

# Gas exchange characteristics of the hybrid *Azadirachta indica* × *Melia azedarach*

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The hybrid *Azadirachta indica* × *Melia azedarach* is a new plant variety obtained through somatic hybridization, showing high concentrations of active insecticidal substances in its seeds. The hybrid could grow normally in regions with an average annual temperature of 15 °C, therefore it will likely become a valuable new tree species in subtropical and warm temperate regions. However, the photosynthetic physiological characteristics of *A. indica* × *M. azedarach* remain unknown. The photosynthetic gas exchange of the hybrid at three different ages (one year old, AM1; three years old, AM3; and five years old, AM5) were measured. The specific leaf mass per area (LMA), leaf N, leaf P, and leaf N/P of tree samples were measured, and the photosynthetic N and P use efficiencies (PNUE and PPUE, respectively) were also assessed. The maximum leaf net photosynthetic rate  $P_n$  (based on area),  $P_m$  (based on mass), light saturation point (LSP), light compensation point (LCP), stomatal conductance ( $g_s$ ), and transpiration rate ( $T_r$ ) of *A. indica* × *M. azedarach* decreased with increasing tree age, whereas the instantaneous water use efficiency (WUE) increased with age. The photosynthetic capacity showed no significant differences between AM3 and AM5, but was significantly higher in AM3 and AM5 when compared with AM1. The  $P_n$ ,  $P_m$ , apparent quantum yield (AQY), LSP,  $g_s$ , and  $T_r$  of *A. indica* × *M. azedarach* were significantly lower than that of the parental species *M. azedarach*, whereas the dark respiration rate ( $R_d$ ) and WUE were significantly higher than that of *M. azedarach*. The reduction in the maximal photosynthetic rate observed with increased age in the hybrid was primarily related to the increased LMA and the decline in leaf nitrogen (N) and leaf phosphorus (P) concentrations. Additionally, the decline in stomatal conductance ( $g_s$ ) was also an important factor leading to age-dependent reductions in the photosynthetic rate. These findings suggest that tree age significantly affects *A. indica* × *M. azedarach* gas exchange during juvenile stages, and the photosynthetic capacity of the hybrid was significantly lower than that of the parental species *M. azedarach*.

**Keywords:** Photosynthesis, Ontogeny, Stomatal Conductance, Leaf Nitrogen, Leaf Phosphorus, Leaf Mass Per Area

## Introduction

*Azadirachta indica* is a valuable multipurpose tree species producing a variety of active insecticidal substances, such as azadirachtin, which can be found at high concentrations in the seeds. *A. indica* is internationally recognized as having the greatest potential for the production of biological pesticide (Zheng et al. 2010, Ogbuewu et al.

2011). Therefore, such species has been introduced into many tropical and subtropical countries and regions. However, *A. indica* exhibits poor growth in regions with an average annual temperature below 20 °C and a minimum temperature below 0 °C (Hegde 1995, Ogbuewu et al. 2011).

Both *Melia azedarach* and *A. indica* belong to the Meliaceae family, and the *M.*

*azedarach* is widely distributed in China within broad latitudinal (18-39°) and altitudinal (600-800 m a.s.l.) ranges (Cheng & Gu 2005). The active insecticidal substances of *M. azedarach* are primarily located in its bark, while seeds and leaf contents are very low, thus limiting its further exploitation. The Nanjing Jiukang Biological Development Co., Ltd. successfully cultivated a hybrid of *A. indica* (from Myanmar) and *M. azedarach* (from Nanjing) for the first time through somatic hybridization technology. This *A. indica* × *M. azedarach* hybrid inherited the excellent features of its parental species, growing normally in Nanjing, which has an average annual temperature of 15 °C, but also producing an active insecticidal substance in its seeds at a markedly higher concentration than that of the local *M. azedarach*. At present, the hybrid has been planted in a small area in Nanjing, China and has produced viable seeds.

Photosynthesis plays a vital role in tree growth, production and regeneration. Previous studies have indicated that hybrids and their parents exhibit differences in their photosynthetic characteristics (Bassman & Zwier 1991, Wu & Campbell 2007). The difference in photosynthesis between hybrids and their parents primarily depended on differences in parental genotype and habitats (Wu & Campbell 2007). *A. indica* × *M. azedarach* is a new variety, and so far no assessment have been carried out of the photosynthetic physiological characteristics of the hybrid or its differences in terms of gas exchange parameters relative to its parents.

