Can Physiological and Anatomical Characters Be Used for Selecting High Yielding Hybrid Aspen Clones?

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Stomatal, CO_2 exchange parameters and several leaf and growth traits were recorded in a five-year-old hybrid aspen clone trial. The field trial consisted of four aspen hybrid clones (*Populus tremula* L. × *P. tremuloides* Michx.) and one local *Populus tremula* seedling source. The mean estimated height of hybrid aspen clones was 1.6 times higher than for *P. tremula*. Similarly, basal diameter was 1.5 times and breast diameter 1.8 times higher in the hybrids. Differences were observed for physiological and growth traits among hybrid clones and *P. tremula*, but, only stomatal characters of hybrid clones differed significantly from those in *P. tremula*. Hybrid clones had larger guard cells (22.9 μm) than *P. tremula* (19.2 μm), whereas *P. tremula* had a higher stomatal density (211.3/mm^2) than the hybrid clones (164.4/mm^2). Among four hybrid clones, net photosynthesis was strongly correlated with foliar nitrogen. Height correlated significantly with foliar nitrogen, but negatively with leaf fresh weight, leaf dry weight and stomatal density. The results suggested that yield components could be controlled by many genes, specific to each clone. No single gas exchange or morphological variable can provide a reliable indicator of yield potential.

Keywords growth, leaf, photosynthesis, stomatal characters
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1 Introduction

Aspen hybrids (*Populus tremula* L. × *P. tremuloides* Michx.), mainly produced from crosses made in the 1950's have displayed hybrid vigor in Finland (Beuker 1989). The phenomenon of hybrid vigor in aspen hybrids is well documented in the US (Li and Wu 1996, 1997, Li et al. 1998). To improve the yield of forest crops, plant and environmental variables that influence and control growth should be fully understood (Michael et al. 1990). Yield is a complex trait, which crop physiologists partition into components. It is important to identify genotypes with traits associated with high yield and to understand the relationships among the traits. Yield involves various morphological, physiological, and biochemical components that are each regulated by different genes. The analysis of yield components and their genetic control in *Populus* has featured centrally in many studies (Hinckley et al. 1989, Stettler and Ceulemans 1993, Stettler et al. 1996). Biomass yields and their physiological basis, as well as the economic importance and practical aspects of intensively cultured poplar plantations, have been thoroughly examined (Zsuffa et al. 1993, Heilman and Stettler 1985).

In tree breeding the use of yield components is new, but essential for understanding why certain breeds and hybrids exhibit superior growth. Significant variation in yield components among *Populus* clones have been found in many studies (Ceulemans et al. 1987, Orlovic et al. 1998, Thomas et al. 1997a, 1997b). These studies have sought for physiological and anatomical measures that could be used to select superior genotypes (Ceulemans and Impens 1983, Ceulemans et al. 1987, Orlovic et al. 1998). However, the correlations between net photosynthesis and growth have been ambiguous (Barigah et al. 1994, Gatherum et al. 1967, Okafo and Hanover 1978, Reighard and Hanover 1990). Most physiological studies have been made in greenhouses, or based on 1–2 year old plants in the field (Ceulemans et al. 1987, Orlovic et al. 1998). The correlation between performance in the greenhouse and in the field has been variously proved significant (Ceulemans et al. 1987) or non-significant (Thomas et al. 1997).

Photosynthetic capacity is dependent on the concentration of N-containing enzymes, pigments, and electron transport components (Evans 1989). An association of foliar nutrient concentration with light-saturated photosynthesis has been found in deciduous and herbaceous species (Field and Mooney 1986, Reich et al. 1995). Foliar nitrogen (N) concentration has been used to estimate net photosynthesis because of the strong coupling between the two (e.g., Field and Mooney 1986).

According to our knowledge, these types of studies have not been undertaken in aspen hybrid clones. We began a study to explore the physiological basis of heterosis by comparing yield components between hybrid aspen clones and a local Finnish *P. tremula* seedling source. The objectives of the study are: 1) to assess variation in physiological and anatomical characters among hybrid aspen clones and local *P. tremula* and 2) to establish relationships among growth, photosynthesis, leaf and stomatal traits and 3) to analyze whether some of these characters could be used to predict aspen hybrid growth.

