0.08
	4	0.86b	0.039	86.73a	2.86	0.290c	0.008	29.83a	2.07	0.73	0.020	0.015	0.0013	-27.26c	0.09
$P_{(clone)}$		**0.0004**		**0.002**		**0.0004**		**0.04**		**0.81**		**0.742**		**<0.0001**	
1	1	1.28	0.104	86.24	9.19	0.31	0.021	22.21	2.33	0.77	0.029	0.013	0.0017	-26.86	0.24
1	2	1.18	0.085	66.58	2.53	0.39	0.031	23.16	0.95	0.72	0.029	0.014	0.0022	-26.66	0.13
1	3	1.21	0.083	66.20	2.73	0.41	0.029	22.48	1.84	0.66	0.073	0.013	0.0015	-26.40	0.11
1	4	0.94	0.049	86.34	3.12	0.31	0.015	24.21	2.87	0.71	0.041	0.014	0.0022	-27.75	0.11
2	1	1.16	0.110	76.93	5.07	0.33	0.026	36.30	4.99	0.71	0.044	0.015	0.0033	-26.67	0.14
2	2	1.13	0.070	73.46	6.31	0.39	0.027	26.99	2.43	0.71	0.041	0.013	0.0021	-26.06	0.13
2	3	1.14	0.052	65.05	3.99	0.43	0.022	23.52	1.38	0.73	0.034	0.014	0.0016	-26.04	0.15
2	4	0.86	0.048	91.73	6.19	0.28	0.008	33.54	3.01	0.74	0.031	0.013	0.0015	-27.05	0.13
3	1	1.17	0.063	60.55	5.41	0.29	0.014	32.29	3.27	0.74	0.040	0.016	0.0027	-26.41	0.18
3	2	1.29	0.095	73.13	2.18	0.37	0.032	27.98	2.41	0.70	0.039	0.014	0.0022	-26.27	0.16
3	3	1.23	0.062	73.31	2.31	0.40	0.025	25.32	2.29	0.74	0.040	0.017	0.0026	-26.01	0.14
3	4	0.77	0.093	82.11	5.07	0.28	0.017	31.74	4.30	0.75	0.034	0.017	0.0030	-26.97	0.09
$P_{(fertxclone)}$		**0.22**		**0.01**		**0.88**		**0.47**		**0.51**		**0.742**		**0.56**	

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Leaves

		Height (cm)		Caliper (mm)		Leaf Weight (g)		Nitrogen (%)		Phosphorus (%)		Potassium (%)		Total Sulfur (%)		Boron (ppm)	
Fertilizer (1=none, 2=N, 3=NPK+)	Clone	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
1		110.04	8.60	13.27	1.09	40.18	7.02	2.32	0.05	0.22	0.006	1.23	0.06	0.41	0.046	34.23	0.99
2		123.78	9.05	15.27	1.42	49.42	9.04	2.44	0.06	0.22	0.007	1.19	0.06	0.36	0.035	30.63	1.11
3		122.57	8.93	15.41	1.35	55.15	9.36	2.37	0.07	0.22	0.005	1.31	0.08	0.41	0.050	32.13	1.18
$P_{(fertilizer)}$		**0.20**		**0.19**		**0.35**		**0.62**		**0.38**		**0.56**		**0.22**		**0.42**	
	1	81.35c	7.50	8.04c	0.49	10.51c	1.93	2.29	0.04	0.21	0.006	1.43	0.09	0.67a	0.026	33.39	1.48
	2	148.23a	4.24	17.28ab	0.71	62.07ab	7.13	2.38	0.07	0.22	0.005	1.25	0.06	0.32b	0.008	32.59	0.85
	3	139.02a	6.82	18.56a	1.00	77.58a	7.91	2.51	0.08	0.23	0.004	1.26	0.08	0.32b	0.009	33.89	1.45
	4	107.95b	7.76	15.00b	1.26	44.71b	8.03	2.34	0.07	0.22	0.009	1.03	0.05	0.26c	0.010	29.59	1.17
$P_{(clone)}$		**0.01**		**0.01**		**0.03**		**0.27**		**0.03**		**0.17**		**<0.0001**		**0.27**	
1	1	76.38	19.07	7.73	1.12	8.94	4.05	2.21	0.05	0.20	0.009	1.41	0.14	0.71	0.044	34.93	3.16
1	2	139.58	5.73	15.15	0.72	47.54	8.58	2.30	0.09	0.22	0.007	1.26	0.15	0.33	0.014	34.45	1.04
1	3	131.93	10.40	17.73	1.73	72.45	12.43	2.44	0.14	0.23	0.005	1.18	0.12	0.32	0.003	34.98	2.41
1	4	92.28	6.32	12.50	0.76	31.79	5.31	2.32	0.09	0.24	0.015	1.07	0.09	0.28	0.008	32.58	1.28
2	1	79.20	12.60	7.71	1.04	10.89	4.32	2.26	0.06	0.19	0.010	1.26	0.16	0.58	0.036	32.40	3.11
2	2	148.38	6.77	17.80	1.41	61.05	16.07	2.56	0.11	0.22	0.008	1.15	0.09	0.30	0.009	31.55	1.71
2	3	152.45	8.67	19.90	1.57	84.26	14.01	2.54	0.10	0.22	0.010	1.31	0.16	0.30	0.013	30.95	1.12
2	4	115.08	14.00	15.67	2.55	41.47	13.94	2.42	0.16	0.22	0.019	1.05	0.09	0.26	0.027	27.60	2.54
3	1	88.48	8.32	8.67	0.36	11.69	2.17	2.40	0.05	0.22	0.007	1.61	0.13	0.71	0.024	32.85	1.79
3	2	156.75	8.38	18.89	0.71	77.62	8.72	2.28	0.12	0.23	0.013	1.34	0.05	0.32	0.014	31.78	1.50
3	3	139.53	17.22	18.31	2.34	75.09	19.90	2.58	0.23	0.23	0.006	1.24	0.13	0.33	0.023	34.23	3.87
3	4	116.50	17.42	16.84	2.66	60.87	18.46	2.29	0.13	0.20	0.009	0.97	0.09	0.25	0.016	28.60	1.54
$P_{(fertxclone)}$		**0.94**		**0.61**		**0.74**		**0.46**		**0.21**		**0.21**		**0.23**		**0.75**	

