Trees (2009) 23:501–508
DOI 10.1007/s00468-008-0296-6

ORIGINAL PAPER

Difference in cuticular transpiration and sclerophylly in juvenile and adult pine needles relates to the species-specific rates of development

Marta Pardos · Rafael Calama · José Climent

Received: 28 July 2008 / Revised: 7 November 2008 / Accepted: 12 November 2008 / Published online: 3 December 2008

Abstract *Pinus* species show remarkable ontogenetic differences in needle morphology (heterophylly) between juvenile and adult vegetative phases. This developmental shift may play an adaptative role in their success under diverse habitats. As a first step to know the functional differences between each vegetative phase, we compared water loss through the cuticles of juvenile and adult needles of 21-month-old nursery-grown seedlings of nine hard pine species. Cuticular transpiration (CT), calculated after complete stomatal closure, was obtained by leaf-drying curves, and was related to leaf, ontogenetic and climatic parameters. The rate of cuticular transpiration (RCT) between juvenile and adult needles differed across pine species, and in particular segregated the Mediterranean species *Pinus canariensis* and *P. halepensis*, from the Eurasian *P. uncinata* and introduced species *P. radiata*. For these species, RCT was always higher in juvenile needles. The different leaf and ontogenic parameters studied were correlated with the variation in RCT among the nine pine species. We discuss this relationship in the light of the species ecology. Besides their possible adaptive interpretation, these results suggest an underlying need to consider the ontogenetic heterophylly when assessing functional traits in hard pine seedlings, in particular those traits that govern water relations.

Keywords Needle age · Water loss · Heteroblasty · Heterophylly · *Pinus*

Communicated by P. von Aderkas.

M. Pardos (✉) · R. Calama · J. Climent
CIFOR-INIA, Ap. Correos 8.111, 28080 Madrid, Spain
e-mail: pardos@inia.es

Introduction

Heteroblasty is a widespread phenomenon in plants, consisting of differences in leaf characteristics, internode dimensions and fate of axillary buds along the plant axis (Jones 1999). In particular, heterophylly refers to the leaf component of heteroblasty, i.e. the variation in the size or shape of leaves produced along the axis of an individual plant. Several conifer genera, such as *Juniperus*, *Cupressus*, *Pinus* and *Cedrus* show a marked heterophylly during their vegetative phase. It is still poorly known as to what extent this process is genetically fixed during ontogeny (Kubien et al. 2007) or related to different adaptive strategies allowing them to cope with the contrasting environmental conditions they encounter (Miller et al. 1995; Winn 1999).

Pinus species, in particular, show remarkable heterophylly and heteroblasty, with distinct juvenile and adult vegetative phases. This plasticity may play an adaptative role in their success across diverse habitats. In the juvenile phase, when growth is free, stem units exclusively bear solitary, juvenile needles and elongate shortly after their formation without forming terminal buds. By contrast, the adult phase is characterised by preformed stem units, baring fascicles of adult needles bundled in dwarf-shoots (Cannell et al. 1976; Lanner 1976). Between these two extreme stages, various transitional stages can be found, characterised by the co-occurrence of juvenile needles and axillary dwarf shoots bearing adult, secondary needles. Differences between the juvenile and adult phases in specific leaf area (SLA), resistance to water loss and resistance to frost damage have been reported in *Pinus canariensis* (Climent et al. 2006, Luis et al. 2007), but these comparisons remain to be investigated in other species.

Both the timing and amplitude of heteroblastic changes can vary greatly between pine species; while most pines form secondary needles after the second vegetative period, some others produce adult needles during the first growing season (Climent et al. 2006 and in preparation). In the Mediterranean species, *Pinus pinea*, *P. canariensis*, *P. halepensis* and *P. brutia*, and to a lesser extent also in *P. pinaster*, the juvenile growth can persist for several years (Klaus 1989). In particular, *P. pinea* and *P. canariensis* have long-lasting juvenile stages which can last for six or more years depending on the genotype and the environment.

One of the theories for the evolution of juvenile forms of heteroblastic woody species suggests that these forms are selected for resistance to water loss; but in fact many juvenile forms of woody plants show characteristics of water spenders (Darrow et al. 2002). In *P. canariensis*, juvenile needles lose water faster than adult needles and seedlings have a higher probability of death than adults when planted under a dry common environment. Yet, provenances from dry regions show a consistently longer juvenile phase (López et al. 2007). This counter-intuitive outcome was also found in *Juniperus occidentalis* (Miller et al. 1995). *Juniperus occidentalis* is a water stress-tolerant species that maintains the juvenile vegetative state as a strategy to enhance its competitive ability. Although such strategy is thought to be a risky compromise, it allows more adaptive biomass allocation in the tree as the transition to the adult form occurs (Miller et al. 1995; Climent et al. 2006). If this is the case, we would expect that prolonged juvenility might be related to greater inter-specific variation in differentiation between juvenile and adult needles.