2 Materials and Methods

2.1 Plant Material

The material used in the study of 1998 was a clone trial established by the Foundation for Forest Tree Breeding and the University of Helsinki. The trial is located in Viikki, Helsinki University (lat. 60°14´N, long. 25°05´E, alt. 10 m). The trial was planted in May 1994 with one-year-old plants, and consists of four aspen hybrid clones (*Populus tremula* × *P. tremuloides*) and one *P. tremula* seedling source (Table 1). Clones 1–4 are known to be species hybrids between *P. tremula* and *P. tremuloides*, but in the case of clones 2, 3 and 4 there is uncertainty about the direction of the cross, and regarding the exact origin of the parental. Each clone was planted as a plot consisting of 4 × 4 plants spaced at 2.5 m × 2.25 m. The plots were laid out in a design of five randomized complete blocks, giving a total of 16 trees × 5 entries (4 clones + one *P. tremula*) × 5 blocks = 400 trees. In each plot, all the characters were measured on the five tallest trees.

2.2 Measurement of Growth

For observation of dynamic seasonal growth patterns, height, breast diameter at height 1.3 m and basal diameter 15 cm from the ground were measured every 2 or 3 weeks (8 measurements) over the period May 31 till October 19 in 1998. Measuring positions were marked on the stems to assure that repeated measurements were taken at exactly the same position. The last measurement on October 19, 1998 was used together with other

Table 1. Material used in the study.

Entries	Mother			Father		
		Latitude	Longitude		Latitude	Longitude
Clone 1	*P. tremula* (E700)	62°22´N	24°59´E	*P. tremuloides* (U2551)	54°06´N	122°03´W
Clone 2 either	*P. tremula* (E295)	62°22´N	24°59´E	*P. tremuloides* (U2502)	43°17´N	78°58´W
or	*P. tremuloides* (U2006)	Unknown	Unknown	*P. tremula* (E294)	62°22´N	24°59´E
Clone 3 either	*P. tremula* (E1732)	62°22´N	24°59´E	*P. tremuloides* (E2554)	45°17´N	78°58´W
or	*P. tremula* (E969)	61°45´N	29°18´E	*P. tremuloides* (E2576)	54°06´N	122°03´W
Clone 4	Same background information as clone 3			Same background information as clone 3		
P. tremula	*P. tremula* open pollinated	60°32´N	24°15´E			

traits for analysis of variance and correlation analysis.

2.3 Measurement of Gas Exchange Parameters

Observations of gas exchange parameters were recorded on July 23, 1998. Net photosynthetic rate (μmol CO_2 $m^{-2} s^{-1}$), stomatal conductance (mol $m^{-2} s^{-1}$), intercellular CO_2 concentration (μmol CO_2/mol), and transpiration rate (mol H_2O $m^{-2} s^{-1}$) were measured with a Li-6400 portable photosynthesis system (Li-Cor Inc.). The measurements of gas exchange parameters were made on a branch on the south side of the lowest third of the crown. The first three fully expanded leaves from the top of the shoot that had emerged in spring were sampled. In measuring, the parameters, we wished to examine the response of the trees to conditions actually prevailing in the field. Accordingly, measurements were made under natural ambient light and temperature levels. The leaves were measured in direct sunlight. The measurement was made between 1100 and 1600 h at 1650–1850 μmol $m^{-2} s^{-1}$ photosynthetically active radiation (PAR). The temperature stayed within the range of 26–27 °C and the relative humidity varied from 62–68%. Flow rate through the sample cell was 500 μmol s^{-1} with a CO_2 concentration of 325–335 μmol mol^{-1}. The measured leaf area taken from near the centre of the lamina was 6 cm^2.

2.4 Measurement of Leaf Characters

Leaf characters were measured immediately after the gas exchange characters. Leaf area (cm^2), and the specific leaf fresh mass (mg/cm^2) and specific leaf dry mass weight/area (mg/cm^2) were recorded. Leaf shape was defined as the ratio of maximum width and maximum length of the leaves. For the measurements of leaf characters, 10 leaves were taken from the same branch that was used to measure gas exchange parameters. Leaf area and shape were measured with a LI-3000A portable area meter (Li-Cor Inc.). Leaf dry mass was obtained after the leaves were dried for 48 h at 105 °C.