Previous studies showed strong differences in photosynthetic characteristics of forest species at different developmental stages. The photosynthetic rate at the leaf level decreases or increases with increasing tree age or size (Abdul-Hamid & Mencuccini 2009, Kenzo et al. 2006, Juárez-López et al. 2008, Nabeshima & Hiura 2008). However, most studies only compared the photosynthetic gas exchange between saplings and adults, while few studies focused on the physiology of trees at younger stages (Thorton et al. 1994, Greenwood et al. 2008, Reinhardt et al. 2009). Photosynthetic characteristics during the juvenile stages are strongly related to early growth and regeneration. Several studies performed at the juvenile stage primarily focused on photosynthetic capacity changes related to age in conifers (Reinhardt et al. 2009). Few studies on broadleaf species showed that the photosynthetic capacity on a mass or leaf area basis increased from sapling to intermediate sized trees (Thomas 2010). Considerable research on age-related changes in leaf gas exchange has focused on the “hydraulic limitation hypothesis”, i.e., the reduction of the leaf specific hydraulic conductance as trees grow in

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height due to an increased path length in roots, stems and branches (Ryan & Yoder 1997, Hubbard et al. 1999, Nabeshima & Hiura 2004). However, recent studies suggested that leaf functional traits (anatomy, morphology, or chemistry) might play important roles in age-related changes in gas exchange (Thomas 2010, Steppe et al. 2011).

The primary purposes of the present study were: (1) to compare the variation in gas exchange parameters of *A. indica* × *M. azedarach* at young stages (seedlings, older seedlings, and saplings); (2) to compare differences in gas exchange parameters between the hybrid *A. indica* × *M. azedarach* and its parents; and (3) to evaluate the relation between the gas exchange parameters of *A. indica* × *M. azedarach* and its leaf mass per area (LMA) and leaf nitrogen (N) and/or phosphorous (P) concentrations with increasing tree age.

## Materials and Methods

### Plant materials

The *A. indica* × *M. azedarach* hybrid and one of its parents (*M. azedarach*) were studied. The other parent species *A. indica* was not included in the study because it cannot grow in the study area. The *A. indica* × *M. azedarach* hybrid was created by somatic hybridization in 2002. In 2005, the Nanjing Jiukang Biological Development Co., Ltd. began to plant *A. indica* × *M. azedarach* hybrid trees in the Qiaoli Village of the Gaochun County. At present, the total planting area covers more than 300 ha in Nanjing. In June 2011, two or three *A. indica* × *M. azedarach* stands with one-year-old (AM1), three-year-old (AM3), and five-year-old (AM5) specimens and two stands with three-year-old *M. azedarach* (MA3) were selected to measure their photosynthetic gas exchange characteristics.

### Study site

The study was conducted in the Qiaoli Village, Gaochun County, Jiangsu Province, China (119° 06' 02" E, 31° 24' 38" N; mean elevation: 26 m a.s.l.). The annual average precipitation at the study site is 1157 mm, annual average temperature 15.4 °C, and the monthly average temperatures in January and July are 4.4 °C and 29 °C, respectively.

**Tab. 1** - Average tree height, diameter at breast height (DBH), crown height, and tree density of AM1 (one-year-old *A. indica* × *M. azedarach*), AM3 (three-year-old *A. indica* × *M. azedarach*), AM5 (five-year-old *A. indica* × *M. azedarach*), and MA3 (3-year-old *M. azedarach*).

Stand	Tree height (m)	DBH (cm)	Crown height (m)	Tree density (tree ha <sup>-1</sup> )
AM1	1.8 ± 0.2	1.2 ± 0.1	1.4 ± 0.1	1667 ± 72
AM3	3.6 ± 0.3	5.3 ± 0.4	2.3 ± 0.2	1458 ± 58
AM5	5.3 ± 0.4	8.3 ± 0.5	3.6 ± 0.2	1046 ± 45
MA3	3.8 ± 0.4	5.7 ± 0.5	2.5 ± 0.3	552 ± 29

The annual sunshine total is 1937 h, with 260 frost-free days throughout the year. The study site belongs to the northern subtropical monsoon climate zone. The soil is loamy with a pH ranging from 5.5 to 6.0. The site conditions of AM1, AM3, and AM5 were similar in that they were all planted in farmland. The slopes of the study area varied from 5% to 10%. The basic characteristics of the stands used in the study are shown in Tab. 1. The understory vegetation coverage in these stands was primarily *Oplismenus undulatifolius* (approximately 80-90%).

### Photosynthetic gas exchange measurements

The photosynthetic gas exchange of the leaves was measured with a LI-6400 portable photosynthesis system (Li-Cor, Inc., Lincoln, NE, USA). According to the mean DBH, tree height, and crown height, four typical sample trees of *M. azedarach* and *A. indica* × *M. azedarach* from three differently aged stands were selected. Four fully expanded and exposed leaves (of similar ages) from the middle layer of the tree canopy in each sample tree were selected for photosynthetic gas exchange measurements. The conditions in the leaf chamber were controlled automatically by the instrument with a CO<sub>2</sub> concentration of 370 ± 10 μmol mol<sup>-1</sup>, and the leaf temperature was maintained at 30 ± 1 °C. The air flow rate was set to 500 mL min<sup>-1</sup>. The photosynthetic light response was obtained by measuring 11 different photosynthetic active radiation (PAR) values (2000, 1500, 1000, 800, 500, 200, 100, 80, 50, 20, and 0 μmol m<sup>-2</sup> s<sup>-1</sup>). Light was provided by a Li-6400 LED red/blue light source. Such measurements were performed from 9:00 to 11:30 a.m. in mid-June 2011. The maximum net photosynthetic rate ( $P_n$ ), the apparent quantum yield (AQY), and the dark respiration ( $R_d$ ) were calculated using the method described by Bassman & Zwier (1991), and the stomatal conductance ( $g_s$ ), transpiration rate ( $T_r$ ), and instantaneous water use efficiency ( $WUE$ ,  $P_n/T_r$ ) were estimated at the maximal photosynthetic rate (eqn. 1):