Drought is an important limiting factor for growth in Mediterranean ecosystems. Thus, woody plants have developed drought resistance to cope with water stress, by either dehydration avoidance or dehydration tolerance strategies (Levitt 1972). Plants that adopt the first strategy avoid stress because their functions are relatively unexposed to tissue dehydration (Blum 2005). Conversely, plants tolerate drought by withstanding some degree of tissue dehydration without protoplasmatic damage (Villar et al. 2004). Under conditions of optimum water availability, cuticular transpiration is relatively insignificant as compared with stomatal transpiration. However, during periods of severe water deficit that causes stomatal closure, the cuticle becomes the most important structure restricting leaf water loss (Tischler and Voigt 1990; Burghardt and Riederer 2003). In particular, a low rate of water loss through the plant cuticle [i.e., cuticular transpiration (CT)] is considered to be an important drought survival mechanism in cereal genotypes (Clarke and McCaig 1982; Clarke et al. 1989; Clarke 1992) and likewise in woody shrubs from the Californian *chaparral* (Davis et al. 2004).

For this study, we chose nine hard pine species that share a circum-Mediterranean geographical distribution area in Western Europe, but presently occupy different ecological niches with marked differences in water availability (Barberó et al. 1998; Gandullo 1994). We tested two hypotheses: first, if juvenile pine needles always lose water faster than adult needles, after complete stomatal closure, irrespective of species; and second, if differences in cuticular transpiration between juvenile and adult needles are greater in those species that delay the formation of adult needles, that is, in the Mediterranean pines (sensu Klaus 1989), compared to Euro-Siberian species. We also expected to find a meaningful relationship between the morpho-physiological characterisation of juvenile and adult needles and the climatic conditions typical of each species origin.

Materials and methods

Plant material

Plant material for this study was obtained from the seedlings of nine pine species. These nine species share a circum-Mediterranean geographical distribution area in Western Europe. They include: (1) five Mediterranean species, subsection *Pinaster* : *P. pinaster*, *P. brutia*, *P. halepensis*, *P. pinea* and *P. canariensis*, (2) three Eurasian pines, subsection *Pinus*: *P. sylvestris*, *P. uncinata*, and *P. nigra*, and (3) one introduced, widely used pine species: *P. radiata*. *P. radiata* is currently widespread in mild and humid climate areas in Northern Spain, but it is also representative of other Mediterranean climate zones (coastal SW California). This species is phylogenetically distant to the other pines, since it is classified in subsection *Australes* of section *Trifoliae*, while all the other studied pines belong to subsection *Pinus* (Gernandt et al. 2005).

Seeds were collected by the Forest Administration (DGB), in natural stands (except in *P. radiata)* from open-pollinated trees at least 100 m apart to reduce consanguinity (Table 1). The nine species were each represented by the provenance which corresponded to the centre of its ecological range, which had shown "typical" or average growth and survival in previous field experiments (Chambel et al. 2007). Seed lots were an equilibrated mixture from 25 to 30 mother trees of the same provenance.

Cultivation conditions and experimental design

Prior to germination, seeds were stratified at 4°C for a month to homogenise their germination rate. In June 2005, two seeds were sown in 300 cc cell Forest-Pot container, and placed in a greenhouse located in NW Spain (42°16′38″ N, 7°37′32″ W, 537 m a.s.l.). During the experimental period, temperatures inside the greenhouse

Table 1 Geographic location of the studied seed source for each species

Species	Provenance	Altitude	Latitude	Longitude
P. brutia	Taurus (Bucak). Turkey	980	37° 29′ N	30° 37′ E
P. canariensis	Tenerife. Spain	1,350	28° 19′ N	16° 44′ W
P. halepensis	Inland Eastern Spain	830	39° 35′ N	0° 58′ W
P. pinea	La Mancha. Spain	690	39° 22′ N	2° 38′ W
P. pinaster	Guadarrama Mountains. Spain	1,230	40° 34′ N	4° 19′ W
P. nigra	Southern Iberian Range. Spain	1,280	40° 43′ N	2° 15′ W
P. sylvestris	Guadarrama Mountains. Spain	1,520	40° 51′ N	4° 00′ W
P. uncinata	Eastern Pyrenees. Spain	1,760	42° 22′ N	2° 17′ E
P. radiata	Vasque Coast. Spain	220	43° 15′ N	2° 05′ W

ranged from −1.2 to 39.4°C (average of 14°C), and mean relative humidity was 80%. Seedlings were grown under natural photoperiod. Water availability was maintained ca. 80% of field capacity, corresponding to 37.3% in volume. Growing media was a mixture of peat and perlite (3:1, v:v), plus 2 g/l of Osmocote. After germination, one germinated seed was removed from each container, leaving those seedlings at the most similar developmental stage. Seedlings were divided in three randomised complete blocks (15 plants per species and block, 135 plants per block, 405 plants in total) to control for fine-scale microenvironmental gradients. In February 2006, 15 seedlings per species were transplanted to individual 7-L containers, and placed at full sunlight (temperatures ranged from −6.0 to 36°C, average temperature of 12°C and mean relative humidity of 72%). Pots were filled with the same growing media and fertiliser. Seedlings were watered regularly, following the nursery's standard cultivation protocol, which implied avoiding both water stress and over-saturation of the pot rooting media.