2.5 Measurement of Foliar Nitrogen

The same 10 leaves used for photosynthetic and leaf character measurements were analysed for total nitrogen using a CHN-900 carbon-hydrogen-nitrogen analyser (LECO Corporation, St. Joseph, MI, U.S.A). Foliar nitrogen was expressed as a percentage of total dry mass in the leaves.

2.6 Measurement of Stomatal Properties

The samples on which stomatal characters were measured were the same as for the measurements of CO_2 exchange parameters, but only one leaf from each individual tree was observed. Leaves were kept fresh until observation. Stomatal density (stomata/mm^2) and mean guard cell length (µm) of fresh leaves were observed with a Scanning Electron Microscope JSM-820 (JIOL, Japan). A piece of leaf about 1 cm^2 in area was cut from the middle of the lamina close to the main vein between two sub-veins. The leaf specimen was coated with platinum, and then observed under 400× magnification. Sixteen fields were observed for stomatal density and one field was transferred to a computer as an image for the measurement of guard cell length per unit area (µm/mm^2). Total guard cell length (µm) was calculated as the mean guard cell length multiplied by stomatal density. Plot means of the observations were used in the data analysis. Stomata occurred only on the abaxial surface of the leaf.

2.7 Statistical Analyses

All calculations were based on plot means. The differences among the hybrid clones and the *P. tremula* were examined by an analysis of variance (ANOVA) using the PROC GLM procedure of the SAS statistical software package (SAS Institute Inc. 1989) with the type III estimation of sum of squares. Entry means of gas exchange parameters, stomatal and leaf characters, and growth traits were separated by Tukey's multiple range test at level of significance of $p \leq 0.05$. Pearson's correlations were calculated using the SAS ®PROC CORR procedure to assess the linear relationships between the studied traits among hybrid clones.

3 Results

3.1 Growth and Gas Exchange Parameters

Seasonal growth patterns are illustrated in Fig. 1 as means of height, breast and basal diameter of the hybrids and *P. tremula*. Height, breast and basal diameter of the four hybrid clones were significantly higher than for the *P. tremula* (Table 2). *P. tremula* gave higher coefficients of variation for growth and gas exchange parameters than the hybrid clones. There were no statistically significant differences in stomatal conductance, intercellular CO_2 concentration or transpiration rate. There was significant difference in net photosynthesis among the four hybrid clones and *P. tremula*, but no difference in net photosynthesis between mean of four hybrid clones and *P. tremula*.

3.2 Stomata and Leaves

Among the stomatal and leaf traits, only stomatal density and total guard cell length of *P. tremula* gave higher coefficients of variation than the hybrid clones (Table 3). Some differences were found among clones for stomatal and leaf characters among four hybrid clones and *P. tremula* (Table 3). However, there were no significant differences in leaf fresh weight or dry weight

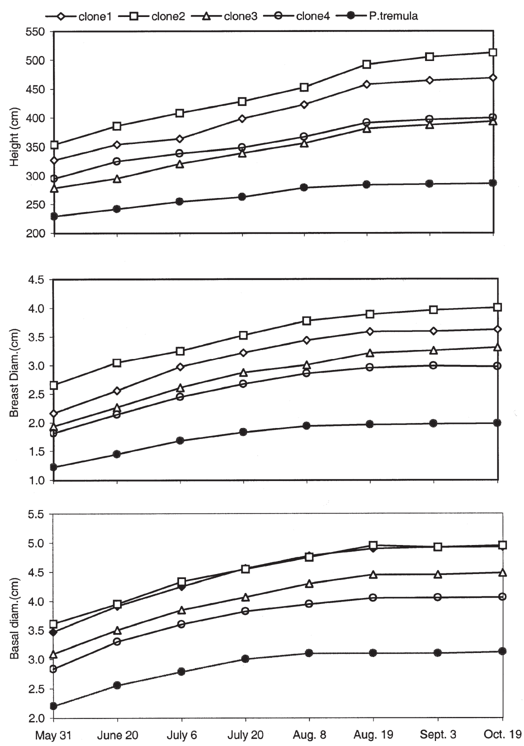

Fig. 1. Growth patterns of mean height, breast diameter and basal diameter for the hybrid clones and *P. tremula*. Each point is based on a plot mean.

