STOMATAL DISTRIBUTION ON LEAVES OF THREE SPECIES OF *CHAMAECYPARIS*

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Abstract: Seedlings of *Chamaecyparis formosensis*, *C. taiwanensis* and *C. lawsoniana* were grown in well-watered nursery conditions. Scale leaves of the three species differed slightly in the general distribution of stomata. In *C. lawsoniana*, size and stomatal characteristics of facial scale leaves were similar to lateral leaves, and similar to facial leaves of other species. In the other two species, lateral leaves were larger than facial leaves, with more stomata but lower stomatal frequency and lower estimated coverage by stomatal pores. Scale leaves from natural seedlings were smaller in *C. formosensis*, and had a lower stomatal frequency in *C. taiwanensis*. Stomatal pore size was smallest in *C. taiwanensis*. Coverage by stomatal pores of *C. lawsoniana* is 0.67% (assuming a 6 μ pore width), compared to 0.52 and 0.49% for the species from Taiwan. Compared to other conifers measured, *Chamaecyparis* is intermediate in its stomatal frequency and coverage by pores, although *C. lawsoniana* has relatively long stomatal pores. Primary leaves of *C. formosensis* are much larger than scale leaves, with more stomata; most stomata are on the abaxial surface, which has a stomatal frequency similar to that of facial leaves.

INTRODUCTION

The stomatal distribution in *Chamaecyparis*, a conifer of the family Cupressaceae, varies with the type of leaf and somewhat with the species (Florin 1931). The scale leaves (Type III of de Laubenfels 1953) found on all but young seedlings have stomata very localized on the leaf, with different patterns on adaxial and abaxial surfaces. Generally a band of stomata occurs on each side of the middle of the adaxial surface; the abaxial surface usually has only two small groups near the base of the leaf. Two types of leaves occur on the flattened mature branchlets. The facial leaves occur on the flattened surfaces and the marginal or lateral leaves occur folded around the edge of the branchlet. Stomatal distribution varies on these leaf types, and may even depend on whether the leaf is on the exposed or shaded side of the branchlet (Florin 1931). Young seedlings produce a flat linear leaf (Type II of de Laubenfels 1953) for a variable time after germination; they may have stomata on the bottom or on both leaf surfaces, depending on the species (Florin 1931).

Stomata are the sites through which most gas exchange of the leaf occurs. The rate of water loss or photosynthesis for a leaf depends on their degree of opening as well as their number and perhaps their location on the leaves. Drought resistance of sources of *Pinus taeda* was highly correlated with their stomatal frequency (Knauf and Bilan 1974) and water loss in *P. strobus* and *P. resinosa* was correlated with stomatal size and number (Davies *et al.*, 1974).

In a study of leaf resistance to water loss, differences among three *Chamaecyparis* species were found. The data on stomatal occurrence presented here were developed to determine to what extent the physiological differences could be caused by differences among the species in

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stomatal pore area. Data presently available (primarily Florin 1931) are qualitative and do not include the frequency of stomata nor their coverage of the leaf, desired for our purposes. Since past work was based on a small sample size and did not include all the species used here, brief qualitative descriptions of stomatal distribution will also be given.

METHODS

Seedlings of several seed sources each of *Chamaecyparis formosensis* Matsum., *C. taiwanensis* Masamune & Suzuki and *C. lawsoniana* (A. Murr.) Parl. were grown together in a forest nursery at San Ping, at 750 m 50 km E of Tainan, Taiwan (22° 59′ N 120° 39′ E). The mean annual temperature is 8 to 13 C higher than in the native range of the species and average relative humidity exceeds 80% in all months. However, *Chamaecyparis* seedlings grow well; *C. lawsoniana*, the one from the most different climate, grows faster here than the two species from Taiwan (Liu *et al*., 1975).

Seeds were planted 15 February 1976, and samples were taken between 6 January and 31 March 1977. Seedlings were watered daily in dry weather, weeded regularly, and grown under screening which provided 50% shade until November 1976; the primary purpose of the screening was to reduce damage from insects and from the torrential summer rains.

The foliage used in the physiological sampling was collected from all plants; all samples included the ends of small branches and thus the youngest foliage. Four sources of each species were chosen for stomatal samples (Table 1), to represent the geographic range and the variety of physiological response. Samples were air-dried after collection and later dried at 70 C; they were first used to determine leaf weight and area for the physiological study. For each species three of the dried samples from each source were chosen randomly; the part of each sample used in this study included the most scale-like leaves. Type II seedling leaves were studied only from *C. formosensis*, which produces them for several months, much longer

Table 1. Location of seed sources for the seedlings examined. Chfo=*C. formosensis*; Chta=*C. taiwanensis*; Chla=*C. lawsoniana*

