Gas exchange, leaf structure, and hydraulic features in relation to sex, shoot form, and leaf form in an evergreen shrub *Sabina vulgaris* in the semi-arid Mu Us Sandland in China

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Abstract

We examined differences in net photosynthetic rate (P_N), transpiration rate (E), water use efficiency (WUE), ratio of sub-stomatal to atmospheric CO_2 concentration (C_i/C_a), cuticle thickness (CT), epidermis cell size (ECS), mesophyll cell size (MCS), vascular bundle size (VBS), tissue density (TD), and coefficient of water loss (k) in *Sabina vulgaris* as related to sex, shoot form, and leaf form. P_N, E, WUE, C_i/C_a, MCS, VBS, and k varied with sex, whereas CT, ECS, and TD did not. These differences in physiology and anatomy between the female and male plants may be closely related with their reproduction behaviour. P_N, E, C_i/C_a, CT, ECS, MCS, and VBS were significantly smaller in the erect shoots than in the prostrate shoots, WUE was just opposite; TD and k did not vary with shoot form. These changes in physiology with shoot form indicate that erect shoots may be more tolerant of water stress than prostrate shoots. P_N, E, C_i/C_a, TD, and k were significantly greater in the spine leaves than in the scale leaves, whereas WUE, CT, ECS, MCS, and VBS followed the opposite trends. The changes in physiology and anatomy with leaf form suggest that scale leaves have higher drought-resistant and water-holding capacities than spine leaves. Measurements of field gas exchange showed that three-year-old seedlings had lower drought-resistance and higher water loss than five-year-old seedlings, which provides some evidence that seedling survival decreases with decreasing plant age.

Additional key words: coefficient of water loss; leaf anatomy; photosynthesis; *Sabina vulgaris*; tissue density; transpiration; water use efficiency.

Introduction

The Mu Us Sandland is a highly vulnerable and multiple-level ecotone, and is characterised by sparse precipitation and poor soil nutrients (Chi 1994, Zhang 1994, Chen and Zhang 1996). The local vegetation coverage is usually especially low (Li 1990, Zhang 1994, Dong and Zhang 2000). However, the community of *Sabina vulgaris*, an evergreen dominant shrub, is an exception. This species tends to grow so densely that it can cover the sandy lands completely, forming a "vast green blanket" (Dong and Zhang 2000).

Gas exchange, leaf structure, and hydraulic features vary with resource regimes (*e.g.* irradiance, CO_2 concentration, soil water and nutrient availability), which in turn can help plants adapt to changing environments through these changes (Strauss-Benedetti and Berlyn 1994, Anderson *et al.* 1995, Roberts *et al.* 1996, Smith *et al.* 1997, Herppich *et al.* 1998, Sims *et al.* 1998, Eamus 1999, Garnier *et al.* 1999, Wright and Westoby 1999). Some recent evidence indicates that the *S. vulgaris* plants in the Mu Us Sandland can adapt to soil water and nutrient shortages through physiological, morphological, and growth acclimation (Jiang and He 1999, Dong and Zhang 2000, He and Zhang 2003).

S. vulgaris is usually dioecious, and is sparsely androgynous (EBFC 1978). In natural stands, it has simultaneously erect and prostrate shoots; its adult plants have

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Abbreviations: C_a – atmospheric CO_2 concentration; C_i – sub-stomatal CO_2 concentration; CT – cuticle thickness; E – transpiration rate; ECS – epidermis cell size; k – coefficient of water loss; MCS – mesophyll cell size; P_N – net photosynthetic rate; RA – reproductive allocation; TD – tissue density; VBS – vascular bundle size; WUE – water use efficiency.

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simultaneously scale leaves and spine leaves, but the former dominate over the latter; its seedlings only have spine leaves, and seedling survival increases with increasing age (He 2000). These characteristics provide an ideal platform to conduct comparative studies within a species. Although the variation in general physiological and anatomical patterns of *S. vulgaris* plants with environments is well documented, the ways of variation within the species remain unclear (He and Zhang 2003). However, complete understanding of these ways is very important to comparative studies between species.

