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Ecophysiology and Biomechanics of *Equisetum Giganteum* in South America

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FLORIDA INTERNATIONAL UNIVERSITY

Miami, Florida

ECOPHYSIOLOGY AND BIOMECHANICS OF *EQUISETUM GIGANTEUM* IN
SOUTH AMERICA

A dissertation submitted in partial fulfillment of the

requirements for the degree of

DOCTOR OF PHILOSOPHY

in

BIOLOGY

by

Chad Eric Husby

2009

To: Dean Kenneth Furton
College of Arts and Sciences

This dissertation, written by Chad Eric Husby, and entitled Ecophysiology and Biomechanics of *Equisetum Giganteum* in South America, having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this dissertation and recommend that it be approved.

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Florida International University, 2009

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ABSTRACT OF THE DISSERTATION

ECOPHYSIOLOGY AND BIOMECHANICS OF *EQUISETUM GIGANTEUM* IN
SOUTH AMERICA

by

Chad Eric Husby

Florida International University, 2009

Miami, Florida

Professor Steven F. Oberbauer, Major Professor

Equisetum giganteum L., a giant horsetail, is one of the largest living members of an ancient group of non-flowering plants with a history extending back 377 million years. Its hollow upright stems grow to over 5 m in height. *Equisetum giganteum* occupies a wide range of habitats in southern South America. Colonies of this horsetail occupy large areas of the Atacama river valleys, including those with sufficiently high groundwater salinity to significantly reduce floristic diversity. The purpose of this research was to study the ecophysiological and biomechanical properties that allow *E. giganteum* to successfully colonize a range of habitats, varying in salinity and exposure. Stem ecophysiological behavior was measured via steady state porometry (stomatal conductance), thermocouple psychrometry (water potential), chlorophyll fluorescence, and ion specific electrodes (xylem fluid solutes). Stem biomechanical properties were measured via a 3-point bending apparatus and cross sectional imaging. *Equisetum giganteum* stems exhibit mechanical characteristics of semi-self-supporting plants, requiring mutual support or support of other vegetation when they grow tall. The mean elastic moduli (4.3 Chile, 4.0 Argentina) of *E. giganteum* in South America is by far the

largest measured in any living horsetail. Stomatal behavior of *E. giganteum* is consistent with that of typical C3 vascular plants, although absolute values of maximum late morning stomatal conductance are very low in comparison to typical plants from mesic habitats. The internode stomata exhibit strong light response. However, the environmental sensitivity of stomatal conductance appeared less in young developing stems, possibly due to higher cuticular conductance. Exclusion of sodium (Na) and preferential accumulation of potassium (K) at the root level appears to be the key mechanism of salinity tolerance in *E. giganteum*. Overall stomatal conductance and chlorophyll fluorescence were little affected by salinity, ranging from very low levels up to half strength seawater. This suggests a high degree of salinity stress tolerance. The capacity of *E. giganteum* to adapt to a wide variety of environments in southern South America has allowed it to thrive despite tremendous environmental changes during their long tenure on Earth.

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1. INTRODUCTION: SIGNIFICANCE OF *EQUISETUM GIGANTEUM*
ECOPHYSIOLOGY AND BIOMECHANICS

Horsetails are unique survivors of a very ancient group of vascular plants, the Sphenophyta, which has a history reaching back to the Upper Devonian, approximately 377 million years ago (Bell and Hemsley 2000). Ancestors of modern horsetails, the giant *Calamites*, prominent elements of Carboniferous coal swamps, may have reached heights of 30 m or more and diameters of up to 1 m (Spatz et al. 1998b, Scagel et al. 1984, Cleal and Barry 1999). Unlike *Calamites*, which had secondary growth, modern horsetails are entirely herbaceous, yet retain the unique stem anatomical features of their extinct ancestors (Niklas 1997). Despite the remarkable conservatism of the architecture and anatomy of *Equisetum* stems, and the small number of species (15) in the modern flora, their ability to thrive under a wide range of conditions is remarkable:

“*Equisetum* grows from sea level all the way up to alpine meadows, and ranges from a primary colonizing weed to a component of climax vegetation. Species of *Equisetum* also occur in a wide range of moisture and edaphic regimes from muddy pond margins to the gravel coverings of railroad beds. These factors make it extremely difficult to regard *Equisetum* as a relict. On the contrary, the extensive range of physical and ecological space occupied by *Equisetum* demonstrates that the genus is extraordinarily successful. Indeed, *Equisetum* is this author’s candidate for the most successful genus of all living vascular plants!”
(Rothwell 1996)