Site	Latitude	Longitude	Elevation (m)	Species Sampled		
				Chfo	Chta	Chla
Taiwan						
Ali Shan	23°32′N	120°47′E	2200-2300	×	×	
Chi Lan Shan	24°41′N	121°49′E	1200-1300	×	×	
Ho Ping	24°15′N	121°35′E	2050	×		
Mou Lin	22°55′N	120°39′E	2300	×		
Hsyun San Shan	24°30′N	121°08′E	1800		×	
Ta Hsueh Shan	24°17′N	121°01′E	2300		×	
United States						
Coos County Area:						
Saunders Lake Dunes	43°32′N	124°13′W	30			×
Coos Co. Forest	43°15′N	124°16′W	70			×
Grayback Area:						
Grayback A	42′09′N	123°23′W	1100			×
Grayback B	42°07′N	123°21′W	1220			×

than the other species (Liu *et al.*, 1975). Three samples of foliage from native saplings of both Taiwanese species were also taken from an old-growth stand at An Ma Shan (2200 m, lat. 24° 17′ N, long. 121° 01′ E), near the "Ta Hsueh Shan" source of *C. taiwanesis.* The dried samples were boiled in 95% alcohol until the chlorophyll disappeared; they were then soaked in 4% NaOH for 1–2 weeks. Photographs (26 x) were taken of one facial and one marginal leaf per sample. Stomata on both sides of the cleared scale leaves could be seen. For the larger, linear type II leaves, the two photographs together included almost the total area of a single leaf. Area of the one leaf surface, percentage of the leaf's projected area bearing stomata, and number of stomata per leaf were determined from the photographs. Leaf area of two surfaces was calculated; the percentage of leaf with stomata and the frequency of stomata (mm^{-2}) were determined on the basis of the total area of both leaf surfaces. Since the samples were not originally collected for stomatal counts, the exposed and shaded side of the branchlets were not distinguished. In the photographs of cleared leaves, stomata on both surfaces were visible but it was often not possible to separate which stomata were on which surface with enough assurance to count the surfaces separately. Some samples were not suitable for use, reducing the total number available.

"t"-tests at the .05 significance level were used to determine whether differences among leaf types or among species were significant for means of total leaf area, total stomata, stomatal frequency, percent of leaf surface bearing stomata, and length of stomatal pore.

Length of the stomatal pore was measured on several enlarged photographs of parts of nursery-grown leaves for each species.

RESULTS

General Patterns of Stomatal Distribution:

The stomatal distribution on leaves of several *Chamaecyparis* spp., including *C. lawsoniana*, has been described in detail by Florin (1931). However, no specific information for the two species from Taiwan was included.

All species displayed considerable variability in the stomatal distribution on type III scale leaves; most leaves, however, generally fit the description for the genus (Florin 1931), with the following exceptions: (1) *C. lawsoniana*: A few leaves lacked at least one of the basal stomatal groups (on the abaxial surface); Florin describes this only from *C. pisifera.* (2) *C. formosensis*: In about half the leaves of both types, adaxial stomatal bands do not merge across the median line of the leaf, as they usually do in *C. lawsoniana.* Again, the basal abaxial stomatal groups in some leaves are greatly reduced or absent. (3) *C. taiwanensis*: The same general pattern was present as in other species. However, the adaxial stomatal bands of lateral leaves were often less regular than in other species, were sometimes incomplete, and seldom merged near the leaf tip; in contrast, this merger occurred in most facial leaves. The basal stomatal groups in facial leaves sometimes merged, unlike our samples of the other species; in other leaves, they were, again, absent.

Most stomata on the primary leaves of *C. formosensis* are on the abaxial side of the leaf, in contrast to *C. lawsoniana*, in which many occur on both surfaces (Florin 1931).

Quantitative Distribution:

Scale leaves: Facial leaves showed no significant differences among species; in contrast, the lateral leaves differed somewhat (Table 2). Lateral leaves of *C. lawsoniana* are smaller than those of the other two species. Those of *C. taiwanensis* have the most stomata, while those of *C. lawsoniana* have stomata distributed over the largest proportion of leaf surface. Stomatal frequency varies, but differences are not significant.

Table 2. Characteristics of leaves by species and leaf type. Standard errors given in parentheses. All data are based on the area of both leaf surfaces. Pictures of primary leaves did not include the whole leaf

Source	Species	Leaf Type	*n*	Leaf Area (mm²)	Total Stomata	Stomatal Frequeny (mm⁻²)	Stomatal Coverage (% of Leaf Area)
Nursery							
—Scale Leaves	*C. formosensis*	Facial	11	2.8(.4)	151(12)	63(5)	14(1)
		Lateral	9	5.0(.7)	167(20)	39(7)	11(1)
	C. taiwanensis	Facial	10	1.8(.3)	128(15)	75(8)	15(1)
		Lateral	11	5.8(.7)	283(29)	51(5)	10(1)
	C. lawsoniana	Facial	12	2.4(.3)	137(13)	60(5)	16(1)
		Lateral	10	3.2(.4)	179(27)	56(4)	16(2)
Nursery							
—Primary Leaves	*C. formosensis*	—	6	>23.8	>688(28)	29(2)	19(1)
Field							
—Scale Leaves	*C. formosensis*	Facial	2	1.2	55	54	15
		Lateral	2	2.6	148	56	11
	C. taiwanensis	Facial	3	2.0	60	42	9
		Lateral	2	5.8	197	35	6

There were too few samples to compare sources within the species from Taiwan. The two Grayback sources of *C. lawsoniana* (Table 1), from the inland end of its range at high elevation, were more similar to each other than they were to those from the coastal populations from Coos County (Table 1); Coos County leaves generally had more stomata than Grayback (Table 3). However, with the small number of samples, the only statistically significant difference (.05 level) is in the percent stomatiferous surface of lateral leaves.