Leaf-drying curves

Cuticular transpiration (CT) after complete stomatal closure was measured by the mass loss in excised needles. When seedlings were 21 months' old (March 2007), fresh juvenile and adult needles from five randomly selected seedlings per species were harvested at 0800 h, placed in wet paper towels, brought into the laboratory and used for water-loss determinations. This period of time following germination was sufficient for all nine species to develop both types of needles in transitional vegetative metamers (Fig. 1). Three adult needles per seedling and an equivalent weight of juvenile needles were selected in the same or adjacent metamers, and their length and projected leaf area were determined after scanning and analysis using the software WinFolia2002a (Régent Instruments Inc., Switzerland). Following an initial weighing, needles were suspended in water overnight. After saturation, needles were reweighed, needle section bases were sealed with paraffin wax to avoid significant water losses, and then needles were placed on a laboratory bench at 26°C and RH of 45%, VPD 1.85 KPa.

Needle weight change was then determined at 5-min intervals for 60 min. Cumulative cuticular transpiration (CCT) was calculated at 5-min intervals during the first 60 min as grams of water lost per gram of leaf dry weight. When representing the leaf–drying curves for the 60-min period we obtain a curve, which started in the origin, corresponding with water loss of zero at time 0. However, we were interested in the linear portion of the curve, which occurred from the 10th minute. Thus, a linear regression

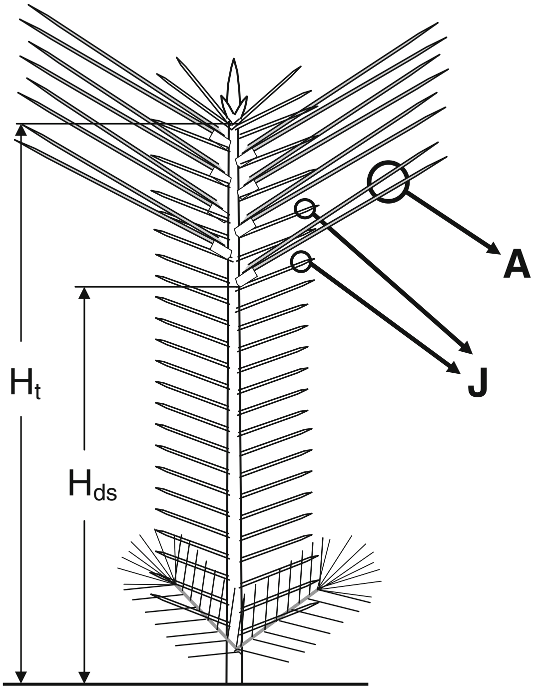

Fig. 1 Position of samples for juvenile needles (*J*) and adult needles (*A*) in transitional vegetative metamers. The proportion of plant height with adult needles in dwarf shoots (DSh) was obtained as the quotient (Ht − Hds)/Ht

was fitted for the relationship between CCT and time between 10 and 60 min. The rate of cuticular transpiration (RCT) was calculated from the slope of the linear regression of time against CCT. To achieve homogeneous error variances and minimise any curvilinear response during the first 60 min, data from the first 5-min increment were not included in the analysis. Using the water-loss data from 10 to 60 min into the drying period represents the best compromise to provide a measure of cuticular transpiration (Tischler and Voigt 1990).

The relative difference of RCT between juvenile and adult foliage was calculated as: $\mathrm{Dif(RCT)} = (\mathrm{RCT}_{\mathrm{juvenile}} - \mathrm{RCT}_{\mathrm{adult}})/\mathrm{RCT}_{\mathrm{juvenile}}$.

Leaf mass area

After the cuticular transpiration test, needle samples were oven-dried for 24 h at 70°C, and this dry mass value was used to determine needle mass per unit area, or LMA (LMA = needle dry mass/needle area, g cm^{-2}). The relative difference in LMA between juvenile and adult foliage was calculate as: $\mathrm{Dif(LMA)} = (\mathrm{LMA}_{\mathrm{juvenile}} - \mathrm{LMA}_{\mathrm{adult}})/\mathrm{LMA}_{\mathrm{juvenile}}$.

We checked the possible bias of using projected area instead of total needle area, (given that pine needles are amphistomatic) by comparing needle cross-sections of juvenile and adult needles per species. Even when the ratio between total needle area and projected leaf area was slightly greater in adult needles, this difference did not affect the results presented, and therefore we preferred to use a simpler measure instead of introducing wider experimental errors.

Ontogeny

Ontogeny was estimated through two variables estimating the timing (acceleration or delay) of development: TB (the percentage of plants with a terminal bud formed after 20 months) and DSh (the proportion of plant height with adult needles, i.e. dwarf shoots, Fig. 1).

Climatic variables

Two climatic variables, Ps (precipitation of the warmest quarter, corresponding to June, July and August) and Dm (the number of dry months, which are the months when $P < 2T$, where P is the precipitation and T is the mean temperature) were chosen to characterise each species. Climatic variables were obtained for the central range of each species studied from the Global Climatic Model WorldClim (Hijmans et al. 2005) implemented in DIVA-GIS version 1.3 at a 30″ resolution.