Our objective was to investigate differences in gas exchange, leaf structure, and leaf hydraulic features in *S. vulgaris* connected with sex, shoot form, and leaf form. Specifically we tried to (*1*) examine whether physiological and anatomical differences between the female and male plants are related with their reproduction, (*2*) determine whether erect shoots are more tolerant of water stress than prostrate shoots, (*3*) find why scale leaves dominate over spine leaves in nature, and (*4*) explore how the difference in photosynthesis affects seedling survival.

Materials and methods

Study species and study site: *Sabina vulgaris* Antione (*Cupressaceae*) is widespread in the northern and northwestern China. It appears in the areas with a mean annual precipitation of 110-570 mm and a mean annual temperature of −1.0 to +9.5 °C, and its growth begins in April and peaks in late July to early August (EBFC 1978, Li 1990). This species is also a typical woody clonal plant (He and Zhang 2003). The study was conducted at the Mu Us Center for Sand Exploitation and Control (MUCSEC) (109°21'–109°17'E, 38°57'–39°10'N, 1 200–1 350 m a.s.l.), where the mean annual precipitation is 360 mm, about 80 % of which falls from mid-June through mid-September, and the mean annual temperature is 6.4 °C (He and Zhang 2003).

Measurements: In mid-August 1999, field measurements were carried out at the MUCSEC. There are diverse landscapes (*e.g.* fixed sandy lands and shifting sandy lands) in the MUCSEC, but most of *S. vulgaris* plants appear in fixed sandy lands. So all chosen materials were from fixed sandy lands rather than from other landscapes.

Net photosynthetic rate (P_N), transpiration rate (E), and CO_2 concentration were measured at 2-h intervals with an open flow gas exchange system (*LCA-4*, *ADC*, Hoddesdon, England) from 08:00 through 18:00. Water use efficiency (WUE) was calculated as the ratio of P_N to E. Four or five replications were made for each measurement. For gas exchange measurements we chose: (*a*) Scale leaves of size-similar female and male plants sharing a very similar microhabitat. (*b*) Scale leaves from the erect and prostrate shoots of the same adult plants. (*c*) Spine and scale leaves from the same adult female plants, which were dominant over male plants in the study site. (*d*) Three- and five-year-old seedlings.

The leaves of adult plants used for gas exchange measurement were fixed in cold formaldehyde–acetic acid–alcohol (FAA) and dehydrated in alcohol for 1 h. This was followed by two 10-min washes of 2,2-dimethoxypropane with 0.1 M HCl (three drops per 25 cm^3), two 5 min acetone washes, and finally a wash with *tert*-butyl alcohol (75 %, v/v) and chloroform. The tissue was progressively embedded in *Paraplast* starting at 35 °C (3–4 d) and continuing at 60 °C (minimum of 3 d). Cross sections, 12 μm thick, were cut with a microtome, and slides with mounted tissue were stained in safranin and fast-green. The sections were viewed through a *Carl Zeiss* microscope. Cuticle thickness (CT), epidermis cell size (ECS), mesophyll cell size (MCS), and vascular bundle size (VBS) were determined.

In order to determine both tissue density (TD) and coefficient of water loss (k) of leaves of *S. vulgaris*, we collected 10 fully developed leaves from the same adult plants chosen for gas exchange measurement. For the detailed procedures regarding TD and k see Wright and Westoby (1999) and He and Zhang (2003), respectively.

Reproductive allocation (RA) was calculated as described by Bazzaz *et al.* (1987).

Data analysis: T-test was employed to compare the differences in gas exchange, leaf anatomy, and hydraulic features of female *vs.* male plants, erect *vs.* prostrate shoots, and spine *vs.* scale leaves, and to compare the differences in gas exchange of three-year-old *vs.* five-year-old seedlings. Spearman correlation was employed to assess the relationship between leaf gas exchange and anatomical features (*SPSS 10.0 for Windows*).