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Leaves

		Copper (ppm)		Zinc (ppm)		Calcium (%)		Iron (ppm)		Magnesium (%)		Manganese (ppm)		Molybdenum (ppm)		Sodium (%)	
Fertilizer (1=none, 2=N, 3=NPK+)	Clone	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
1		8.53	0.69	140.26	11.30	1.45	0.07	199.81	19.85	0.40	0.022	33.33	2.38			0.018	0.0014
2		8.14	0.72	115.99	8.49	1.35	0.09	176.29	17.83	0.36	0.026	34.68	1.84			0.019	0.0014
3		7.24	0.55	90.71	9.80	1.39	0.08	177.05	24.78	0.36	0.027	32.49	2.28			0.020	0.0017
$P_{(fertilizer)}$		**0.34**		**0.10**		**0.26**		**0.63**		**0.47**		**0.31**		**0.4188**		**0.742**	
	1	6.97	0.61	86.78	11.19	1.47a	0.07	170.20ab	22.87	0.35b	0.016	32.25	3.40			0.019	0.0023
	2	8.98	0.77	125.60	8.18	1.59a	0.04	167.33ab	16.65	0.45a	0.014	35.36	2.02			0.019	0.0019
	3	9.07	0.92	132.66	12.39	1.56a	0.02	149.55b	19.54	0.46a	0.020	35.71	2.05			0.019	0.0009
	4	7.01	0.61	121.08	15.12	0.99b	0.06	248.17a	26.07	0.26c	0.015	30.94	2.08			0.019	0.0015
$P_{(clone)}$		**0.01**		**0.07**		**0.03**		**0.01**		**0.01**		**0.68**		**0.0347**		**0.989**	
1	1	6.33	0.49	90.03	18.17	1.56	0.19	177.30	43.13	0.37	0.019	29.88	6.78	<0.7		0.020	0.0041
1	2	10.13	1.56	143.00	19.02	1.58	0.08	176.50	19.81	0.46	0.021	37.88	4.76	<0.7		0.015	0.0029
1	3	10.48	1.56	151.25	17.02	1.52	0.03	184.70	48.29	0.49	0.030	35.70	4.16	<0.8		0.020	0.0000
1	4	7.20	0.41	176.75	15.67	1.13	0.03	260.75	40.36	0.30	0.019	29.88	3.21	<0.8		0.018	0.0025
2	1	7.78	1.65	102.28	17.15	1.38	0.09	202.38	48.99	0.34	0.025	37.63	4.44	<0.7		0.020	0.0041
2	2	9.28	1.59	120.75	4.66	1.56	0.04	145.00	23.70	0.46	0.031	33.13	0.78	<0.7		0.020	0.0041
2	3	9.13	7.81	139.75	17.98	1.60	0.03	130.30	20.05	0.43	0.015	38.58	3.52	<0.8		0.020	0.0000
2	4	6.38	0.25	101.18	21.70	0.87	0.17	227.50	29.40	0.23	0.035	29.38	4.09	<0.8		0.018	0.0025
3	1	6.80	0.87	68.03	23.48	1.48	0.11	130.93	24.83	0.34	0.038	29.25	6.87	<0.8		0.018	0.0048
3	2	7.55	0.61	113.05	13.84	1.62	0.10	180.50	42.92	0.43	0.023	35.08	4.22	<0.8		0.023	0.0025
3	3	7.13	0.88	90.98	27.20	1.56	0.06	123.75	15.38	0.46	0.062	32.88	2.15	<0.8		0.018	0.0033
3	4	7.45	1.91	85.30	14.33	0.96	0.05	256.25	69.05	0.25	0.016	33.58	4.13	<0.8		0.023	0.0025
$P_{(fertxclone)}$		**0.72**		**0.41**		**0.37**		**0.89**		**0.55**		**0.30**		**0.477**		**0.294**	