Lateral leaves differ from facial leaves in the two species from Taiwan (Table 2). Lateral leaves are larger and have more stomata, although stomata occupy a smaller percentage of leaf surface (the preceding are significant at .01 level for *C. taiwanensis*); their stomatal frequency is lower (significantly at .05 for both species). No leaf type differences were significant for *C. lawsoniana.*

Leaves from field-grown plants were too few to test statistically. Leaves of *C. formosensis* were smaller, and those of *C. taiwanensis* had fewer stomata on leaves of a similar size, than those from the nursery stock. Field-grown leaves showed differences between lateral and facial leaves similar to those of nursery-grown leaves (Table 2).

Primary Leaves: Primary leaves (Type II) of *C. formosensis* were much larger, with many more stomata and a larger percentage of stomatiferous leaf surface than scale leaves (Table 2). Their stomata are more widely separated: although stomatal frequency on the most heavily stomated surface was not significantly different from that of facial scale leaves, the total leaf-surface frequency (Table 2) is much lower.

Stomatal Pore Size: Stomatal pore length was measured on photographs of 3-4 different leaves of each species. Among these few samples, leaf type and origin (field or nursery)

Table 3. Variation in characteristics between seed sources of *C. lawsoniana*. (Standard errors in parentheses)

Seed Origin	Leaf Type	*n*	Leaf Area (mm^2)	Total Stomata	Stomatal Frequency (mm^{-2})	Stomatal Coverage (% of leaf)
Grayback	Facial	6	2.5(.4)	125(18)	51(5)	13(3)
	Lateral	4	3.5(.3)	167(23)	47(4)	12(3)
Coos County Forest	Facial	6	2.3(.4)	150(21)	69(14)	19(4)
	Lateral	6	3.0(.6)	188(44)	61(10)	19(3)

made no consistent difference in pore size; all data were pooled by species. *Chamaecyparis lawsoniana* had the largest pore, *C. formosensis* is nearly as large, but stomata of *C. taiwanensis* are somewhat smaller (Table 4). Differences are significant.

DISCUSSION AND CONCLUSIONS

From the data presented above, one can estimate the coverage of stomatal pore area as a percentage of the leaf surface. This should represent the most effective area for water loss. Assumptions are (1) that the pore is elliptical and (2) all stomata are open to $6\,\mu$ wide (to allow comparison with other published data). The facial leaves of the three species are very similar in the estimated coverage of stomatal pore area, .64 to .70% (Table 4); in *C. taiwanensis* a larger frequency compensates for a smaller pore length. However, the lateral leaves vary more; a lower stomatal frequency in the species from Taiwan reduces pore coverage to about 0.4%, whereas frequency in *C. lawsoniana* remains more similar to the facial leaves (Table 2, 4). Taking into account the different sizes of the two types of leaves on the various species, the overall scale leaf surface coverage by stomatal pores is calculated to be 0.52% and 0.49% for the two species from Taiwan and 0.67% for *C. lawsoniana*. Such a difference in pore area (about 34 percent) may have some physiological significance by allowing higher maximal rates of gas exchange. However several unobserved factors could compensate for, or reinforce, such a difference: e. g. the maximal pore width actually attained, the number of leaf-surface papillae or the size of the air space between leaves, and the overlap of other leaves over the stomatal groups (Oppenheimer 1970).

Stomatal frequency and size often vary with environment (Levitt 1972); the data presented here are comparable in that they reflect an equivalent environmental influence. The few field-grown leaves examined show that either stomatal frequency or leaf size can be much different under conditions in the native habitat.

Among the conifers measured these three species of *Chamaecyparis* are intermediate in

Table 4. Pore size and estimated pore area as a percentage of leaf area, scale leaves. A pore width of $6\,\mu$ is assumed

Species	*n*	Pore Length(μ) (+ standard error)	Pore Area (% of leaf area)	
			Facial	Lateral
C. formosensis	96	23.2(.25)	0.68	0.43
C. taiwanensis	71	18.1(.31)	0.64	0.44
C. lawsoniana	112	24.6(.25)	0.70	0.64

stomatal frequency and pore area relative to total leaf surface (Meidner & Mansfield 1968, Napp-Zinn 1966); pore lengths of *C. formosensis* and *C. lawsoniana* are quite large, similar to *Cedrus deodora* and *Pinus strobus*, but smaller than *P. resinosa* (Meidner & Mansfield 1968, Davies *et al.*, 1974).

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