Statistical analysis

Each relationship between CCT and time was assumed to be linear between 10 and 60 min, so linear regression was fitted to the whole data set, jointly including all species and needle ages (Eq. 1).

$$\mathrm{CCT} = a + b \times t \tag{1}$$

where t is time (in minutes), a represents the intercept and b the slope of the regression. The slope, b, can be interpreted as the rate of cuticular transpiration (RCT). To test differences in CCT and RCT due to species and needle age, we first assumed that both the intercept and slope parameters in Eq. 1 are expressed as an effect of *species* (a_S and b_S), an effect of needle type (a_N and b_N), and an effect of the interaction between species and needle type ($a_{S\times N}$ and $b_{S\times N}$), all of them were assumed to be fixed factors. Inherent correlation among measurements coming from the same plant prevented us from solving the proposed model using ordinary least squares; thus, a mixed model was used, adding random effect factors (u and v) specific to each plant. The model for CCT is:

$$\mathrm{CCT} = [a_S + a_N + a_{S\times N} + u] + [b_S + b_N + b_{S\times N} + v] \times t \tag{2}$$

The level of significance for fixed effect factors was evaluated using Type-III F tests. Empirical best linear unbiased predictors (EBLUP's) for random parameters were predicted for each plant, thus individual plant-level responses could be represented. Differences in CCT were tested by computing least squared differences among pairs of species, needle age and interactions, jointly for the whole interval as well as separately for initial (minute 10) and final moments (minute 60). Tests of equality of slopes between species, needle age and interactions were also carried out to test for significant differences in RCT. All analyses were done using SAS (version 8.2) procedure MIXED.

The relationship between variables (RCT, Dif(RCT), LMA, Dif(LMA),TB, DSh, Ps and Dm) was assessed using the Pearson (r) coefficient, using the mean values for each species.

Results

The mixed model showed highly significant effects ($P < 0.0001$) of the species, needle type, and species × needle type in the rate of cuticular transpiration, RCT (slope of CCT vs. time) (Table 2), with higher RCT values corresponding to juvenile needles, (26% greater than adult needles). Neither the species ($P = 0.759$)

Table 2 Type III *F* tests for fixed effects included in the linear regression model of CCT versus time

Effect	Num DF	F	Pr > F
a_S	8	0.62	0.7591
a_N	1	108.89	<0.0001
$a_{S \times N}$	8	0.57	0.8058
b_S	8	13.63	<0.0001
b_N	1	39.29	<0.0001
$b_{S \times N}$	8	7.14	<0.0001

Parameters b_i represent the contribution of each source of variation to the slope of the line, this is, the rate of residual transpiration RCT while a_i represent intercepts (s species, N needle type). $CCT = [a_S + a_N + a_{S\times N} + u] + [b_S + b_N + b_{S\times N} + v] \times t$

nor the interaction between species and needle type ($P = 0.806$) significantly affected the intercept parameter, *a* (Table 2).

Juvenile needles had a significantly higher RCT than adult needles for five species (*P. canariensis* and *P. radiata* with $P < 0.001$, *P. halepensis* and *P. uncinata* with $P < 0.01$ and *P. pinaster* with $P < 0.1$), while three species showed no significant differences between foliage types (*P. pinea*, *P. brutia* and *P. nigra*), and only one, *P. sylvestris*, showed a significantly ($P < 0.1$) lower RCT for juvenile needles than for adult needles (Fig. 2, Table 3).

The cumulative cuticular transpiration (CCT) between 10 and 60 min (Table 4) was higher in juvenile than adult needles (Fig. 3). Again, these differences in CCT were species-related. For example, after 60 min the CCT of both juvenile and adult needles of *P. canariensis* was lowest, and highest in *P. radiata*.

Adult needles were distinct from juvenile needles in their higher LMA ($P < 0.0001$), and such differences were also species-dependant ($P_{\text{species}\times\text{needle type}} < 0.0001$, Fig. 4). The LMA of juvenile (but not adult) needles was negatively correlated to the rates of cuticular transpiration (RCT) of both juvenile and adult needles (Table 5). The precipitation of the warmest quarter at the seed source (Ps) was significantly and positively correlated with RCT for both juvenile ($P = 0.072$) and adult ($P = 0.063$) needles, and negatively with LMA of both juvenile ($P = 0.009$) and adult ($P = 0.099$) needles (Table 5). The number of dry months (Dm) at the seed source was negatively correlated with RCT of adult needles ($P = 0.038$) and positively to LMA of juvenile needles ($P = 0.003$). Meaningfully, the relative difference of RCT between juvenile and adult needles [Dif(RCT)] was negatively correlated with both the proportion of budset per species (TB, $P = 0.006$) and the proportion of plant height with adult needles (DSh, $P = 0.052$, Table 5).