Although the ancestors of horsetails thrived in the warm Carboniferous coal swamps 345 million years ago (Stewart and Rothwell 1993), modern horsetail species diversity is mostly concentrated in the north temperate zone between 40 and 60 degrees of latitude (Hauke 1990). Indeed, horsetails are found from extremely cold climates on northern Ellesmere Island on the Arctic Ocean (82 degrees north) to warm tropical climates in Central and South America and are found on every continent except Australia. This extensive geographic range evinces a remarkable degree of adaptability in an apparently quite archaic group. By studying modern horsetails, botanists can gain a deeper understanding of the adaptive potential inherent in such an ancient and morphologically conservative plant group. Such studies can lead to surprising results. For example, Marsh et al. (2000) found that *Equisetum* species contributed disproportionately to nutrient cycling in an Alaskan shrub wetland (e.g., *Equisetum* litter provided 55% of ecosystem phosphorus over a two year period), despite making up only 5% of the ecosystem biomass. This contribution was partly due to the *Equisetum* rhizomes and roots penetrating more deeply in the soil than other wetland plants. Such behavior unexpected in “relic” plant groups and provides important perspective on interpreting adaptive strategies of more recent plant groups, such as leptosporangiate ferns and seed plants. The aim of the current study is to elucidate some of the key structural and functional adaptations of the giant horsetail, *Equisetum giganteum* L., growing in contrasting climatic conditions in South America, ranging from the Atacama Desert of northern Chile to mesic river valleys in northwest Argentina.

Key questions addressed in this research

1. *What biomechanical adaptations allow E. giganteum to thrive and grow tall in environments as harsh and exposed as the Atacama Desert?*

A previous study of the biomechanics of a greenhouse-grown giant horsetail in Europe (Spatz et al. 1998a) found a low value for the flexural stiffness of *E. giganteum* stem tissue. However, the identity of the plant used in this study is uncertain (see Chapter 3) and the environment of a glasshouse in Germany is very different from those under which giant horsetails normally grow (Hauke 1963). The findings of Spatz et al. (1998a) were utilized to estimate the support tissue mechanical properties of stems of *Calamites* (Spatz et al. 1998b). However, since Spatz et al. (1998a) studied a plant growing under conditions of minimal wind stress and extended periods of low light during northern European winters, their measurements of stem tissue properties may not represent the actual biomechanical properties of giant horsetail stems in the field. In my study, the biomechanical properties of *E. giganteum* stems were studied in the most environmentally stressful conditions under which this species grows, which included high wind, sun and salinity stress.

2. *How do giant horsetail stomata behave under field conditions?*

Control of transpirational water loss is key for plant living in environments with high evaporative demand. Stomata are the crucial valves that regulate the rate of transpiration in vascular plants (Raven 2002). Although horsetails are generally wetland plants with significant moisture requirements, they can grow in arid areas as long as there is a moist “oasis” of some kind where groundwater is plentiful (Hauke 1963, C. Husby,

personal observations). *Equisetum* stomata are uniquely complex in structure among vascular plants. A guard cell pair is overlain by a pair of subsidiary cells with ridge-like thickenings on the inner walls (Dayanandan and Kaufman 1972, Hauke 1957). Until now, *Equisetum* stomatal behavior only has been studied to a limited extent under controlled cultivated conditions on the small-statured species *E. hyemale* (Dayanandan 1977). Here, I discuss the behavior of *E. giganteum* stomata under a variety of field conditions in contrasting environments in South America.

3. How does Equisetum giganteum cope with high groundwater salinity stress in the valleys of the Atacama Desert?

Soil salinity poses a significant physiological challenge to plant life, both for wild plants growing in naturally saline environments and for cultivated plants growing on the ~20% of the world's cultivated land that is affected by salinity (Rhoades and Lovejoy 1990). Altogether salinity impacts ~7% of the world's land (Szabolcs 1994), nearly a billion hectares (Yensen 2006), and this area is increasing (Ghassemi et al. 1995). Natural plant communities in saline habitats often are less productive and less diverse than otherwise similar habitats with lower soil salinity (Orcutt and Nilsen 2000). Even mildly saline lands create agricultural losses amounting to trillions of dollars per year (Yensen 2006).