Results

There were significant differences in P_N, E, WUE, and C_i/C_a between the female and male plants of *S. vulgaris* (Table 1). Significant differences in CT, ECS, and TD between the female and male plants were not observed, but the opposite directions were true in MCS, VBS, and k between the both (Table 1). There were significantly negative relationships between P_N and both MCS and VBS ($p < 0.05$). The female's RA was 0.61 ± 0.04 while the male's RA was 0.24 ± 0.04, which means that reproductive cost was much greater in the female than male plants of *S. vulgaris*.

Leaf P_N, E, and C_i/C_a were significantly smaller in the

Table 1. Comparison of diurnal gas exchange, leaf anatomy, and hydraulic features of female and male plants of *S. vulgaris* [means ± 1 SE, $n = 5$ (gas exchange) or 10]. Different superscripts within a column represent the values significant at $p = 0.05$.

	Female	Male
P_N [μmol m^{-2} s^{-1}]	6.44 ± 0.18^{a}	5.56 ± 0.16^{b}
E [mmol m^{-2} s^{-1}]	2.33 ± 0.16^{b}	3.55 ± 0.27^{a}
WUE [mmol mol^{-1}]	2.68 ± 0.17^{a}	1.85 ± 0.18^{b}
C_i/C_a	0.52 ± 0.03^{b}	0.63 ± 0.02^{a}
CT [μm]	3.9 ± 0.2^{a}	4.2 ± 0.2^{a}
ECS [μm]	13.5 ± 1.0^{a}	15.5 ± 1.5^{a}
MCS [μm]	23.3 ± 2.2^{b}	33.4 ± 2.4^{a}
VBS [μm]	60.5 ± 5.3^{b}	130.2 ± 12.3^{a}
TD [kg kg^{-1}]	0.29 ± 0.02^{a}	0.28 ± 0.02^{a}
k	0.84 ± 0.03^{a}	0.72 ± 0.02^{b}

Table 2. Comparison of diurnal gas exchange, leaf anatomy, and hydraulic features of erect and prostrate shoots of *S. vulgaris* [means ± 1 SE, $n = 5$ (gas exchange) or 10]. Different super scripts within a column represent the values significant at $p = 0.05$.

	Erect	Prostrate
P_N [μmol m^{-2} s^{-1}]	5.31 ± 0.18^{b}	6.57 ± 0.16^{a}
E [mmol m^{-2} s^{-1}]	1.61 ± 0.10^{b}	2.53 ± 0.18^{a}
WUE [mmol mol^{-1}]	3.43 ± 0.16^{a}	2.62 ± 0.18^{b}
C_i/C_a	0.45 ± 0.02^{b}	0.56 ± 0.03^{a}
CT [μm]	3.8 ± 0.2^{b}	4.5 ± 0.2^{a}
ECS [μm]	15.3 ± 1.0^{b}	19.7 ± 1.1^{a}
MCS [μm]	28.5 ± 1.7^{b}	36.1 ± 1.5^{a}
VBS [μm]	58.9 ± 5.3^{b}	153.8 ± 14.6^{a}
TD [kg kg^{-1}]	0.29 ± 0.02^{a}	0.28 ± 0.02^{a}
k	0.78 ± 0.02^{a}	0.74 ± 0.02^{a}

erect shoots than in the prostrate shoots of *S. vulgaris*, whereas WUE was greater in the former than in the latter (Table 2). CT, ECS, MCS, and VBS were significantly smaller in the erect shoots than in the prostrate shoots, whereas TD and k showed no significant differences between the both (Table 2). There were significantly positive relationships between P_N and both MCS and VBS ($p < 0.05$).