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Stems

		Stem Weight (g)		Nitrogen (%)		Phosphorus (%)		Potassium (%)		Total Sulfur (%)		Boron (ppm)		Copper (ppm)		Zinc (ppm)	
Fertilizer (1=none, 2=N, 3=NPK+)	Clone	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
1		44.48	7.50	0.86	0.040	0.13	0.0057	0.77	0.045	0.10	0.0097	15.89	0.50	6.55	0.41	80.65	7.03
2		63.66	10.09	0.94	0.049	0.12	0.0058	0.72	0.044	0.09	0.0074	15.71	0.63	5.67	0.24	76.59	4.16
3		74.39	11.40	0.92	0.054	0.13	0.0074	0.79	0.048	0.10	0.0086	16.03	0.59	5.44	0.34	68.11	6.11
$P_{(fertilizer)}$		**0.15**		**0.17**		**0.29**		**0.28**		**0.10**		**0.85**		**0.05**		**0.18**	
	1	13.63c	1.94	1.15	0.050	0.16	0.0090	0.84a	0.049	0.14a	0.0071	19.04a	0.48	6.04	0.50	66.88b	4.93
	2	80.72a	8.08	0.83	0.031	0.13	0.0042	0.85a	0.042	0.09b	0.0044	15.30b	0.40	6.18	0.36	64.68b	2.83
	3	91.20a	9.09	0.86	0.034	0.12	0.0043	0.79a	0.047	0.09b	0.0039	14.73b	0.46	6.01	0.29	61.33b	3.22
	4	57.83b	10.22	0.79	0.029	0.11	0.0026	0.56b	0.021	0.06c	0.0025	14.70b	0.29	5.28	0.43	106.43a	5.20
$P_{(clone)}$		**0.04**		**0.06**		**0.07**		**0.02**		**0.01**		**0.02**		**0.08**		**0.005**	
1	1	13.13	4.04	1.06	0.073	0.15	0.0120	0.78	0.069	0.15	0.0170	18.27	1.43	6.70	1.74	65.13	12.69
1	2	54.63	7.24	0.83	0.043	0.13	0.0085	0.92	0.082	0.10	0.0063	15.23	0.74	6.48	0.44	67.90	6.98
1	3	77.13	15.73	0.85	0.053	0.13	0.0103	0.81	0.071	0.10	0.0087	15.33	0.98	6.53	0.56	68.43	6.21
1	4	33.05	6.95	0.71	0.028	0.11	0.0048	0.58	0.031	0.06	0.0000	15.33	0.25	6.53	0.96	117.25	9.26
2	1	11.35	4.16	1.21	0.020	0.15	0.0111	0.79	0.041	0.13	0.0048	19.28	0.79	6.30	0.60	74.50	4.31
2	2	82.00	12.20	0.81	0.068	0.11	0.0025	0.71	0.037	0.08	0.0075	14.60	0.35	5.15	0.30	68.53	3.83
2	3	102.05	14.14	0.89	0.042	0.12	0.0071	0.85	0.121	0.09	0.0025	14.55	0.99	6.10	0.30	65.75	2.79
2	4	59.23	12.15	0.83	0.069	0.10	0.0048	0.53	0.052	0.06	0.0071	14.40	0.69	5.13	0.43	97.58	9.82
3	1	16.40	1.79	1.18	0.131	0.16	0.0229	0.94	0.110	0.14	0.0132	19.38	0.54	5.28	0.25	60.58	9.49
3	2	105.53	9.18	0.86	0.063	0.14	0.0050	0.91	0.040	0.10	0.0065	16.08	0.85	6.90	0.79	57.63	0.23
3	3	94.43	18.81	0.83	0.086	0.12	0.0063	0.72	0.047	0.09	0.0085	14.30	0.47	5.40	0.54	49.80	0.63
3	4	81.23	23.75	0.83	0.010	0.11	0.0041	0.58	0.028	0.06	0.0041	14.38	0.39	4.18	0.18	104.45	7.00
$P_{(fertxclone)}$		**0.29**		**0.95**		**0.96**		**0.14**		**0.85**		**0.62**		**0.16**		**0.52**	