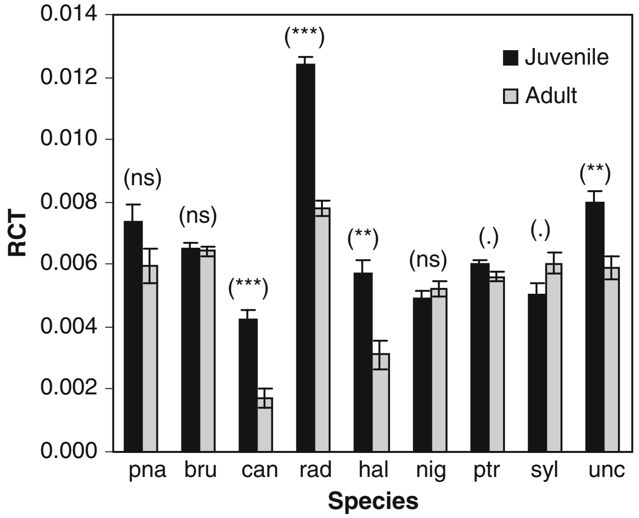

Fig. 2 Mean rate of cuticular transpiration (RCT, g g^{-1} min^{-1}) and *P* value for the effect of needle type within species. (*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, $^{\cdot}$ $P < 0.1$, *ns* non-significant). ($n = 5$ per needle type and species) pna: *P. pinea*, bru: *P. brutia*, can : *P. canariensis*, rad : P. radiata; hal: *P. halepensis*, nig: *P. nigra*, ptr: *P. pinaster*, syl: *P. sylvestris*, unc: *P. uncinata*

Table 3 Equality test of slopes (RCT) between species × needle-type

	pna	bru	can	hal	ptr	nig	syl	unc	rad	*Juvenile*
pna	ns	ns	**	ns	ns	*	*	ns	***	**pna**
bru	ns	ns	·	ns	ns	ns	ns	ns	***	**bru**
can	***	***	***	ns	ns	ns	ns	**	***	**can**
hal	***	***	·	**	ns	ns	ns	·	***	**hal**
ptr	ns	ns	***	**	·	ns	ns	·	***	**ptr**
nig	ns	ns	***	*	ns	ns	ns	**	***	**nig**
syl	ns	ns	***	***	ns	ns	·	*	***	**syl**
unc	ns	ns	***	**	ns	ns	ns	**	***	**unc**
rad	·	·	***	***	**	**	*	*	***	**rad**
Adult										

Values above the diagonal show inter-specific differences for juvenile needles. Values below the diagonal show inter-specific differences for adult needles. Diagonal values represent intra-specific differences between juvenile and adult needles

pna: *P. pinea*, **bru**: *P. brutia*, **can** : *P. canariensis*, **rad** : P. radiata; **hal**: *P. halepensis*, **nig** : *P. nigra*, **ptr**: *P. pinaster*, **syl**: *P. sylvestris*, **unc**: *P. uncinata*

*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, $^{\cdot}$ $P < 0.1$, *ns* non-significant

Discussion

Our experiment is one of the very few to give experimental evidence of the functional differences linked to heterophylly in different hard pines, by comparing the rate of

Table 4 Type III *F* tests of the linear regression model for CCT between 10 and 60 min, for the effect of needle type (juvenile and adult needles) within species

Eff	*P. sylvestris*		*P. nigra*		*P. uncinata*		*P. canariensis*		*P. pinaster*		*P. radiata*		*P. pinea*		*P. brutia*		*P. halepensis*	
	F	Pr > *F*	*F*	Pr > *F*	*F*	Pr > *F*	*F*	Pr > *F*	*F*	Pr > *F*	*F*	Pr > *F*	*F*	Pr > *F*	*F*	Pr > *F*	*F*	Pr > *F*
a_S	10.7	0.009	42.5	0.0003	64.2	<0.0001	19.3	0.0033	62.2	0.0001	28.6	0.0007	11.1	0.0103	11.2	0.0015	10.4	0.0093
b_S	2.2	0.1460	1.9	0.1726	8.1	0.0053	22.6	<0.0001	3.1	0.0821	82.9	<0.0001	4.4	0.0390	0	0.9459	7.2	0.0085

Linear regression was fitted to the data using a mixed model. CCT $= [a_S + a_N + a_{S\times N} + u] + [b_S + b_N + b_{S\times N} + v] \times t$

