Response of four *Salix* species to soil water deficit

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Abstract

Growth and water-use efficiency were measured over twelve weeks in potted cuttings of two tree willow species, *Salix matsudana* × *alba* 'Tangoio' and *S. lucida spp lasiandra*, and two shrub willow species, *S. schwerinii* 'Kinuyanagi' and *S. lasiolepis* grown in a glasshouse under three soil water regimes: well watered (WW), moderate deficit (MD) and severe deficit (SD). The shrub willows are possible alternatives to the tree willows for use in riverbank stabilisation in regions where willow sawfly *Nematus oligospilus* has defoliated and killed tree willows, and may be preferable for some industrial applications requiring efficiency in removing water and nitrogen from soil. Dry mass accumulation was greatest in *Salix matsudana* × *alba* 'Tangoio' and lowest in *S. lasiolepis* in WW treatment, but differences were less in the MD and SD treatments. Whole-plant water-use efficiency (W_p), measured as dry mass accumulation divided by plant water-use, was not significantly different between MD and WW treatments. Mean dry weight gain was negative in all plants surviving the SD treatment. Carbon allocation to root growth was lowest in *S. schwerinii* 'Kinuyanagi' which may account for its inability to survive the SD treatment. Carbon allocation to leaf growth was significantly higher in the shrub species and allocation to stem growth was correspondingly lower.

Additional keywords: water-use efficiency, drought response, stomatal conductance, carbon allocation, willows.

Introduction

Members of the Genus *Salix* L. are dioecious trees or shrubs. Tree willows grow into large trees up to 20 m high, nearly always with a single trunk which can be 60 to 90 cm in diameter. The leaves are long and lance shaped, with a long tapered point and a finely toothed margin. Shrub willows, or sallows, are low shrubs to small trees with multiple stems and stout branches. The leaves are oval to roundish in outline, hairy and with conspicuous veins. Osier willows, or basket willows, are medium-sized shrubs forming several stems up to 20 cm in diameter, with slender branches and narrow linear leaves with a smooth leaf margin (Argus, 2004)

Most *Salix* species will vary phenotypically in response to moisture, nutrients, shade and wind. Sometimes normally prostrate species growing in a protected environment may be erect, and leaves of an usually small-leaved species may be very large in nutrient rich sites, or under shade. It is not normal for tree and shrub willow species to hybridise.

Tree willows have a wide-spreading root system and are excellent trees for the protection of alluvial flats during floods. In New Zealand several *Salix matsudana* × *alba* hybrids have been developed and released for riverbank stabilisation along alluvial rivers and for hillslope stabilisation. These habitats can be subject to extremes of available soil moisture, particularly in drought susceptible East Coast regions.

The arrival in New Zealand of the willow sawfly *Nematus oligospilus* in 1997 threatens the integrity of the tree willows now being used for riverbank stabilisation as the larvae of this insect are capable of completely defoliating the trees several times in a single season, particularly in the favourable climate along the east coast regions of the North Island (Charles *et al.*, 1998). The woody parts of defoliated trees become very brittle making them dangerous to work with, and many trees have died. This has prompted plantings of non-tree willows including *S. schwerinii* 'Kinuyanagi' (Ky).

Incursion of intensive dairy farming into regions with lighter, shallower and more free-draining soils in recent years has meant that there is increased loading of dairy shed effluent onto pasture with the possibility of high N concentration in the drainage water. The ability of willows to coppice repeatedly makes them promising candidates for use in effluent irrigated systems, either as vegetation to be directly irrigated by the effluent, or as riparian buffer zone plantings to capture nitrogen from seepage or re-irrigated tile drainage. A tree willow clone *Salix matsudana* × *alba* 'Tangoio' has recently been trialed as an alternative to pasture for removing large amounts of water and nitrogen from irrigated dairy shed effluent (Snow *et al.*, 2003).

Materials and Methods

Plant material and growth conditions

Two tree willow species, *Salix matsudana* × *alba* 'Tangoio' (Tn) and *S. lucida spp lasiandra* (Ln), and two shrub-like willow species, *S. schwerinii* 'Kinuyanagi' (Ky) and *S. lasiolepis* (Lp) were selected for comparison. *S. schwerinii* 'Kinuyanagi' (Ky) is an osier willow. Stem cuttings were taken from trees in the HortResearch nursery at Aokautere, near Palmerston North, New Zealand, in October 2004 and immersed in water to a depth of 20 cm for 72 hours. After 72 hours single 40 cm cuttings of each species were struck directly into 20 dm^{-3} pots containing a mixture of sand, peat and fine bark (ratio 1:1:1) watered to full capacity and with three month slow release fertiliser, and arranged randomly inside a glasshouse under natural light at HortResearch, Palmerston North, New Zealand (40.4 °S, 175.6 °E).