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Stems

		Calcium (%)		Iron (ppm)		Magnesium (%)		Manganese (ppm)		Molybdenum (ppm)		Sodium (%)	
Fertilizer (1=none, 2=N, 3=NPK+)	Clone	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
1		0.63	0.039	138.09	19.94	0.18	0.011	11.79	0.56	0.80	0.010	0.013	0.0012
2		0.63	0.040	108.08	14.60	0.16	0.011	12.73	0.67	0.78	0.011	0.014	0.0015
3		0.65	0.045	112.56	16.12	0.17	0.011	12.66	0.73	0.79	0.017	0.012	0.0010
$P_{(fertilizer)}$		**0.65**		**0.35**		**0.13**		**0.36**		**0.41**		**0.123**	
	1	0.86a	0.034	190.27a	8.70	0.22a	0.008	13.86	0.93	0.81	0.021	0.020a	0.0013
	2	0.52c	0.012	83.22b	5.39	0.17b	0.005	11.75	0.64	0.78	0.011	0.010b	0.0000
	3	0.52c	0.019	77.49b	9.11	0.18b	0.007	12.15	0.53	0.81	0.008	0.011b	0.0008
	4	0.64b	0.032	131.66b	25.83	0.12c	0.005	11.98	0.84	0.76	0.015	0.011b	0.0008
$P_{(clone)}$		**0.01**		**0.002**		**0.01**		**0.06**		**0.10**		**0.002**	
1	1	0.85	0.052	211.67	17.84	0.23	0.018	11.57	1.13	0.80	0.000	0.020	0.0000
1	2	0.52	0.014	93.05	8.13	0.18	0.012	12.55	1.72	0.80	0.000	0.010	0.0000
1	3	0.52	0.023	83.13	21.33	0.19	0.013	11.90	0.49	0.83	0.025	0.010	0.0000
1	4	0.67	0.063	182.90	50.72	0.12	0.009	11.10	1.14	0.78	0.025	0.013	0.0025
2	1	0.87	0.042	194.75	10.18	0.22	0.013	15.45	1.25	0.78	0.025	0.023	0.0025
2	2	0.52	0.029	80.43	10.78	0.17	0.005	10.75	0.80	0.78	0.025	0.010	0.0000
2	3	0.52	0.029	74.43	17.99	0.17	0.004	13.48	1.27	0.80	0.000	0.013	0.0025
2	4	0.59	0.023	82.70	19.13	0.11	0.010	11.23	0.73	0.75	0.029	0.010	0.0000
3	1	0.86	0.087	169.75	13.02	0.22	0.014	14.00	1.89	0.85	0.050	0.018	0.0025
3	2	0.54	0.020	76.18	9.26	0.17	0.007	11.95	0.56	0.78	0.025	0.010	0.0000
3	3	0.52	0.049	74.93	10.90	0.17	0.017	11.08	0.54	0.80	0.000	0.010	0.0000
3	4	0.67	0.075	129.38	52.19	0.12	0.009	13.63	2.15	0.75	0.029	0.010	0.0000
$P_{(fertxclone)}$		**0.98**		**0.31**		**0.99**		**0.09**		**0.59**		**0.672**	