$$P_n = P_{max} \left( 1 - e^{-\frac{-AQY \cdot PAR}{P_{max}}} \right) - R_d$$

where  $P_n$  is the net photosynthetic rate (μmol

m<sup>-2</sup> s<sup>-1</sup>),  $P_{max}$  is the maximum net photosynthetic rate (μmol m<sup>-2</sup> s<sup>-1</sup>), AQY is the apparent quantum yield (μmol m<sup>-2</sup> s<sup>-1</sup>), PAR is photosynthetically active radiation (μmol m<sup>-2</sup> s<sup>-1</sup>), and  $R_d$  is dark respiration (μmol m<sup>-2</sup> s<sup>-1</sup>). The light compensation point (LCP) was obtained by performing a linear regression with data taken at PPFDs of 0, 20 and 50 μmol m<sup>-2</sup> s<sup>-1</sup> (Hieke et al. 2002). The light saturation point (LSP) was estimated according to the trends in the  $P_n$ -PAR curves (Zhang & Xu 2000).

### Measurements of the LMA and leaf N and P concentrations

After measuring the photosynthetic gas exchange, all the leaves were collected and scanned to measure the leaf area using a WinFOLIA Analysis System (Regent Instruments Inc., Canada). Sampled leaves were then dried at 65 °C until a constant mass was reached, in order to calculate the specific leaf mass per area (LMA, g m<sup>-2</sup>). The dried leaves were then ground and passed through a 2-mm sieve. The total nitrogen (N) concentration was determined by Kjeldahl method. The phosphorus (P) concentration was determined by the colorimetric method described by Murphy & Riley (1962), with the absorbance wave length set at 880 nm. These measurements were used to determine the leaf N/P ratio for *A. indica* × *M. azedarach* and *M. azedarach*. The leaf N and P concentrations are presented on a dry-mass basis. The photosynthetic use efficiencies for N (PNUE) and P (PPUE) were calculated as the ratio of the mass-based maximum photosynthetic capacity ( $P_m$ ) to the leaf N and P concentrations, respectively.

### Statistical analysis

One-way analysis of variance (ANOVA) followed by *post-hoc* Duncan's test ( $\alpha = 0.05$ ) was carried out to test for differences in photosynthetic gas exchange parameters, LMA, leaf N, leaf P, leaf N/P, PNUE, and PPUE between the *A. indica* × *M. azedarach* specimens of different ages. Pearson's correlation between the photosynthetic parameters and the LMA, leaf N, leaf P, and leaf N/P was also calculated. A linear regression analysis was performed to test the contribution of several photosynthetic gas exchange parameters and leaf trait parameters to maximal net photosynthetic rate. All statistical analyses were performed using the software package SPSS® 13.0 (SPSS Inc., Chicago, Illinois, USA).

## Results

### Photosynthetic gas exchange

The maximal net photosynthetic rate  $P_n$ ,  $P_m$ , LSP, and  $g_s$  of *A. indica* × *M. azedarach* decreased with increasing tree age (Tab. 2). These parameters (except LSP) were signifi-

cantly higher for AM1 than for AM3 and AM5 ( $P < 0.05$ ). No significant difference were found between AM3 and AM5 ( $P > 0.05$ ). The  $AQY$  of AM1 was significantly higher than that of AM3 ( $P < 0.05$ ), whereas that of AM5 was between the values obtained for AM1 and AM3. The  $R_d$  of AM1 and AM3 were significantly higher than that of AM5 ( $P < 0.05$ ). The  $WUEs$  of AM3 and AM5 were significantly higher than that of AM1 ( $P < 0.05$ ).

In comparison with MA3, the maximal net photosynthetic rate ( $P_a$ ) of AM3 was only 75.6% of that observed for MA3. In addition, the  $AQY$ ,  $LSP$ ,  $g_s$  and  $T_r$  of AM3 were significantly lower ( $P < 0.05$ ), and the  $R_d$  and  $WUE$  were significantly higher than those of MA3 ( $P < 0.05$ ). No significant differences in the  $LCP$  were detected between AM3 and MA3 ( $P > 0.05$ ).