In November 2004 a soil water stress experiment was initiated. For each species, the experiment comprised three treatments and each treatment was replicated three times. The experiment comprised well-watered (WW, 90 % of field capacity), moderate deficit (MD, 60 % of field capacity) and severe deficit (SD, 40 % of field capacity) treatments, where field capacity was defined as the soil water content after 24 hours drainage following saturation of the soil. The initial weight at field capacity was measured for each pot at the beginning of the experiment, and pots were then allowed to dry down to the required soil water content in each treatment. The water treatments were maintained by weighing each pot on a top loading balance (Mettler Toledo Viper, SW 35) every two days, and the transpired water was recorded and replaced. Evaporation from the soil was minimised by covering the soil surface with plastic.

Each week the pot positions were rotated to minimise the effect of environmental heterogeneity inside the glasshouse. The experiment ran for 82 days, and at the conclusion all cuttings, except for one WW and two MD Tangoio, were harvested to determine the biomass of leaf, stem and root components. The three Tangoio plants were used to gather data on sap flow and water use. Prior to drying, leaf area samples from each species were measured with a planimeter (LiCor LI-3100, LiCor Inc, Lincoln NE, USA). All plant components were dried in a forced draught oven at 70 °C for 2 days prior to weighing on an electronic balance. W_p was determined as the ratio of total biomass accumulation to total water transpired (g biomass kg^{-1} water). For the RW/SW ratio SW was calculated by summing leaf and stem mass, ignoring changes in the cutting mass.

Just prior to the harvest, measurements of stomatal density were made on leaves from each treatment. Stomatal impressions were made by placing a drop of 'superglue' on the axial and abaxial surface of leaf, covering with a glass microscope slide, then removing the slide taking the impression with it. Stomata were viewed and counted under a compound microscope, and the density calculated as the counted number divided by the area of the field of view. This method was not possible for 'Kinuyanagi' because of the proliferation of leaf hairs on the abaxial surface. Instead an estimate of relative stomatal density was made from a comparison of stomatal counts for all species made from leaf cross-sections viewed under LM (x400), and SEM photos.

Leaf transpiration measurements were taken for the WW treatments on days 66 and 68. Five leaves in full sun were measured at 90 minute intervals from 0900 hours till 1630 hours with a porometer (LiCor LI-1600, LiCor Inc, Lincoln NE, USA).

Results

Biomass accumulation, partitioning, water-use and W_p

The watering treatment had a significant effect on dry mass accumulation, with 15-37 % reductions in the MD compared to the WW treatment and a 2-14% loss of dry mass in the SD compared to the mass of the initial cutting (Table 1). A significant species difference in dry mass accumulation was apparent in the WW where the mean dry mass of Tn was 38% higher, and in the MD treatment where the dry mass of Ls was about 34 % lower than that of the other species. Tn and Ln, the tree willow species, consistently ranked higher than Ky and Ls, the shrub willow species in the MD and SD treatments. Ky did not survive the SD treatment dying before the soil reached SD, though all three plants had produced considerable stem and leaf material during the drying down stage. Only one Ls plant survived the SD treatment.

There were significant species differences in the partitioning of dry mass between roots and shoots. In both WW and MD treatments, Ky had a lower *RW:SW* ratio than the other species, although the differences were not significant.. Over all species, the watering treatment did not affect the *RW:SW* ratio, and there was no species by treatment interaction. The cuttings of all plants in the SD treatment and 7 of 12 plants in the MD lost biomass over the course of the experiment. One cutting of Ls lost biomass in the WW treatment.

The watering treatment had a large effect on total water-use of all species, with 74 % and 99 % reductions in water-use of the MD and SD treatments compared to the WW treatment (Table 1). Total water-use was highest in the species Tn in all watering treatments, although the mean differences were relatively small except in the case of Ls. The large difference in dry mass accumulation combined with a smaller difference in water-use resulted in a significantly higher W_p in the species Tn compared with the others in the WW treatment. Although not significant, W_p was also higher for the tree species in the MD treatment.

Stomatal density, distribution and influence on leaf transpiration

The density and distribution of stomata for the four species are given in Table 3. The two tree species Tn and Ln have stomata on both leaf surfaces (amphistomatous), whereas Ky and Ls have stomata on the lower surface only (hypostomatous). Mean transpiration rate for the four species (measured for the WW treatment only) is given in Table 4. Leaf areas for the four trees for which transpiration rate was measured were very similar at harvest. The contribution of the two leaf surfaces to the leaf transpiration was 21% upper/79% lower in Tn and 7% upper/93% lower in Ln. Considering both leaf surfaces calculated leaf transpiration rates for Tn were 1.4 g H_2O m^{-2} and for Ln were 0.9 g H_2O m^{-2}. The transpiration rate from the upper surface in relation to the lower surface is proportionally lower than the stomatal density.