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Roots

		Root Weight (g)		Nitrogen (%)		Phosphorus (%)		Potassium (%)		Total Sulfur (%)		Boron (ppm)		Copper (ppm)		Zinc (ppm)	
Fertilizer (1=none, 2=N, 3=NPK+)	Clone	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
1		12.51	2.13	0.82	0.036	0.17	0.013	0.85	0.06	0.12	0.006	12.70	1.19	10.49	1.16	68.62	6.23
2		19.47	3.50	0.94	0.029	0.16	0.012	0.77	0.08	0.12	0.006	13.92	1.27	10.29	1.05	71.05	7.16
3		17.97	3.44	0.89	0.036	0.17	0.009	0.83	0.05	0.13	0.006	14.16	0.97	11.56	1.05	75.48	9.25
$P_{(fertilizer)}$		**0.18**		**0.07**		**0.74**		**0.69**		**0.30**		**0.58**		**0.49**		**0.77**	
	1	2.28b	0.38	0.90	0.039	0.15	0.013	0.76	0.05	0.14	0.007	16.55a	2.00	11.16	1.71	62.21	8.89
	2	18.95a	2.06	0.81	0.043	0.18	0.011	0.95	0.07	0.12	0.009	13.83ab	0.82	10.78	1.41	61.71	4.81
	3	25.31a	2.43	0.92	0.036	0.19	0.008	0.91	0.06	0.13	0.006	14.13ab	0.58	9.69	1.02	79.11	12.47
	4	19.66a	4.23	0.91	0.042	0.14	0.016	0.64	0.06	0.11	0.005	10.31b	1.12	11.78	1.08	82.36	6.02
$P_{(clone)}$		**0.02**		**0.38**		**0.09**		**0.07**		**0.15**		**0.33**		**0.70**		**0.06**	
1	1	2.18	0.88	0.85	0.079	0.18	0.023	0.82	0.07	0.13	0.018	13.43	3.17	10.63	2.32	76.35	19.27
1	2	14.38	2.80	0.76	0.082	0.18	0.003	0.96	0.11	0.12	0.011	12.80	1.76	12.93	3.55	59.15	5.49
1	3	21.38	3.25	0.89	0.069	0.21	0.022	0.92	0.12	0.12	0.009	14.80	1.61	8.60	1.85	62.68	4.79
1	4	12.10	2.94	0.78	0.069	0.18	0.043	0.69	0.14	0.10	0.008	9.78	2.80	10.43	2.33	76.30	16.44
2	1	2.08	0.79	0.96	0.020	0.13	0.040	0.64	0.01	0.14	0.009	19.15	7.05	5.70	.	46.80	3.10
2	2	21.50	4.32	0.92	0.076	0.17	0.021	0.90	0.18	0.13	0.020	14.98	1.72	9.85	1.87	57.33	7.76
2	3	32.23	3.55	0.91	0.067	0.19	0.012	0.91	0.12	0.11	0.006	13.03	0.65	9.35	1.91	82.85	19.83
2	4	20.88	7.51	0.98	0.071	0.14	0.027	0.58	0.15	0.11	0.006	11.15	2.28	12.83	1.73	85.10	7.08
3	1	2.58	0.38	0.89	0.090	0.13	0.014	0.78	0.11	0.15	0.011	18.38	2.49	12.93	2.72	55.78	9.98
3	2	20.98	3.10	0.75	0.044	0.20	0.025	0.99	0.04	0.13	0.017	13.73	0.68	10.10	2.60	68.65	11.82
3	3	22.33	4.19	0.97	0.060	0.18	0.005	0.90	0.08	0.15	0.010	14.55	0.32	11.13	1.81	91.80	33.85
3	4	26.00	9.94	0.97	0.037	0.16	0.010	0.64	0.04	0.12	0.007	10.00	0.65	12.08	1.81	85.68	7.68
$P_{(fertxclone)}$		**0.12**		**0.21**		**0.88**		**0.98**		**0.60**		**0.54**		**0.78**		**0.51**	