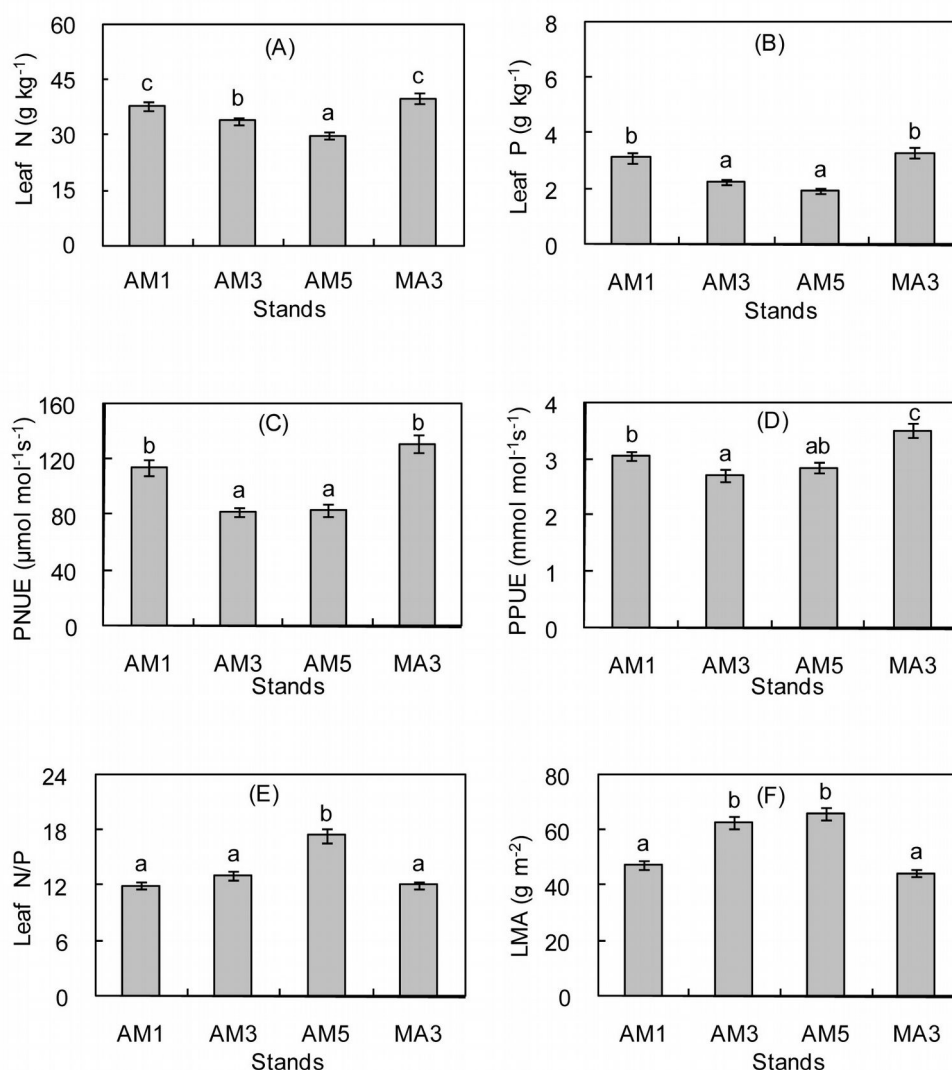
#### Changes in the LMA, leaf N, and leaf P

As the age of *A. indica* × *M. azedarach* increased, the leaf N and leaf P concentrations decreased gradually, whereas the leaf N/P and LMA gradually increased (Fig. 1A-F).

**Tab. 2** - Photosynthetic gas exchange parameters  $P_a$  (based on area),  $P_m$  (based on mass),  $AQY$ ,  $LSP$ ,  $LCP$ ,  $R_d$ ,  $g_s$ ,  $T_r$ , and  $WUE$  of AM1 (one-year-old *A. indica* × *M. azedarach*), AM3 (three-year-old *A. indica* × *M. azedarach*), AM5 (five-year-old *A. indica* × *M. azedarach*), and MA3 (three-year-old *M. azedarach*). Different letters between values along the same row indicate significant differences after the *post-hoc* Duncan's test ( $P \leq 0.05$ ;  $n=13-16$ ; means  $\pm$  SE).