P_N, E, and C_i/C_a were significantly greater in the spine leaves than in the scale leaves of *S. vulgaris*, whereas WUE of scale leaves was significantly smaller than that of scale leaves (Table 3). There was no significant difference in P_N between the three-year-old and five-year-old seedlings, but E, WUE, and C_i/C_a followed the opposite trends (Table 3). CT, ECS, MCS, and VBS were significantly smaller in the spine leaves than in the scale leaves, whereas the opposite was true in TD and k between both (Table 3). There were significantly negative rela-tionships between P_N and both MCS and VBS ($p < 0.05$).

Table 3. Comparison of diurnal gas exchange of spine and scale leaves or three-year-old and five-year-old seedlings of *S. vulgaris*, and of leaf anatomy and hydraulic features of spine and scale leaves (means ± 1 SE, $n = 5$ for gas exchange of leaf types, 4 for gas exchange of plant age differences, 10 for other determinations). Different superscripts within a column represent the values significant at $p = 0.05$.

	Spine	Scale	Three-years-old	Five-years-old
P_N [μmol m^{-2} s^{-1}]	3.63 ± 0.09^{a}	3.08 ± 0.08^{b}	2.79 ± 0.28^{a}	2.85 ± 0.33^{a}
E [mmol m^{-2} s^{-1}]	1.64 ± 0.07^{a}	1.23 ± 0.09^{b}	2.49 ± 0.25^{a}	1.68 ± 0.19^{b}
WUE [mmol mol^{-1}]	1.89 ± 0.08^{b}	2.37 ± 0.09^{a}	1.08 ± 0.09^{b}	1.76 ± 0.08^{a}
C_i/C_a	0.57 ± 0.03^{a}	0.19 ± 0.02^{b}	0.66 ± 0.03^{a}	0.42 ± 0.04^{b}
CT [μm]	3.7 ± 0.3^{b}	6.7 ± 0.4^{a}		
ECS [μm]	12.5 ± 0.6^{b}	15.5 ± 0.5^{a}		
MCS [μm]	36.3 ± 1.9^{b}	43.6 ± 2.3^{a}		
VBS [μm]	82.5 ± 2.3^{b}	273.6 ± 8.4^{a}		
TD [kg kg^{-1}]	0.29 ± 0.004^{a}	0.26 ± 0.006^{b}		
k	0.89 ± 0.02^{a}	0.81 ± 0.01^{b}		

Discussion

In this paper many obvious changes in physiology and anatomy with sex, shoot form, and leaf form were found (Tables 1 to 3). C_i/C_a is a good indicator to reflect the physiological drought-resistant capacity of leaves, the higher value indicating lower drought-resistance (Hu and Wang 1998). CT and ECS are positively related with non-stomatal transpiration; increased MCS and VBS are beneficial to enhance the potential of photosynthesis and water transport (Murray 1997). The higher values of TD and k reflect lower anatomical drought-resistance (Wright and Westoby 1999, He and Zhang 2003).

RA is among important components of fitness (Bazzaz *et al.* 1987). RA is greater in the female than in the male, which may be closely related with higher reproductive cost in the female and may have evolution significance (Antos and Allen 1999). In this respect our results from *S. vulgaris* support this well-documented pattern. Recent study pointed out that the differences in leaf structure and function between female and male plants are closely related with their reproductive process (Jones *et al.* 1999). For example, P_N is significantly greater in the female than in the male, which helps the female accumulate more organic products to meet their higher reproductive cost (Jones *et al.* 1999). Our results support completely this viewpoint. Diurnal WUE is significantly greater in the female plants than in the male plants of *S. vulgaris* (Table 1). This implies that the females have greater organic production per unit of water, consequently being beneficial to higher reproductive effort in female plants. Unfortunately, the exact mechanism of physiological differences between female and male plants is still not clear due to very limited studies (Jones *et al.* 1999).