Table 2. Means and coefficients of variation (CV) of growth and gas exchange parameters among the hybrid clones and *P. tremula* seedling source.

Clone	Growth traits			Gas exchange parameters			
	Height (cm)	Basal diameter (cm)	Breast diameter (cm)	Net photosynthetic rate (μmol CO_2 m^{-2} s^{-1})	Stomatal conductance (mol H_2O m^{-2} s^{-1})	Intercellular CO_2 concentration (μmol CO_2/mol)	Transpiration rate (mol H_2O m^{-2} s^{-1})
Clone 1	468.72ab	4.92a	3.63ab	20.19a	0.37a	224.46a	5.04a
CV	8.35	9.89	11.28	4.77	10.23	4.43	7.40
Clone 2	510.36a	4.99a	4.00a	20.38a	0.35a	217.99a	5.17a
CV	16.02	12.14	15.63	5.83	14.07	4.22	7.12
Clone 3	394.88bc	4.50a	3.33ab	17.11b	0.33a	233.06a	4.93a
CV	12.91	10.81	17.09	8.17	9.14	2.63	6.81
Clone 4	399.28abc	4.06ab	2.98b	19.57ab	0.36a	225.11a	5.20a
CV	11.64	12.64	15.73	5.52	8.78	4.10	7.02
Mean of hybrids	443.31(a)	4.61(a)	3.48(a)	19.31(a)	0.35(a)	225.14(a)	5.10(a)
P. tremula	285.64c(b)	3.11b(b)	1.98c(b)	17.52ab(a)	0.33a(a)	223.48a(a)	4.87a(a)
CV	21.52	17.04	29.41	13.68	17.70	5.72	13.35

Means followed by the same letters are not significantly different at $P<0.05$ (Tukey´s HSD test). The mean over hybrid clones and for *P. tremula* are followed by letters in brackets, which indicate whether or not these means are significantly different at $P<0.05$.

Table 3. Means and coefficients of variation (CV) of the stomatal and leaf traits among the hybrid clones and *P. tremula* seedling source.

Clone	Stomatal traits			Leaf traits				
	Length of stomata (μm)	Stomatal density (mm^{-2})	Total guard cell length (μm)	Leaf size (cm^2)	Leaf width/ length (cm)	Fresh weight/ area (mg/cm^2)	Dry weight/ area (mg/cm^2)	Foliar nitrogen (%)
Clone 1	25.47a	159.75c	4066.64a	13.21c	0.948a	17.91a	7.25a	2.52b
CV	5.94	4.08	5.71	8.53	2.93	5.78	6.31	10.92
Clone 2	21.19b	163.63bc	3405.84b	18.73b	0.842b	16.55a	6.89a	2.89a
CV	8.58	7.84	2.96	12.00	3.30	10.14	14.00	4.33
Clone 3	20.32b	182.59b	3707.68ab	25.76a	0.908a	17.36a	7.30a	2.25b
CV	6.35	7.52	3.21	6.87	17.39	4.00	6.69	1.81
Clone 4	24.77a	151.70c	3737.70ab	16.06cb	0.900ab	18.47a	7.47a	2.78a
CV	7.99	8.96	4.02	14.79	4.65	6.05	7.68	9.74
Mean of hybrids	22.93(a)	164.41(b)	3740.7(b)	18.44(a)	0.899(a)	17.57(a)	7.23(a)	2.61(a)
P. tremula	19.24b(b)	211.31a(a)	4066.42a(a)	13.17c(a)	0.904a(a)	16.55a(a)	7.63a(a)	2.59ab(a)
CV	4.89	10.16	11.89	6.00	3.88	10.63	5.17	4.19

Means followed by the same letters are not significantly different at $P<0.05$ (Tukey´s HSD test). The mean over hybrid clones and for *P. tremula* are followed by letters in brackets, which indicate whether or not these means are significantly different at $P<0.05$.

per unit area. Compared the mean of the four hybrid clones with *P. tremula*, only the stomatal characters differ between hybrid clones and *P. tremula*. Hybrid clones had larger mean guard cell length (22.9 μm) than *P. tremula* (19.2 μm), whereas *P. tremula* had a higher stomatal density (211.3/mm^2) than hybrid clones (164.4/mm^2).