Parameters	AM1	AM3	AM5	MA3
$P_a$ ( $\mu\text{mol m}^{-2}\text{s}^{-1}$ )	14.94 $\pm$ 0.37 <sup>b</sup>	12.71 $\pm$ 0.25 <sup>a</sup>	11.89 $\pm$ 0.31 <sup>a</sup>	16.82 $\pm$ 0.46 <sup>c</sup>
$P_m$ ( $\mu\text{mol g}^{-1}\text{s}^{-1}$ )	312.08 $\pm$ 7.77 <sup>b</sup>	200.93 $\pm$ 3.95 <sup>a</sup>	179.32 $\pm$ 4.68 <sup>a</sup>	376.29 $\pm$ 9.13 <sup>c</sup>
$AQY$ ( $\mu\text{mol mol}^{-1}$ )	0.055 $\pm$ 0.002 <sup>b</sup>	0.051 $\pm$ 0.001 <sup>a</sup>	0.053 $\pm$ 0.002 <sup>ab</sup>	0.056 $\pm$ 0.001 <sup>b</sup>
$R_d$ ( $\mu\text{mol m}^{-2}\text{s}^{-1}$ )	1.249 $\pm$ 0.025 <sup>b</sup>	1.287 $\pm$ 0.031 <sup>b</sup>	1.146 $\pm$ 0.018 <sup>a</sup>	1.119 $\pm$ 0.021 <sup>a</sup>
$LSP$ ( $\mu\text{mol m}^{-2}\text{s}^{-1}$ )	1284.8 $\pm$ 21.6 <sup>b</sup>	1195.1 $\pm$ 15.8 <sup>ab</sup>	1049.9 $\pm$ 17.2 <sup>a</sup>	1425.5 $\pm$ 25.3 <sup>c</sup>
$LCP$ ( $\mu\text{mol m}^{-2}\text{s}^{-1}$ )	28.54 $\pm$ 1.62 <sup>a</sup>	27.94 $\pm$ 1.87 <sup>a</sup>	27.31 $\pm$ 1.49 <sup>a</sup>	33.87 $\pm$ 2.05 <sup>a</sup>
$g_s$ ( $\text{mol m}^{-2}\text{s}^{-1}$ )	0.065 $\pm$ 0.005 <sup>b</sup>	0.044 $\pm$ 0.003 <sup>a</sup>	0.040 $\pm$ 0.003 <sup>a</sup>	0.077 $\pm$ 0.006 <sup>c</sup>
$T_r$ ( $\text{mmol m}^{-2}\text{s}^{-1}$ )	2.496 $\pm$ 0.213 <sup>b</sup>	1.538 $\pm$ 0.156 <sup>a</sup>	1.435 $\pm$ 0.138 <sup>a</sup>	2.417 $\pm$ 0.251 <sup>b</sup>
$WUE$ ( $\mu\text{mol mmol}^{-1}$ )	5.986 $\pm$ 0.305 <sup>a</sup>	8.264 $\pm$ 0.231 <sup>c</sup>	8.286 $\pm$ 0.294 <sup>c</sup>	6.959 $\pm$ 0.263 <sup>b</sup>

**Fig. 1** - Comparison of leaf N (A), leaf P (B), PNUE (C), PPUE (D), leaf N/P (E), and LMA (F) among AM1 (one-year-old *A. indica* × *M. azedarach*), AM3 (three-year-old *A. indica* × *M. azedarach*), AM5 (five-year-old *A. indica* × *M. azedarach*), and MA3 (three-year-old *M. azedarach*). Different letters indicate significant differences across age classes (AM1-AM5) and MA3 after the Duncan's test ( $P \leq 0.05$ ). Error bars represent standard errors ( $n=4$ ).



**Tab. 3** - Correlations between photosynthetic gas exchange parameters and leaf traits with tree age. (\*):  $P \leq 0.05$ ; (\*\*):  $P \leq 0.01$ .

	$P_a$	$P_m$	AQY	$R_d$	LSP	LCP	$g_s$	$T_r$	WUE	PNUE	PPUE	LN	LP	N/P	LMA
$P_a$	1	0.963**	0.426	0.287	0.858**	0.433	0.948**	0.972**	-0.964**	0.820**	0.315	0.900**	0.987**	-0.965**	-0.975**
$P_m$	-	1	0.574	0.411	0.933**	0.591*	0.974**	0.962**	-0.940**	0.726**	0.203	0.945**	0.974**	-0.908**	-0.976**
AQY	-	-	1	0.100	0.498	0.827**	0.644*	0.557	-0.527	0.057	-0.286	0.469	0.411	-0.287	-0.489
$R_d$	-	-	-	1	0.584*	0.308	0.329	0.281	-0.143	0.162	0.029	0.574	0.402	-0.228	-0.274
LSP	-	-	-	-	1	0.631*	0.884**	0.851**	-0.805**	0.533	-0.004	0.970**	0.906**	-0.784**	-0.879**
LCP	-	-	-	-	-	1	0.685*	0.577*	-0.542	-0.076	-0.562	0.612*	0.476	-0.298	-0.498
$g_s$	-	-	-	-	-	-	1	0.984**	-0.966**	0.647*	0.091	0.919**	0.951**	-0.875**	-0.954**
$T_r$	-	-	-	-	-	-	-	1	-0.983**	0.720**	0.188	0.892**	0.963**	-0.912**	-0.956**
WUE	-	-	-	-	-	-	-	-	1	-0.739**	-0.204	-0.849**	-0.943**	0.922**	0.967**
PNUE	-	-	-	-	-	-	-	-	-	1	0.775**	0.605*	0.792**	-0.881**	-0.792**
PPUE	-	-	-	-	-	-	-	-	-	-	1	0.094	0.269	-0.386	-0.280
LN	-	-	-	-	-	-	-	-	-	-	-	1	0.945**	-0.815**	-0.915**
LP	-	-	-	-	-	-	-	-	-	-	-	-	1	-0.956**	-0.976**
N/P	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0.942**
LMA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1

