Phenotypic plasticity of Pinus pinaster to water stress: biomass allocation and root architecture

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ABSTRACT

In a context of climate change, adaptation of perennial plantations to water constraints becomes a major concern for wood productivity. Scenario of climate change predict severe summer drought in south-western France. Our project plans to describe adaptive mechanisms of the species.
The phenotypic plasticity of *Pinus pinaster* ait. to water availability is tested in the field by planting 1-years-old trees under a greenhouse open at its borders for rainwater exclusion. Water is provided to compensate evapo-transpiration to half the plants by aerial irrigation. One well growing and one slow growing half-sib family, both of the local improved provenances were compared. The soil is a sandy spodosol. The soil water content, water table level, air temperature and humidity were monitored. Shallow soil water content decreased to 6% in the dry treatment in late summer. After an inventory of height and diameter, 40 saplings were uprooted in March July September and March for aerial and root biomass and architecture assessment.
Surprisingly, water shortage did not affect total biomass of the saplings. However, the water stressed trees showed slimmer stems, more biomass allocation to needles (+18%), and distinctly less allocation to roots (-30%), especially distal roots. Water constraints did not affect taproot length and rooting depth or maximal root radial distance. Selection for height at age 10-years resulted in saplings with a better harvest index for the stem.
We hypothesize that *P. pinaster* saplings stop root growth when the soil is too dry, but maintain their productivity by setting more needles.
KEYWORDS: root system architecture, water-stress, plasticity, genomics, *Pinus pinaster*

.1 INTRODUCTION

In a context of climate change, adaptation of perennial plantations to water constraints becomes a major concern for wood productivity. Scenario of climate change predict severe summer drought in south-western France. This part of France is covered by a 1 million hectare *Pinus pinaster* ait. monospecific forest, the "Landes Forest" grown using intensive management techniques, it produces nearly 25% of French wood. This project is part of a larger project involving the same type of experiment in Congo in *Eucalyptus* plantation and in Brazil in *Coffea* plantation.

Usually, water stress experiments are made with plants grown in pots, in this way, the control of the environment and the yield of underground parts is easy. Most of the water-stress experiences are conducted within one growth season only.

Our project intends to assess the plasticity of perennial plants during two growth seasons so as to test the consequences of a water stress of the preceding year on the plant. We also chose to study plants grown in the field, to work in more natural conditions and to get a full expression of root architecture. We plan to describe adaptive mechanisms, in particular the consequences on root architecture and root biomass.

.2 MATERIAL AND METHODS

P. pinaster from a polycross family which get a good index and one family which get a bad index based on stem height and stem straightness at age 10-years were sawn in turf plugs in may 2007 in a nursery with irrigation. Rainfall in the Landes area is nearly 1000 mm per year, 300 mm is provided during the summer months, and thus water exclusion had to be installed to obtain a controlled water-stress. Exclusion was obtained by installing a 9 m x 58 m plastic tunnel green house in the nursery on a flat ground on sandy spodosols mid November 2007. The greenhouse was completely open at its ends and on one meter height on its sides. The greenhouse was divided into three zones: irrigated, non-irrigated, irrigated the second year only. The sprinklers irrigated during the night twice a week to provide 5 mm water per day on may-october period. The both family seedlings were also installed outdoor with a similar design. The entire greenhouse could be irrigated if necessary.

The seedlings were planted in January 2008, after pruning all roots outside the turf plugs, in a 40 x 40 square spacing leaving a 2 m buffer zone on each side planted with non-improved seedlings. "+" and "-" family seedlings were alternated so they suffered from the same water-stress. The water-table level was monitored manually each week as well as the soil water content (deltaT PR2 profile soil probe) with 10 tubes. Temperature and air humidity was also monitored, with 4 hobo devices.

The experiment was weeded regularly so as to avoid asymmetric competition, an electric fence was installed against rabbits. The experiment was flooded end June 2008 by an unusually strong thunderstorm, and severely damaged by the Klaus storm in January 2009. The greenhouse was completely destroyed and rebuilt in May 2009.

In March, July and September 2008 and March 2009 an inventory of stem length, collar diameter and status was made on the whole experiment. At each date, 10 seedlings per treatment were destructively sampled to get needles and meristems for stomate counting, RNA expression studies, anatomy observations, above- and belowground architecture and biomass measurements. Predawn leaf water potential was also measured. Only trees which were not damaged by Klaus were used in the 2009 destructive sampling.

Roots were excavated using shovels and small gardening tools as well as compressed air. Horizontal surface roots were followed as far as possible to get the meristem, which is used for anatomy but not for RNA expression. The 44 root systems of the trees sampled in March 2009 were 3D digitised using the methods set-up by Danjon et al. (1999) and analysed like Danjon et al. (2005). They were stored at 4°C. Horizontal surface roots could reach more than 2 m and were supple. To measure them, we built a sort of 5 m x 5 m "ring" with 4 levels of horizontal ropes attached around four trees at 10 cm vertical spacing. The tree collar was attached to a wooden jaw screwed to a large wooden board planted obliquely in the soil. Trees were oriented in the same way than in the field. Each long horizontal root was clamped with a small paper clip attached to a 2m bicycle tyre tube strap, itself tied to the appropriate horizontal rope. Sinker roots were attached with thin wires to strings attached to a breeze block. Root cross sectional diameters were recorded using a Mitutoyo plastic electronic calliper ref. 700-127. We used the PiafDigit software to drive the Polhemus 3Ddigitiser and capture the architecture in “.mtg” files (Danjon and Reubens 2008). The number of fine roots on a given segment (Khuder et al. 2007) as well as the point where the colour of a given root change from brown to black were recorded in the “.mtg” file. All roots with a base diameter larger than 0.7 mm were measured. 5 root systems were measured per day.