3.3 Relationship of Gas Exchange Parameters with Foliar Nitrogen and Growth

Non-significant correlation was found between gas exchange parameters and growth traits of height, breast and basal diameter. Net photosynthesis correlated significantly with foliar nitrogen,

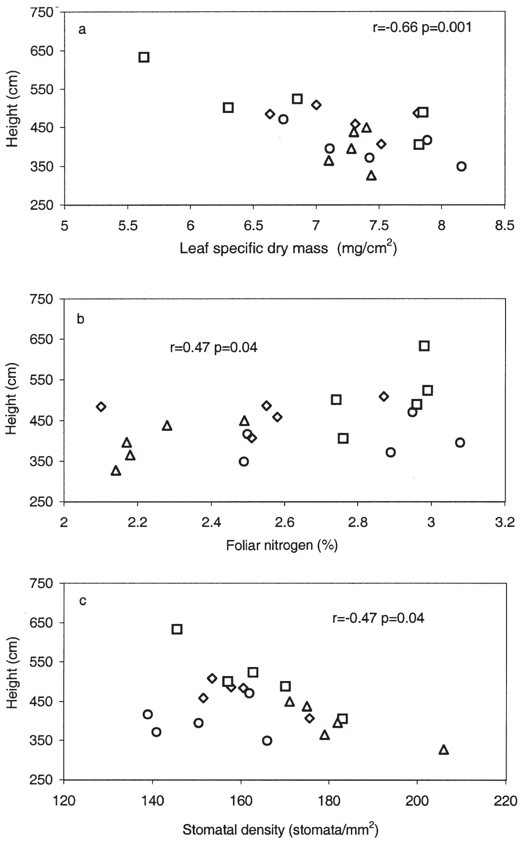

Fig. 2. Relationship between height and leaf specific dry mass (a), foliar nitrogen (b), and stomatal density (c) for the hybrid clones. Each point is based on a plot mean. The symbols are as in Fig. 1.

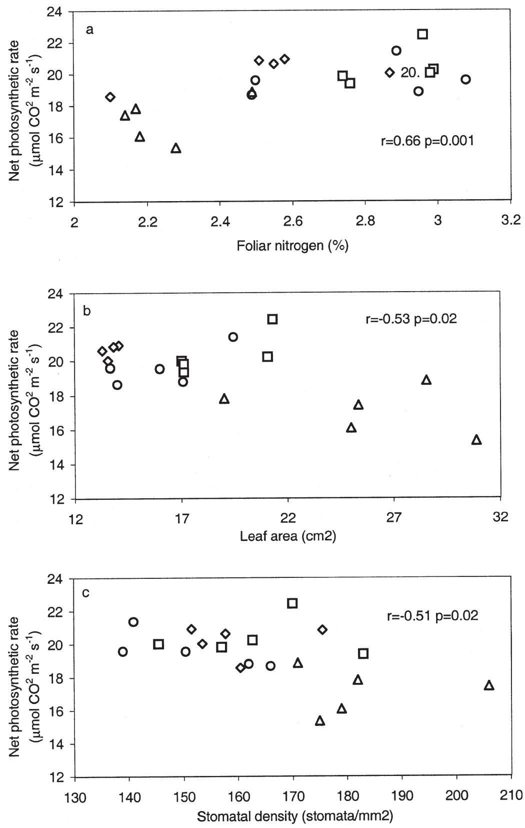

Fig. 3. Relationship between net photosynthetic rate and foliar nitrogen (a), leaf area (b) and stomatal density (c) for the hybrid clones. Each point is based on a plot mean. The symbols are same as Fig. 1.

negatively with leaf area and stomatal density (Fig. 2). Foliar nitrogen was correlated with transpiration rate ($r=0.49$, $P=0.02$), and negatively correlated with stomatal density ($r=-0.55$, $P=0.01$).