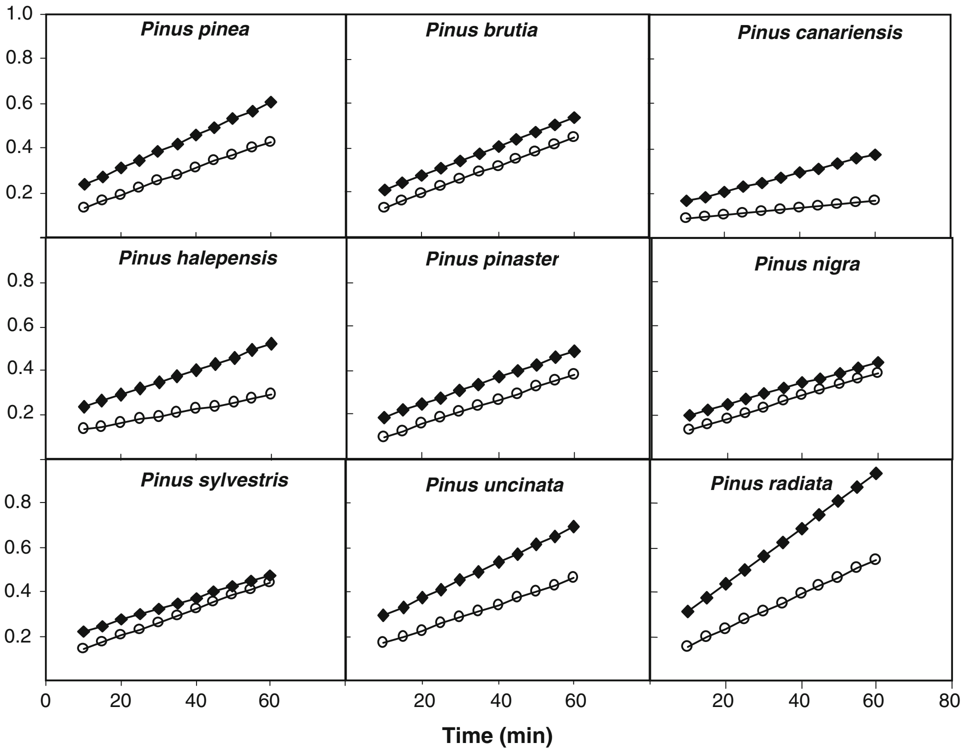

Fig. 3 Marginal response for CCT (g of water loss/g leaf dry weight) (random parameters in Eq. 2 set to zero) between 10 and 60 min, for the effect of needle type [juvenile (*square*) and adult (*circle*) needles] within species. ($n = 5$ per needle type and species) CCT $= [a_S + a_N + a_{S\times N}] + [b_S + b_N + b_{S\times N}] \times t$

cuticular transpiration in juvenile and adult leaves in nine species growing under a common cultivation regime.

The results partially confirm our first hypothesis that juvenile needles lose water faster than adult needles once stomatal closure is complete. As juvenile needles contain more water (higher initial cumulative cuticular transpiration, CCT), they can maintain a higher rate of water loss for a longer period of time before limiting factors come into play, to reduce their rate of water loss (Tischler and Voigt 1990). Moreover, a lower rate of cuticular transpiration (RCT) might be attributed to a greater resistance to water vapour diffusion through the external epidermal walls (Premachandra et al. 1992) and may help seedlings to spend less water and maintain better water status under drought conditions (Villar et al. 2004). As mentioned above, differences in rate of cuticular transpiration (RCT) between juvenile and adult needles were related to the species considered, with *P. canariensis, P. halepensis, P. pinaster, P. radiata* and *P. uncinata* showing the differences in the hypothesised direction (juveniles losing more water per time unit than adults), while three species showed no significant differences, and only one (*P. sylvestris*) showed a difference in the opposite direction.

A clear divergence was found for cuticular transpiration and LMA not only among species but more importantly between juvenile and adult needles. Such results demonstrate species-specific differences in cuticular transpiration and LMA among the nine pine species studied in a common environment. Intuitively, it might be assumed, that differences in cuticular permeability should be directly related to a variation in the thickness of the cuticular membrane (Riederer and Schreiber 2001). However, it is difficult to relate cuticular permeances with cuticle thickness (Burghardt and Riederer 2003). Accordingly, our results only showed a negative correlation (Pearson coefficient ~ −0.6) between LMA of juvenile,

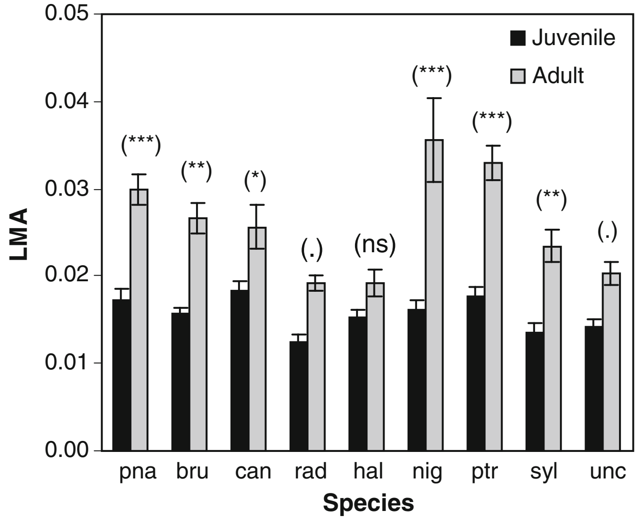

Fig. 4 Mean leaf mass area (LMA = needle dry mass/needle area, g cm^{-2}) for each species and needle type and P value for the effect of needle type within species. ($n = 5$ per needle type and species). (*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, ˙ $P < 0.1$, *ns* non-significant). Intervals are standard errors. pna: *P. pinea*, bru: *P. brutia*, can : *P. canariensis*, rad: P. radiata; hal: *P. halepensis*, nig: *P. nigra*, ptr: *P. pinaster*, syl: *P. sylvestris*, unc: *P. uncinata*

but not adult, needles and rate of cuticular transpiration (RCT) and cumulative cuticular transpiration (CCT). Thus, LMA of juvenile needles was species-dependent, but the role of this trait in differentiation between juvenile and adult needles is not evident.