Discussion and conclusions

Whole-plant water-use efficiency did not change significantly between the WW and MD treatments in any of the tested species except for Ln. The plants responded to the SD treatment by utilising water stored in the cutting to compensate for the reduced available water, so W_p in this treatment was confused by the net loss in biomass of the surviving plants while still producing stems, roots and leaves.

Table 1. Total water-use, dry mass accumulation, water-use efficiency and root weight to shoot weight ratio in the four willow species *Salix schwerinii* 'Kinuyanagi', *S. lasiolepis*, *S. matsudana × alba* 'Tangoio' and *S. lucida spp lasiandra* subjected to three soil water regimes: well-watered (WW), moderate soil water deficit (MD), and severe soil water deficit (SD). Values are means of three plants ± the standard error of the mean, except for SD Ky (N=0) and SD Ls (N=1). Means within treatments followed by the same letter are not significantly different (P < 0.05).

***Salix* species**	**Treatment**	**Water Use Kg**	**DW gaing**	**W_p g mass /Kg H_2O**	RW:SW
Kinuyanagi	WW	100.15± 6.35	555.08±28.5 ^{a}	5.56±0.46 ^{e}	0.23±0.03 ^{a}
Lasiolepis	WW	84.01± 10.25	470.39±69.5 ^{a}	5.60±0.43 ^{e}	0.31±0.05 $^{a\,b}$
Tangoio	WW	108.49±4.42	718.77±46.8 ^{b}	6.66±0.24 ^{f}	0.30±0.03 $^{a\,b}$
Lasiandra	WW	100.71±0.75	528.79±41.6^{a}	5.25±0.42 ^{e}	0.31±0.04 ^{b}
Kinuyanagi	MD	29.76 ± 5.03	182.88±26.5 ^{c}	6.23±1.11 ^{e}	0.22±0.01 ^{a}
Lasiolepis	MD	12.72±7.03	70.12±42.5 ^{d}	5.29±0.83 ^{e}	0.40±0.14 ^{a}
Tangoio	MD	31.36±2.31	197.95±9.52^{c}	6.54±0.53 $^{e\,f}$	0.26±0.01 ^{a}
Lasiandra	MD	27.35±2.02	198.15±14.2 ^{c}	7.43±0.85 ^{f}	0.23±0.02 ^{a}
Kinuyanagi	SD	*	*	*	
Lasiolepis	SD	0.28	-7.21	-25.75	0.42
Tangoio	SD	2.13±0.82	-11.91±10.7	-6.80±7.94	0.35±0.05 ^{b}
Lasiandra	SD	0.15±0.20	-93.35±25.2	-84.7±80.8	0.76±0.16 ^{c}

Table 2. Dry mass allocation in the four willow species *Salix schwerinii* 'Kinuyanagi', *S. lasiolepis*, *S. matsudana* × *alba* 'Tangoio' and *S. lucida spp lasiandra* subjected to three soil water regimes: well-watered (WW), moderate soil water deficit (MD), and severe soil water deficit (SD). Values are means of three plants ± the standard error of the mean, except for SD Ky (N=0) and SD Ls (N=1). Means within treatments followed by the same letter are not significantly different ($P < 0.05$).

Salix Species	Treat-ment	Biomass allocation g			% biomass allocation		
		Stem	Leaf	Root	Stem	Leaf	Root
Ky	WW	257.3±10.2 ^{a}	161.3±6.5 ^{a}	97.4±8.4 ^{a}	49.9	31.3	18.9
Ls	WW	179.7±28.5 ^{b}	167.9±37.1 ^{ab}	108.6±34.2 ^{a}	39.4	36.8	23.8
Tn	WW	358.0±14.1 ^{c}	135.2±8.8 ^{b}	143.2±24.2 ^{b}	56.3	21.2	22.5
Ln	WW	234.0±35.2 ^{ab}	120.9±13.4 ^{b}	111.7±22.2 ^{a}	50.2	25.9	23.9
Ky	MD	72.7±7.5 ^{a}	75.7±6.7 ^{a}	32.3±2.4 ^{b}	40.2	41.9	17.9
Ls	MD	30.9±22.5 ^{b}	40.0±15.4 ^{b}	24.6±5.1 ^{a}	32.4	41.9	25.7
Tn	MD	118.1±3.1 ^{c}	49.0±2.0 ^{b}	43.0±0.9 ^{c}	56.2	23.3	20.5
Ln	MD	94.1±4.3 ^{d}	68.3±6.0 ^{a}	36.9±3.3 ^{b}	47.2	34.3	18.5
Ky	SD	*	*	*			
Ls	SD	26.9	4.2	13.0	61.0	9.4	29.6
Tn	SD	25.4±5.5 ^{b}	7.2±0.5 ^{b}	11.3±0.9 ^{b}	57.9	16.4	25.7
Ln	SD	4.2±1.7 ^{a}	2.6±1.2 ^{a}	5.1±0.6 ^{a}	35.4	22.1	42.5