The difference in C_i/C_a between the female and the male plants of *S. vulgaris* suggests that the former has higher physiological drought-resistant capacity than the latter. But the difference in k between the female and male plants reflects that the females have lower anatomical drought-resistant capacity than the males. This disparity between physiological and anatomical drought-resistant capacities may indicate at least three possible explanations: (*1*) There may exist a trade-off between the two types of drought-resistant capacities. (*2*) There may be a diverse expression of the same characteristic (*i.e.* drought-resistant capacity). (*3*) There may be other mechanisms determining the two capacities. Beyond all doubt, the drought-resistant capacity at whole leaf level is essential to alleviate water stress during the longer reproductive period of the female (Eamus 1999). There are significantly positive relationships between E and both MCS and VBS ($p<0.05$). This supports the viewpoint that increased MCS and VBS are beneficial to enhance the potential of water transport, which may be related to higher transpiration of the male (Murray 1997). In our study no significant difference in non-stomatal transpiration between the female and male plants of *S. vulgaris* was observed owing to similarity in CT and ECS.

Soil water availability was relatively favourable for the prostrate shoots of *S. vulgaris* in that they usually have plenty adventitious roots that can take in enough water from top wet sandy soil (He 2000). P_N and E were significantly greater in the prostrate shoots than in the erect shoots, which may be related with the differences in soil moisture regime surrounding the two types of shoots. There were significant positive correlations between P_N and E or MCS and VBS ($p<0.05$). The differences in WUE, CT, and ECS between the erect and prostrate shoots suggest that the erect shoots respond to relatively unfavourable soil moisture through increasing WUE, whereas the prostrate shoots can try to reduce water loss through decreasing non-stomatal transpiration. The responses of C_i/C_a, TD, and k to contrasting soil water regime indicate that the physiological drought-resistant capacity is greater in the erect shoots than the prostrate shoots, whereas the anatomical drought-resistant capacity is similar to each other. So it may be speculated that the erect shoots of *S. vulgaris* are more tolerant of water stress than the prostrate shoots based on our findings.

In the nature, leaf form of *S. vulgaris* varies with changing soil water availability. For example, the spine leaves in adult plants are plentiful in wetter microhabitats, but are very limited in drier microhabitats (He 2000). Spine leaves had higher P_N than scale leaves, which means that diurnal photosynthetic product accumulation is greater in the former than in the latter. The differences in E, WUE, and C_i/C_a (Table 3) indicate that the scale leaves have lower water loss and higher both WUE and physiological drought-resistant capacity than spine leaves. Cuticle and epidermis cells are important protective structures, thickness and size of which increase with increased stress (Strauss-Benedetti and Berlyn 1994, Rocas *et al.* 1997). The differences in CT, ECS, TD, and k (Table 3) suggest that the anatomical drought-resistance and water-holding capacities are much greater in the scale leaves than in the spine leaves. Soil water regime is the most fundamental factor limiting plant growth and productivity in the Mu Us Sandland (Li 1990, Zhang 1994). Scale leaves may be more useful to help the *S. vulgaris* plants survive in the semi-arid environments with strong evapo-transpiration than the spine leaves. These results explain why scale leaves of *S. vulgaris* are dominant over spine leaves in nature from the physiological and anatomical points of view.

In natural stands the seedlings of *S. vulgaris* are very limited (He and Zhang 2003). Field gas exchange measurements (Table 3) show that (*1*) there is not significant difference in diurnal photosynthetic product accumulation between three-year-old and five-year-old seedlings; (*2*) three-year-old seedlings exhibit higher water loss and lower both WUE and physiologically drought-resistant capacity than five-year-old seedlings. These findings may provide some physiological evidence that survival of younger seedlings of *S. vulgaris* is lower than that of the older ones. Of course, there must be some other mechanisms explaining this phenomenon. Unfortunately, in this respect the direct evidence is still very limited. Full understanding of seedling phase, which is essential to understand the process of population regeneration (Davis 1998), needs further effort.

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