Confirming our second hypothesis, the relative difference of rate of cuticular transpiration (RCT) was significantly and negatively correlated to each species ontogeny, evaluated through budset and the proportion of plant height with adult needles. The later secondary needles formed in particular species, the greater the difference between their cuticular transpiration and that of juvenile needles. Therefore, our results confirm that in general, the behaviour, in terms of transpirational water loss, of juvenile and adult needles is more similar in the Euro-Siberian pines, which have accelerated ontogeny than in the Mediterranean pines, with a much longer delay before the formation of their adult foliage. A typical representative of this pattern is *P. canariensis* which needs 2–6 years before undergoing a heteroblastic change in the main shoot (Climent et al. 2006), but once adult needles are formed, they show the lowest rate of cuticular transpiration (RCT) of all the pines studied. Both greenhouse and field studies have demonstrated the high capacity of this species to withstand drought and the existence of high intra-specific variation in survival under drought stress (Climent et al. 2002; López et al. 2007).

The two exceptions to this link between ontogenetic and functional heterophylly were *P. uncinata* and *P. radiata,* which combine the very precocious appearance of adult needles with a high differentiation between these and juvenile needles in terms of cuticular transpiration rates. However, in *P. radiata*, budset occurs much later than in *P. uncinata*. More research is needed to understand the adaptive cues governing this decoupling between different developmental features in pine seedlings.

Different studies have shown that developmental patterns at the seedling stage can be linked to different life history strategies in pines (Strauss and Ledig 1985; Grotkopp et al. 2002). Accordingly, the different developmental features of the studied pine species (either undergoing an early heteroblastic change or maintaining a prolonged juvenile phase) are consistent with contrasting stress-resistance strategies. Juvenile needles spend more water, but they can be less expensive to construct as compared with adult needles, due to their lower LMA (Villar and Merino 2001). This risky but low-cost strategy can be advantageous in limiting environments, given that some plants can undergo the transition to the adult, more stress-tolerant form

Table 5 Correlation values (Pearson's r) of different parameters derived from the leaf drying curves, ontogeny parameters and climatic variables

	RCT_A	LMA_J	LMA_A	Dif(LMA)	DSh	TB	Dm	Ps
RCT_J	0.700 (0.018)	−0.627 (0.035)						0.528 (0.072)
RCT_A		−0.639 (0.032)		0.550 (0.063)	0.597 (0.045)		−0.620 (0.038)	0.548 (0.063)
Dif(RCT)			−0.559 (0.059)	−0.837 (0.002)	−0.576 (0.052)	−0.792 (0.006)		
LMA_J					−0.476 (0.097)		0.833 (0.003)	−0.758 (0.009)
LMA_A				0.792 (0.006)				−0.474 (0.099)
Dif(LMA)					0.493 (0.089)	0.589 (0.047)		
DSh						0.714 (0.015)	−0.814 (0.004)	
TB							−0.537 (0.068)	

RCT_J rate of residual transpiration in juvenile needles, *RCT_A* rate of residual transpiration in adult needles, Dif(RCT) = (RCT_J − RCT_A)/RCT_J; *LMA_J* leaf mass area of juvenile needles, *LMA_A* leaf mass area of adult needles, Dif(LMA) = (LMA_J − LMA_A)/LMA_J; *TB* percentage of plants with terminal bud formed after 20 months, *DSh* proportion of plant height with adult needles, *Ps* precipitation of the warmest quarter, *Dm* number of dry months

Probability values are shown in parenthesis. Only correlations with $P < 0.1$ are shown

(Miller et al. 1995; Darrow et al. 2002). Our results support that the species from drier habitats tend to delay heteroblastic change, and this is generally related to lower cuticular transpiration of adult needles and higher differentiation between juvenile and adult foliage types (see correlations in Table 5). This inter-specific trend mimics the variation observed among provenances of Canary Island pine (Climent et al. 2006), which suggests that ontogenetic delay could have been selected in dry environments.

In addition to these ecological implications, deserving further investigation, the results of this research underline the need to account for ontogenetic shifts in needle morphology (heterophylly) when assessing the functional traits of pine seedlings, in particular those traits implicated in water relations.

Acknowledgments We wish to thank Dr. Mathew Robson for critical reading and English revision of the manuscript. This study was supported by project AT05-002-INIA.

References

Barberó M, Loisel R, Quézel P, Richardson DM, Romane F (1998) Pines of the Mediterranean basin. In: Richardson DM (ed) Ecology and biogeography of *Pinus*. Cambridge University Press, London, pp 153–170

Blum A (2005) Drought resistance, water-use efficiency, and yield potential- are they compatible, dissonant, or mutually exclusive? Aust J Agric Res 56:1159–1168. doi:10.1071/AR05069

Burghardt M, Riederer M (2003) Ecophysiological relevance of cuticular transpiration of deciduous and evergreen plants in relation to stomatal closure and leaf water potential. J Exp Bot 54(389):1941–1949. doi:10.1093/jxb/erg195

Cannell MGR, Thompson S, Lines R (1976) An analysis of inherent differences in shoot growth within some north temperate conifers. In: Cannell MGR, Last FT (eds) Tree physiology and yield improvement. Academic Press, London, pp 173–205