3.4 Relationship of Stomatal Characters with Gas Exchange Parameters, Growth and Leaf Characters

There was a strong negative correlation between stomatal density and mean guard cell length (Fig. 4). Height correlated significantly with foliar nitrogen, but negatively with leaf dry mass and stomata density (Fig. 3). Leaf dry mass also correlated negatively with breast and basal diameter ($r=-0.56$, $P=0.01$; $r=-0.50$, $P=0.02$). Likewise, leaf fresh mass correlated negatively with height ($r=-0.61$, $P=0.004$), breast diameter ($r=-0.55$, $P=0.01$) and basal diameter ($r=-0.49$, $P=0.02$). Leaf size correlated positively with stomata density ($r=0.51$, $P=0.02$), but negatively with mean guard cell length ($r=-0.62$, $P=0.003$).

4 Discussion

In the present study, significant variations were found in the wide array of growth, photosynthetic, stomatal and leaf traits among the hybrid

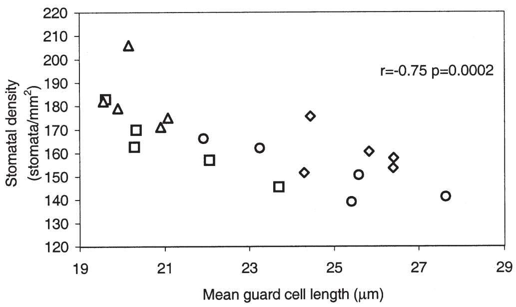

Fig. 4. Relationship between mean guard cell length and stomatal density for the hybrid clones. Each point is based on a plot mean.The symbols are as in Fig. 1.

clones and *P. tremula*. The existence of such variation indicates that clones could rather easily be selected for further breeding and practical cultivation. High levels of clonal variation have been found for morphological, growth and molecular traits in trembling aspen (Chong et al. 1994, Lund et al. 1992, Reighard and Hanover 1990; Thomas et al. 1997a, 1997b). Nelson and Ehlers (1984) noted that net photosynthesis was under strong genetic control in two hybrid *Populus* clones.

Parameters for gas exchange and growth traits in *P. tremula* showed higher variation than parameters in hybrid clones. Because the *P. tremula* was a half-sib family, the coefficient of variation of *P. tremula* was higher than for the hybrid clones. A similar pattern was found for stomatal density and total guard cell length, but not for length of stomata or any of the leaf traits. It appears, characters like growth and gas exchange parameters, including length of stomata and total guard cell length, that show a higher coefficient of variation for the *P. tremula* than for hybrid clones, are better predictors of yield than characters (e.g. leaf traits) where the coefficient of variation for *P. tremula* is smaller than in the hybrids.

The relationships between carbon fixation and yield for aspen indicate that no correlation or occasionally a negative one, exists between leaf or whole plant net photosynthesis and productivity (Okafo and Hanover 1978, Reighard and Hanover 1990, Thomas et al. 1997a). However, a significant positive correlation between net photosynthesis and biomass production was reported in hybrid poplars (*P. euramericana*) and *P. deltoides* by Orlovic et al. (1998). For aspen, in particular, previous studies reported a poor or negative correlation between either leaf or net photosynthesis of whole plants and productivity (Okafo and Hanover 1978; Reighard and Hanover 1990).

Our results showed that net photosynthesis was not correlated with height, breast diameter or basal diameter. For aspen clones in growth chamber experiments, Thomas et al. (1997a) showed a positive relationship between net photosynthesis and dry mass or height growth, but correlations were not significant between physiological characters measured in the growth chamber and field measurements of either physiological or growth characters.

In our study, a significant correlation was found between foliar nitrogen and net photosynthesis among the four hybrid clones. This indicates that foliar nitrogen might be used to predict net photosynthesis in aspen. The result is in agreement with a finding by Field and Mooney (1986). Foliar nitrogen has proved a good predictor of net photosynthesis (Reich et al. 1995). The biochemical basis for the relationship between foliar N and net photosynthesis is known (Evans 1989). For C_3 species, Reich et al. (1995) described the relationship between photosynthetic capacity and leaf nitrogen concentration with one general equation.