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Roots

		Calcium (%)		Iron (ppm)		Magnesium (%)		Manganese (ppm)		Molybdenum (ppm)		Sodium (%)	
Fertilizer (1=none, 2=N, 3=NPK+)	Clone	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
1		0.99	0.06	999.43	145.10	0.18	0.013	31.94	5.40	0.82	0.04	0.06	0.012
2		1.12	0.07	1001.00	95.92	0.18	0.011	35.55	3.43	0.74	0.03	0.05	0.004
3		1.18	0.07	1231.50	163.43	0.20	0.011	42.36	5.54	0.78	0.01	0.05	0.003
$P_{(fertilizer)}$		**0.14**		**0.58**		**0.13**		**0.41**		**0.10**		**0.55**	
	1	1.09	0.04	864.10	72.54	0.17	0.009	39.11	7.95	0.82	0.08	0.08	0.018
	2	0.96	0.05	996.25	89.14	0.20	0.010	31.02	3.11	0.79	0.01	0.04	0.002
	3	1.03	0.05	1346.58	139.49	0.23	0.011	42.04	4.57	0.78	0.01	0.05	0.003
	4	1.30	0.11	1079.49	249.28	0.14	0.009	34.90	7.22	0.75	0.03	0.04	0.005
$P_{(clone)}$		**0.15**		**0.65**		**0.10**		**0.63**		**0.96**		**0.15**	
1	1	1.08	0.10	768.50	119.04	0.16	0.015	40.18	18.70	0.95	0.15	0.10	0.045
1	2	0.89	0.11	1005.00	158.74	0.19	0.018	26.03	3.65	0.80	0.00	0.05	0.003
1	3	0.96	0.04	1222.00	197.57	0.24	0.018	35.30	2.86	0.80	0.00	0.05	0.009
1	4	1.03	0.19	1002.23	555.32	0.13	0.023	26.28	12.82	0.73	0.08	0.04	0.008
2	1	1.12	0.08	727.50	102.50	0.16	0.010	27.05	5.25	0.60	0.20	0.06	0.000
2	2	1.03	0.06	973.75	136.95	0.21	0.023	35.58	5.59	0.78	0.03	0.04	0.006
2	3	0.94	0.03	1262.75	162.26	0.20	0.010	44.38	7.47	0.78	0.03	0.04	0.005
2	4	1.40	0.15	903.25	232.34	0.13	0.006	30.95	6.42	0.75	0.03	0.05	0.012
3	1	1.10	0.05	1028.00	91.83	0.19	0.012	44.08	9.31	0.80	0.00	0.06	0.010
3	2	0.97	0.08	1010.00	207.84	0.21	0.011	31.45	6.80	0.80	0.00	0.04	0.003
3	3	1.18	0.11	1555.00	355.91	0.25	0.019	46.45	11.92	0.75	0.03	0.05	0.005
3	4	1.48	0.18	1333.00	536.11	0.15	0.015	47.48	16.88	0.78	0.03	0.05	0.005
$P_{(fertxclone)}$		**0.05**		**0.97**		**0.41**		**0.62**		**0.31**		**0.67**	

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		Evapotranspiration ($mol/m^2/s$)		Stomatal Conductance (mmol $H_20/m^2/s$)		Net Assimilation ($umol/m^2/s$)		Water Use Efficiency ($umolCO_2/mmol\ H_20$)	
Fertilizer (1=none, 2=N, 3=NPK+)	Clone	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
1		5.54	0.10	670.70	15.85	33.42	1.00	6.07	0.17
2		5.34	0.10	647.73	16.26	32.82	0.93	6.18	0.16
3		5.46	0.08	662.68	14.75	33.61	0.78	6.20	0.13
$p_{(fertilizer)}$		**0.20**		**0.47**		**0.74**		**0.74**	
	1	5.52b	0.13	679.53	20.68	25.47c	1.01	4.58b	0.14
	2	5.27c	0.10	619.07	16.51	33.83b	0.79	6.47a	0.13
	3	5.13c	0.09	618.33	15.70	34.50b	0.64	6.78a	0.12
	4	5.85a	0.09	724.56	15.30	39.33a	0.84	6.76a	0.13
$p_{(clone)}$		**0.03**		**0.07**		**0.0001**		**<0.0001**	
1	1	5.57	0.26	690.61	37.35	24.04	1.81	4.29	0.25
1	2	5.27	0.21	612.78	29.55	33.45	1.55	6.42	0.26
1	3	5.50	0.16	683.30	29.00	36.89	1.20	6.78	0.24
1	4	5.81	0.17	696.12	28.83	39.31	1.60	6.80	0.23
2	1	5.29	0.22	637.01	38.87	24.42	1.91	4.54	0.26
2	2	5.23	0.17	622.58	32.87	33.15	1.24	6.40	0.24
2	3	4.79	0.14	580.68	23.89	33.19	0.87	7.01	0.21
2	4	6.03	0.13	750.66	20.04	40.51	1.24	6.75	0.21
3	1	5.70	0.18	710.98	30.40	27.94	1.43	4.92	0.22
3	2	5.32	0.15	621.84	24.13	34.89	1.33	6.58	0.19
3	3	5.10	0.14	591.02	23.41	33.41	1.10	6.56	0.15
3	4	5.71	0.16	726.90	29.47	38.19	1.51	6.73	0.26
$p_{(fertxclone)}$		**0.08**		**0.09**		**0.18**		**0.27**	