The leaf N, leaf P, PNUE, and PPUE of AM1 were significantly higher than those of AM3 and AM5 (with the exception of the PPUE in AM5 -  $P < 0.05$ ), whereas the leaf N/P and LMA of AM1 were significantly lower than those of AM3 and AM5 ( $P < 0.05$ ), with the exception of the leaf N/P of AM3. The Leaf P, LMA, PNUE, and PPUE exhibited no significant difference between AM3 and AM5 ( $P > 0.05$ ). The leaf N of AM3 was significantly higher than that of AM5 ( $P < 0.05$ ), whereas the leaf N/P of AM3 was significantly lower than that of AM5 (Fig. 1A and Fig. 1E -  $P < 0.05$ ). The leaf N, leaf P, PNUE, and PPUE of AM3 were significantly lower than those of MA3 ( $P < 0.05$ ), and the LMA of AM3 was significantly

higher than that of MA3 ( $P < 0.05$ ). No significant difference in the leaf N/P was observed between AM3 and MA3 ( $P > 0.05$ ).

#### Correlation analysis

Correlations between the photosynthetic gas exchange parameters and leaf traits with age for *A. indica* × *M. azedarach* are shown in Tab. 3. The  $P_a$ , LSP, and LCP were significantly and positively correlated with the leaf N concentration. The  $P_a$ ,  $P_m$ , LCP,  $g_s$ , and  $T_r$  were significantly and positively correlated with the leaf P concentration. Significantly negative correlations were observed between the LMA and  $P_a$ ,  $P_m$ ,  $g_s$ , and  $T_r$ . The WUE was significantly and positively correlated with the LMA. The LSP and LCP were

significantly and positively correlated with the PNUE, and the LSP was negatively correlated with the PPUE. No significant correlation was observed between the other gas exchange parameters and PPUE or PNUE.

#### Regression analysis

Based on the result of the correlation analysis, nine variables showing significant correlations were further analyzed as possible predictors of  $P_a$  using a simple linear regression analysis. Linear regression parameters for these relations are shown in Tab. 4. All the models between  $P_a$  and LSP,  $g_s$ ,  $T_r$ , WUE, PNUE, TN, TP, N/P or LMA were significant ( $P < 0.001$ ). The standardized partial regression coefficient was highest for LMA, followed by  $g_s$  and TP, the  $T_r$  and TN concentration also had important effects on  $P_a$ , and other variables had a relatively small impact on  $P_a$ .

**Tab. 4** - Linear regression analysis between the maximal net photosynthetic rate (based on area) and LSP,  $g_s$ ,  $T_r$ , WUE, PNUE, TN, TP, N/P or LMA with tree age. (Coefficient): partial regression coefficient; (Std coeff.): standardized partial regression coefficient.

Variable	Coefficient	SE	Std coeff.	Whole model			
				P	F	$r^2$	P
LSP	0.012	0.001	0.933	<0.001	66.789	0.857	<0.001
Constant	-1.067	1.751	0.000				
$g_s$	107.890	7.942	0.974	<0.001	184.552	0.943	<0.001
Constant	7.828	0.407	0.000				
$T_r$	2.538	0.227	0.962	<0.001	124.526	0.918	<0.001
Constant	8.550	0.432	0.000				
WUE	-1.131	0.130	-0.940	<0.001	76.134	0.872	<0.001
Constant	21.739	0.993	0.000				
PNUE	1.156	0.145	0.930	<0.001	63.828	0.851	<0.001
Constant	5.408	0.986	0.000				
TN	0.366	0.035	0.958	<0.001	112.219	0.910	<0.001
Constant	0.729	1.182	0.000				
TP	2.595	0.191	0.974	<0.001	184.238	0.943	<0.001
Constant	6.800	0.481	0.000				
N/P	-0.846	0.123	-0.908	<0.001	47.202	0.808	<0.001
Constant	25.168	1.755	0.000				
LMA	-0.159	0.011	-0.976	<0.001	197.31	0.947	<0.001
Constant	22.557	0.675	0.000				