Variations in stomatal density and mean guard cell length might reflect plant growth. A positive correlation was found between stomatal density and fast growth in *Betula pendula* Roth (Wang et al. 1995) and *Azadirachta indica* A. Juss (Kundu and Tigerstedt 1998). Likewise, a strong correlation was demonstrated between the number of stomata on the leaf adaxial surface and biomass in *Populus* hybrids (Orlovic et al. 1998). However, Ceulemans et al. (1987) found no correlation between stomatal density (either adaxial or abaxial) and growth. In our study stomatal density was correlated negatively with height, but did not correlate with breast or basal diameter. Stomatal density decreased linearly with mean guard cell length (Fig. 4). The phenomenon is known for many plants, e.g. oak trees (Abrams and Kubiske 1990) and peach (Loreti et al. 1993). Since stomatal density increased as fast as the mean guard cell length decreased, there was no significant difference between total guard cell length of the mean of four hybrid clones and *P. tremula*. Nonetheless, significant differences existed between hybrids and *P. tremula* for mean guard cell length and stomatal density. The hybrid clones had a lower stomatal density but a larger mean guard cell length compared to *P. tremula*. For example, the hybrid clone 1 and *P. tremula* both have almost the same values of total guard cell length, but stomatal density of *P. tremula* was the highest, and mean guard cell length was the smallest among the entries (Table 3). Leaf anatomical characters were affected by variations in the light regime (Garcia Nunez et al. 1995). At high light intensities stomatal density normally increased with the time the leaves are exposed to continuous light (Koike et al. 1998, Zacchini et al. 1997). The high stomatal density and small mean guard cell length in *P. tremula* may be associated with the adaptation of this species to long day-length during the summer. In addition, stomatal characters could be also affected by different soil type. Guzina et al. (1995) showed that the differences in the numbers of stomata between *Populus deltoides* and hybrid poplar (*Populus* × *euramericana*) were statistically significant, and a genotype × environment interaction (GE) was shown in field experiments on three types of soil.

In our study, most leaf traits failed to correlate with growth traits. However, leaf fresh mass and leaf dry mass showed a moderate negative correlation with height, breast diameter and basal diameter. Wang et al. (1995) found a negative correlation between leaf specific dry mass and net photosynthesis in birch. They suggested that leaves with high dry mass might have high photosynthetic accumulates that inhibit photosynthetic efficiency. Cain and Ormrod (1984), studying two hybrids and two *Populus* clones, observed no significant differences among clones in ratio of leaf mass to leaf area. In our study, there were no significant differences among the clones for leaf area, leaf fresh or leaf dry mass.

It is apparent that the correlation between net photosynthesis and growth rate in forest trees is complex and is dependent on many factors. These include the tree population being studied, the age of the trees, the time(s) of year that net photosynthesis is measured, and site conditions (Johnsen and Major 1995).

5 Conclusions

The mean estimated height of hybrid aspen clones was 1.6 times higher than for *P. tremula*. Similarly, basal diameter was 1.5 times and breast diameter 1.8 times higher in the hybrids. Because the *P. tremula* was a half-sib family, the coefficient of variation of *P. tremula* was higher than for the hybrid clones for most traits in this study. Among the physiological and morphological characters examined here, only stomatal characters of hybrid clones differ from *P. tremula*. Leaf fresh and dry weight was correlated negatively with these growth traits (height, breast diameter and basal diameter). None of the other traits showed significant correlations with all the growth traits. Considering the complex interactions among factors governing yield, it is doubtful whether any single gas exchange or morphological variable can be a useful and reliable indicator of yield potential. Our analyses were based on a small number of clones measured over only five years. The results of the study need to be followed up by larger and longer experiments. Further studies are needed to confirm results found in this study.

In a separate paper concerning the same material (Yu et al. 2001) we investigated the relationships between growth and phenological traits. We found that the correlation between growth period and yield was highly significant. The growth period varied from 143–158 days for the four hybrid clones, but was only 112 days for *P. tremula*. Thus so called hybrid vigor seems here to be mainly attributable to a longer growth period rather than to physiological, leaf morphological or stomatal characters. The long growth period of the hybrids can be at least partly explained by the relatively southern ecotypes of *P. tremuloides* used in the hybrids (Table 1). Physiological processes are important in accounting for hybrid vigor, but their expression is in turn regulated by daylength and temperature responses of the genotype.

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