Chambel MR, Climent J, Alía R (2007) Divergence among species and populations of Mediterranean pines in biomass allocation of seedlings grown under two watering regimes. Ann For Sci 64:87–97. doi:10.1051/forest:2006092

Clarke JM (1992) Phenological variability: effect on determination of leaf water loss in wheat. Crop Sci 32:1457–1459

Clarke JM, McCaig TN (1982) Excised-leaf water retention capability as an indicator of drought resistance of Triticum genotypes. Can J Plant Sci 62:571–578

Clarke JM, Romagosa I, Jana S, Srivastava JP, McCaig TN (1989) Relationship of excised-leaf water loss rate and yield of *Durum* wheat in diverse environments. Can J Plant Sci 69:1075–1081

Climent J, Gil L, Pérez E, Pardos J (2002) Efecto de la procedencia en la supervivencia de plántulas de *Pinus canariensis* Sm. en medio árido. Investig Agrar Sist Recursos For 11:171–180

Climent J, Chambel MR, López R, Mutke S, Alía R, Gil L (2006) Population divergence for heteroblasty in the Canary Island pine (*Pinus canariensis*, Pinaceae). Am J Bot 93(6):840–846. doi:10.3732/ajb.93.6.840

Darrow HE, Bannister P, Burrit DJ, Jameson PE (2002) Are juvenile forms of New Zealand heteroblastic trees more resistant to water loss than their mature counterparts? NZ J Bot 40:313–325

Davis SD, Pratt RB, Ewers EW, Bowen TJ (2004) Interactions between water stress and freezing regulates chaparral distribution patterns in coastal California. In: Arianoutsou M, Papanastasis V (eds) Medecos, 10th International Conference Millpress, Rhodes, Greece, pp 15

Gandullo J, Sánchez Palomares O (1994) Estaciones ecológicas de los pinares españoles. ICONA, Ministerio de Agricultura, Pesca y Alimentación, Madrid

Grotkopp E, Rejmanek M, Rost TL (2002) Toward a causal explanation of plant invasiveness: seedling growth and life-history strategies of 29 pine (*Pinus*) species. Am Nat 159:396–419. doi:10.1086/338995

Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A (2005) Very high resolution interpolated climate surfaces for global land areas. Int J Climatol 25:1965–1978. doi:10.1002/joc.1276

Jones CS (1999) An essay on juvenility, phase change, and heteroblasty in seed plants. Int J Plant Sci 160:S105–S111. doi:10.1086/314215

Klaus W (1989) Mediterranean pines and their history. Plant Syst Evol 162:133–163. doi:10.1007/BF00936915

Kubien DS, Jaya E, Clemens J (2007) Differences in the structure and gas exchange physiology of juvenile and adult leaves in *Metrosideros excelsa*. Int J Plant Sci 168(2):563–570

Lanner RM (1976) Patterns of shoot development in *Pinus* and their relationship to growth potential. In: Cannell MGR, Last FT (eds) Tree physiology and yield improvement. Academic Press, London, pp 223–243

Levitt J (1972) Responses of plants to environmental stresses. Academic, New York

López R, Zehavi A, Climent J, Gil L (2007) Contrasting ecotypic differentiation for growth and survival in *Pinus canariensis* (Pinaceae). Aust J Bot 55:1–11. doi:10.1071/BT07016

Luis V, Taschler D, Hacker J, Jiménez MS, Wieser G, Neuner G (2007) Ice nucleation and frost resistance of *Pinus canariensis* seedlings bearing needles in three different developmental states. Ann For Sci 64:177–182. doi:10.1051/forest:2006102

Miller PM, Eddleman LE, Miller JM (1995) *Juniperus occidentalis* juvenile foliage: advantages and disadvantages for a stress-tolerant, invasive conifer. Can J For Res 25:470–479. doi:10.1139/x95-052

Premachandra GS, Saneoka H, Fujita K, Ogata KS (1992) Leaf water relations, osmotic adjustment, cell membrane stability, epicuticular wax load and growth as affected by water deficits in *Sorghum*. J Exp Bot 43:1569–1576. doi:10.1093/jxb/43.12.1569

Riederer M, Schreiber L (2001) Protecting against water loss: analysis of the barrier properties of plant cuticles. J Exp Bot 52:2023–2032. doi:10.1093/jexbot/52.363.2023

Strauss SH, Ledig FT (1985) Seedling architecture and life-history evolution in pines. Am Nat 125:702–715. doi:10.1086/284373

Tischler CR, Voigt PW (1990) Variability in leaf characteristics and water loss in the weeping love grass complex. Crop Sci 30:111–117

Villar R, Merino J (2001) Comparison of leaf construction costs in woody species with differing leaf life-spans in contrasting ecosystems. New Phytol 151:213–226. doi:10.1046/j.1469-8137.2001.00147.x

Villar P, Planelles R, Oliet J, Peñuelas JL, Jacobs DF, González M (2004) Drought tolerance and transplanting performance of holm oak (*Quercus ilex*) seedlings alter drought hardening in the nursery. Tree Physiol 24:1147–1155

Winn AA (1999) The functional significance and fitness consequences of heterophylly. Int J Plant Sci 60:S113–S121. doi:10.1086/314222