#### Discussion

The photosynthetic gas exchange is affected by ontogeny. The  $P_a$ ,  $P_m$ , LSP, LCP,  $g_s$ , and  $T_r$  of *A. indica* × *M. azedarach* decreased with increasing tree age, whereas the instantaneous WUE increased. In general, the photosynthetic gas exchange parameters of AM3 and AM5 were significantly lower than the parameters of AM1 ( $P < 0.05$ ), and no significant differences in these parameters were observed between AM3 and AM5 ( $P > 0.05$ ). Thorton et al. (1994) reported that the  $P_m$  of high-elevation red spruce (*Picea rubens*) was reduced by 32% from seedling to sapling. Greenwood et al. (2008) also showed that the  $P_m$  of the 12-year age class of red spruce trees exhibited a significant decrease compared with the three-year-old trees, and the  $P_a$  and  $g_s$  were similar between the three-year age and 12-year age classes. Reinhardt et al. (2009) found that the photosynthetic gas exchange parameters at the leaf

level in *Abies fraseri* exhibited no significant difference between seedlings (<3 cm in height), older seedlings (>three years of age and ~0.15-1 m in height), and saplings (>10 years of age and ~1-2 m in height). However,  $P_a$  or  $P_m$  of *Acer saccharum*, *Tsuga canadensis*, and *Betula alleghaniensis* increased from the sapling (~1 cm dbh) to later stages of larger size (~2-6 cm - Thomas 2010). Many studies have indicated that a reduction of photosynthesis with increasing tree age (or size) is primarily caused by the increase in the structural complexity and tree height, which lead to hydraulic constraints and further results in lower stomatal conductance (Bond 2000, Drake et al. 2010). However, age-related (or size-related) hydraulic limitations in younger trees do not act on photosynthesis as a key driving variable. Other factors, such as leaf characteristics (anatomy, morphology or chemistry), may also play a key role in age-related (or size-related) decline (Steppe et al. 2011).

In this study, a decline in the maximum photosynthetic rate of *A. indica* × *M. azedarach* with increasing tree age may result in part from the decreased stomatal conductance, leading to a reduction in available CO<sub>2</sub> in the leaf cells. A positive correlation between the photosynthetic rate ( $P_a$  or  $P_m$ ) and the  $g_s$  was observed (Tab. 3). Previous studies have also shown that the  $g_s$  typically decreases with tree age or height (Steppe et al. 2011). When the stomata are open and CO<sub>2</sub> diffuses into the leaves, moisture evaporates through transpiration. The decline in stomatal conductance with increasing tree age leads to a decline in the transpiration rate. Although the photosynthesis rate also decreased with age, the magnitude of the decrease in  $g_s$  was higher than that of  $P_a$ , which indicates that increased *WUE* occurs in older saplings. Nabeshima & Hiura (2004) also confirmed that the increased *WUE* with an increase in plant size is related to the relatively higher magnitude of the decrease in stomatal conductance.

The photosynthesis of plants varied among different genotypes and habitats. Therefore, hybrids showing a photosynthetic physiology that differs from their parental species are better suited to certain habitats (Ludwig et al. 2004). Some studies reported that the physiological traits of hybrids had parental-like and transgressive traits (Ludwig et al. 2004, Wu & Campbell 2007, Silim et al. 2001). In this study, the maximal net photosynthetic rate (14.94  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , based on area) of one-year-old *A. indica* × *M. azedarach* trees was close to that of one-year-old *A. indica* (15.13  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , provenance from Myanmar - Kundu & Tigerstedt 1998). The introduced *A. indica* (from India) in the southwestern region of China showed that the maximal  $P_a$ , *AQY*, *LSP*, *LCP*, and  $R_d$  of 10-year-old *A. indica* varies from 9.46 to

11.58  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , from 0.044 to 0.056  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , from 1021.82 to 1205.31  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , and from 1.19 to 1.34  $\mu\text{mol m}^{-2} \text{s}^{-1}$  depending on its source (Zheng et al. 2011). In the current study, the gas exchange parameters of five-year-old *A. indica* × *M. azedarach* were close to the results of the above-described study, but the maximal net photosynthetic rate and *LSP* were significantly lower than those of local *M. azedarach* ( $P < 0.05$ ). This finding suggests that the gas exchange characteristics of *A. indica* × *M. azedarach* are more similar to those of the parental *A. indica*, and its light use efficiency is lower than that of the local parental species *M. azedarach*. Bassman & Zwier (1991) showed that the values of gas exchange parameters in *Populus trichocarpa* × *P. deltoides* were in the range between those of the two parent species. The photosynthetic rate of the hybrid poplar *Populus* × *euramericana* Dode (Guinier) was generally higher than that of eastern cottonwood (*Populus deltoides* Bartr - Orlovic et al. 1998). Wu & Campbell (2007) revealed that the gas exchange traits in *Ipomopsis* can be both intermediate and transgressive relative to those in the parental species. The performance of hybrids in terms of their photosynthetic physiology depended on the parental cytotype (Wu & Campbell 2007).