Table 3 Stomatal and leaf hair density, and transpiration % for the four willow species *Salix schwerinii* 'Kinuyanagi', *S. lasiolepis*, *S. matsudana* × *alba* 'Tangoio' and *S. lucida spp lasiandra.* Values are the means of 10 HP fields for each of three leaves ± the standard error of the mean.

Species	Leaf surface	Stomatal density /mm^2	%	Leaf Hairs /mm^2	Transpiration %
Kinuyanagi	Upper	0	0	79.4	
	Lower	>300 est.	100	∞	
Lasiolepis	Upper	0	0	3.0	
	Lower	261± 48	100	0.5	
Tangoio	Upper	87± 8	35	11.5	21
	Lower	161± 2	65	2.9	79
Lasiandra	Upper	92± 19	25	0	7
	Lower	273± 29	75	0	93

Table 4. Transpiration and stomatal conductance measured for five leaves of the four willow species *Salix schwerinii* 'Kinuyanagi', *S. lasiolepis*, *S. matsudana* × *alba* 'Tangoio' and *S. lucida spp lasiandra.* at 0900, 1200, 1400 and 1600 on two days. Values given are means and were measured for the lower leaf surface on WW treatment plants only.

Willow Species	Leaf area m^2	Mean transpiration ($g\ H_2O\ m^{-2}$)	Mean stomatal conductance ($mmol\ m^{-2}\ s^{-1}$)
Kinuyanagi	1.99	1.7±0.9	69.7±35.8
Lasiolepis	1.84	2.3±2.0	98.7±91.2
Tangoio	1.95	1.1±0.9	54.2±90.3
Lasiandra	1.75	0.8±0.4	33.9±19.3

While W_p with the WW treatment was significantly higher in Tn this was not so for the MD treatment. Despite the difficulty in defining a W_p for the surviving plants in the SD treatment all the plants belonging to the two tree willow species survived and were able to utilise the small amount of water available in the soil and in the cutting to produce new leaves, stems and roots. Storage of photosynthate in new tissue varied between the four species. In particular, Ky allocated a lower % in root biomass and this may partly explain the greater stress experienced by Ky in the SD treatment.

Blake *et al.* (1984) showed that variation in stomatal morphology, including ledges, cuticular ridges and trichomes, were related to increased W_p in several *Populus* clones. They also reported that one *P. alba* clone (A-499) with a very high stomatal density had the highest W_p. In this study there is not a close correlation between stomatal density and W_p. Of the four species Tn had the lowest stomatal density but the highest W_p in the WW treatment, the nearest treatment to a control experiment that would test this hypothesis. The exact mechanism for an increase in W_p with stomatal density is unclear, since transpiration rate is often proportional to stomatal number (Ceulemans *et al.*, 1978, Blake, 1980), although not always (Blake *et al.*, 1984). This study does not support the hypothesis that transpiration rate is proportional to stomatal number alone.

In this experiment the tree species allocated their photosynthate differently to shrub species with a higher proportion going to form new stems and roots and a lesser proportion going to leaves. In periods of water stress the tree species will have a greater stem volume in which to draw stored water and a lower leaf area reducing water loss through evapotranspiration. In contrast, where the primary purpose of the willow is to transpire water and remove N, as is the case in the application of willows to manage irrigated dairy shed effluent, then the greater allocation of photosynthate to new leaves is advantageous.

The tree willows had a lower transpiration rate, a greater allocation of biomass to stem and root production and a greater capacity to cope with water stress than did the shrub willows particularly Ky. For these reasons tree willows should always be favoured. In those parts of New Zealand where willow sawfly *Nematus oligospilus* is defoliating and killing tree willows Ky could be considered, being the far more vigorous of the two shrub willow species. However, it is likely that under severe water stress as happens with low river flows and high temperatures in mid to late summer in eastern regions of the North Island mortality may be a factor with Ky that would not normally be expected with the deeper rooting tree willows. The relationship between W_p measured on potted cuttings must also be compared to the same clones growing in the field to ensure that the results are not due to the experimental conditions such as confined access for roots (Ismail *et al*., 1994).

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