.3 RESULTS

The first year, the water table was unusually low in winter and rose very rapidly in June to reach -50 cm after the thunderstorm. Thus, the difference between treatments was low in July 2008. End August, the water table was below 1.5 m deep, and the shallow part of the soil reached the wilting point in the dry treatment, it raised to 20% water content after the Klaus storm.

From the inventories (not shown), the collar diameter increment was high from March to June 2008 and 15%*** larger in the dry treatment, but it was half less*** in the dry treatment in summer 2008, as well as spring 2009***. Conversely, stem length increment was 30%*** larger in spring 2008 and 20 %*** larger in summer 2008. The "+" family showed a *** larger stem increment for both periods with no interaction.

The total biomass of seedlings in the dry treatment was 40% larger in July 2008, but not significantly different in September. In September, water stressed plants showed 13%* more relative needle biomass, 13%* less branch biomass in the aerial biomass, 12%*** smaller root partitioning coefficient, 20% less relative lateral root biomass, but the same relative biomass of sinkers (not shown). In September 2008, no active root apex was found in the dry treatment whereas each irrigated plant had more than 30 active root apex.

In March 2009, neither the water stress nor the family had affected the total biomass of sampled trees, which is very surprising (table 1). However both factors influenced the biomass allocation. The "-" family had more branch biomass, stockier stems and less stem wood. The water stressed plants allocated much more to the leaves, less to the stump and much less to the roots (-30%). They showed slimmer stems, and they allocated less root volume to horizontal shallow roots beyond ZRT (Zone of Rapid Taper) and sinkers beyond ZRT. No differences in maximal rooting depth, maximal radial distance or root number could be detected.

Individual roots generally tapered constantly until they reached a diameter of about 1 mm. After this limit, they could extend 1.5 m with a diameter which could vary but without a distinct tapering. They could even reach 2 or 3 mm diameter in section of black colour, irregular shape and with small holes which are certainly absorbing parts of the root. As a consequence, the full length of a root could not been extrapolated from the tapering of its proximal part.

.4 DISCUSSION

The large stem height of "+" family, despite low juvenile-mature genetic correlations in *P. pinaster*, have resulted in an improved harvest index for the stem even in 2-years old trees. High water stress could not be completely established the first year on the whole soil profile, despite more than one year rain exclusion. Surprisingly, *P. pinaster* did not react to water shortage in setting up more roots or more sinker roots. Young *P pinaster* trees could assimilate biomass even with low water availability. However they seemed to stop root growth to increase their photosynthetic system. We hypothesized that when the soil is too dry for root growth, a larger amount of needles is grown to produce the same amount of biomass in water stressed conditions. The strategy of this pioneer species was appropriate to maintain its global productivity in 2008, will it maintain it in 2009? Despite the Klaus storm, two additional sample periods are planned in July and September 2009.

Table 1. Characteristics of saplings measured in March 2009. The irrigated family with potential bad growth is the control. Effect of factors are given in % compared to the control, for the “+” family, the water stressed trees, the outside trees and finally the “+” family in dry conditions (root types according to Danjon et al. 2005).

Variable	unit	mean irrigated Fam -	SD	Factor effect % Fam +	Water Stress	outside	interaction Dry Fam +	bloc
root + shoot dry weight	g	51.7	9.43	-3.36	+10.1	+10.7	+3.75	
stem length	cm	36.1	5.06	+15*	+21.5*	-9.39*	+12.8	
collar diameter	cm	1.08	0.148	-2.5	-6.65**	+10.3	-4.87	*
stem length/collar diameter		35.5	2.61	+16*	+18.4***	-17.8**	+9.82	
leaf in total DW	%	41.6	3.15	-1.82	+18***	-3.5****	+9.9*	
branches in shoots DW	%	44.8	7.84	-13*	+3.19	+3.85	+1.8	
stem density	g/cm3	0.421	0.0424	-6.32	-0.067	+5.77	-4.58	
RPC (root in total DW without stump)	%	30.9	3.68	+2.62	-30.2****	-6.95**	-22.5	
RPC stump	%	14.3	1.72	-2.83	-11.3**	+9.62**	-2.08	
RPC stem wood	%	0.119	0.0145	+11.9*	+12.5	+6.82	+10	
Max. radial distance	cm	126	39.2	-2.81	+37	-5.98	+41.5	
Max. depth	cm	-47.2	7.13	-1.81	-1.59	+0.601	-0.963	
Root length	cm	1550	368	+1.75	-34.8**	-8.05	-37.6	*
Relative root number af	n/m	9.89	3.57	+20.4	+10.9	+8.03	+2.52	
relative deep root number	%	12.2	10.1	-21.1	+81.9*	-30.1*	+81.3	
Root type	Root volume (%) by root type in whole root volume excluding the stump							
(1) Stump	%	37.3	4.43	-3.47	+20.5*	+14.8	+22.2	*
taproot	%	16.9	6.1	-10.6	+83.2*	+32.1	+158	
Sinkers below ZRT	%	1.76	2.29	-22.7	-12.1	+18.1	-83.2	
(6) Sinkers beyond ZRTt	%	5.76	4.06	+23.6	-37*	-68.2	-42.2	
(9) Oblique	%	4.97	3.34	+56.1	+41.4	-15.5	+53.9	
(3) ZRT	%	7.61	1.64	-17.2	+1.6	+50.8**	+4.99	
(4) horizontal shallow beyond ZRT	%	51	10.4	+4.93	-27.2*	-0.127	-35.1	**
(8 + 9) Intermediate & Oblique	%	14.2	3.33	+8.86	+34.5	-23.2	+53.4	
(7) Deep roots	%	2.81	3.1	-14.2	+64.2	-37.9	+109	

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