Changes in the gas exchange with increasing tree age (or size) are closely related to the leaf structure and biochemical properties. It is well known that the maximal photosynthetic rate is strongly affected by various leaf characteristics, such as the LMA, N and P concentrations, and N/P (Reich et al. 1998, Hidaka & Kitayama 2009). In the current study, the leaf N and P concentrations of the hybrid exhibited a decreasing trend with increasing tree age, whereas the leaf N/P and LMA increased with age. The maximum photosynthetic rates ( $P_a$  or  $P_m$ ) were positively correlated with the leaf N and leaf P concentrations and were negatively correlated with the leaf N/P and LMA (Tab. 3). A linear regression analysis also indicated that leaf traits (*i.e.*, LMA, TP and TN) played a leading role in age-dependent changes in hybrid photosynthesis (Tab. 4). A reduction in the nutrient availability with increasing tree age has been suggested to affect the age-dependent decrease in photosynthesis (Gower et al. 1996). Some studies have indicated that leaf N concentrations decrease as the tree size increases (Merilo et al. 2009, Wright et al. 2004). In contrast, other studies suggested that leaf N concentrations do not vary with increasing tree age (or size - Niinemets 2002, Nabeshima & Hiura 2008) or may even decrease with increasing tree age (or size - Nabeshima & Hiura 2004). A reduced N content clearly limits the formation of a high-capacity photosynthetic apparatus,

and this effect is partly responsible for the size-dependent reductions in photosynthetic potentials (Merilo et al. 2009, Woodruff et al. 2009).

Although the LMA typically increases with increasing tree age, marked increases in LMAs were observed across age classes of less than 40 years, and the rate of change decreases across the mature age classes (Steppe et al. 2011). A similar result was observed in this study. In conifers, the age- and size-dependent increase in LMA (LMA = leaf density × thickness) is primarily associated with enhanced leaf density, whereas both the thickness and density are increased in broad-leaved species (England & Attiwill 2006, Juárez-López et al. 2008, Niinemets et al. 2009). Many studies have reported that age-dependent (or size) increases in the LMA were possibly caused by greater water stress in the leaves of large trees (Ambrose et al. 2009, Niinemets et al. 2009). The photosynthetic capacity is also influenced by the increased LMA, and this effect has been related to changes in the ratios of internal air space and mesophyll area to the total area. In addition, a larger LMA may also imply an increased respiration rate per leaf surface area, which is associated with a reduction in the net carbon gain per unit leaf area (Koch et al. 2004).

The PNUE and PPUE of the *A. indica* × *M. azedarach* were also found to decrease with increasing tree age. No significant differences in these two parameters were observed between AM3 and AM5 ( $P > 0.05$ ), but they were significantly higher in AM1 ( $P < 0.05$ ). On a global scale, the PPUE of trees increases when P availability decreases (soil P and leaf N/P), whereas the PNUE significantly increases with the decline in the photosynthetic rate through a larger LMA (Wright et al. 2005). When the N and P availabilities are low, a higher LMA would lead to a decline in the PNUE (Hidaka & Kitayama 2009) because a larger proportion of N is invested in non-photosynthetic compounds. As a result, the N allocated to photosynthesis enzymes is reduced, leading to a decline in photosynthesis (Onoda et al. 2004, Takashima et al. 2004). A higher LMA may also reduce photosynthesis through another mechanism, *i.e.*, by extending the CO<sub>2</sub> diffusion path from the stomata to mesophyll cells and then to chloroplasts, leading to a decrease in the light received by the chloroplast and a decline in the photosynthetic rate (Parkhurst 1994, Hanba et al. 1999). Our results show a decline in the maximal photosynthetic rate of *A. indica* × *M. azedarach* with increasing tree age, which is consistent with the above-mentioned processes of age-related reductions in stomatal conductance, leaf N, and leaf P and increased LMA.



## Conclusions

The present study was aimed at investigating the photosynthetic physiological characteristics of *A. indica* × *M. azedarach* in its juvenile stages. Our results indicate that most photosynthetic gas exchange parameters of *A. indica* × *M. azedarach* decreased with increasing tree age, whereas the instantaneous water use efficiency (*WUE*) increased with age. The photosynthetic gas exchange parameters of AM3 and AM5 were significantly lower than the AM1 parameters. The reduced maximal photosynthetic rate of *A. indica* × *M. azedarach* with age was primarily related to the increase in the leaf mass per area (LMA) and a decrease in the leaf nitrogen (N) and leaf phosphorus (P) concentrations. Additionally, the decline in stomatal conductance (*g<sub>s</sub>*) was also an important factor leading to age-dependent reductions in the photosynthetic rate. The gas exchange characteristics of *A. indica* × *M. azedarach* were more similar to those of the parental *A. indica*, and its light use efficiency was lower than that of the local parental species *M. azedarach*.

These results may be useful in the management of cultivated stands of the hybrid *A. indica* × *M. azedarach*. For example, pruning and thinning must be conducted after the stands reach three years of age in order to enhance tree growth and seed output. Indeed, the high photosynthetic rate also implies that the hybrid needs higher light conditions in its juvenile stages and may be unsuitable for growth in the understory.

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