As freshwater sources diminish and population pressure to utilize saline areas and salt affected water resources increase, there is an ever greater need to understand how plants tolerate and adapt to salinity (Yensen 2006). Because global sea level is expected to rise considerably due to global warming (Meier and Wahr 2002), it has become

increasingly important to understand how plant species will respond to increasing salinity levels (Rooth and Stevenson 2000). Researchers have found that most crop species have little genetic basis for salinity tolerance.

Glenn *et al.* (1999) advocate focusing future studies on the broad taxonomic array of plants naturally adapted to salinity stress in order “to define a minimal set of adaptations required for tolerant germplasm.” Duarte *et al.* (1995) and Meinzer (2003) also advocate comparisons among a broad range of plant types in order to better detect general patterns and constraints in plant ecology and physiology. Furthermore, because physiological adaptations of plants to salinity stress have important similarities to adaptations to water, cold, and oxidative stress (Bohnert and Jensen 1996, Munns 2001), studies of salinity tolerance can lead to improved understanding of other aspects of plant stress physiology. Therefore, a better understanding of how diverse types of plants cope with high soil salinity has potential benefits for our understanding of global change, agriculture, and plant ecophysiology. Since horsetails are morphologically (Niklas 1997) and physiologically (Hoffman and Hillson 1979) unique among vascular plants, studies of how they tolerate salinity stress represent a significant expansion of the phylogenetic breadth of salinity tolerance knowledge. Thus, a goal of this research is to investigate how *E. giganteum* has adapted to a range of groundwater salinities in South America.

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2. LITERATURE REVIEW: BIOLOGY OF *EQUISETUM* AND THE GIANT HORSETAILS

The genus *Equisetum*

The genus *Equisetum* is the only remaining representative of the once abundant and diverse subdivision Sphenophytina. The 15 living species of the genus comprise the plants commonly known as horsetails. The genus name is derived from the Latin equis, meaning horse and seta, meaning bristle, in reference to the coarse black roots of *E. fluviatile* which resemble a horse's tail (Hauke 1993). The horsetails range in size from the diminutive *E. scripoides* (stems averaging 12.9 cm tall and 0.5 - 1.0 mm diameter, Hauke 1963) to the giant horsetails, *E. giganteum* and *E. myriochaetum*, the latter reaching heights of eight or more meters and the former 5 or more meters (Hauke, 1963) and stem diameters of ~4 cm (C. Husby, unpubl. data). *Equisetum* species are vascular plants that reproduce sexually by means of gametophytes that arise from spores which are borne on cones. Hence, together with the other spore-bearing vascular plants, the Lycophytes (club mosses), Psilophytes (whisk ferns) and Pterophytes (true ferns), *Equisetum* species are classified as pteridophytes. However, recent molecular phylogenetic studies suggest that perhaps *Equisetum* should be classified within the true ferns and Psilotaceae (Pryer et al. 2001, Smith et al. 2006, Qiu et al. 2007), a conclusion also supported by spermatozoid structure (Renzaglia et al. 2002, Renzaglia et al. 2000). *Equisetum*, ferns and Psilophytes appear to be closer to seed plants (to which they form a sister group) than they are to Lycophytes, making the traditional classification of “pteridophyte” mainly one of convenience (Kranz and Huss 2005, Qiu et al. 2007).

However, enigmas remain in the placement of *Equisetum* within vascular plants, because inclusion of fossil taxa in the phylogeny creates a different picture, with *Equisetum* not nested within ferns, but still allied more closely to ferns than Lycophytes (Rothwell and Nixon 2006). Furthermore, *Equisetum* cell walls have recently been shown to contain a hemicellulose, (1 → 3, 1→ 4)-b-D-glucan, thought to be unique to the Poales, and not shared by eusporangiate ferns (its putative closest pteridophyte relative according to molecular data), *Psilotum*, or Lycophytes (Fry et al. 2008a). This hemicellulose may be linked to silica accumulation and deposition (Fry et al. 2008a). In addition, *Equisetum* contains a cell wall remodeling enzyme that it appears to share only with charophytic algae, not with other pteridophytes (even the basal most pteridophytes, the Lycophytes) (Fry et al. 2008b). In any case, these observations are intriguing evidence of the extreme isolation of the genus and its retention of ancient features, along with its many unique morphological features (see below). *Equisetum* has a history stretching back to the Cretaceous and possibly as far back as the Triassic (Hauke 1978). As a result, *Equisetum* may perhaps be the oldest living genus of vascular plants (Hauke 1963).

Morphology of *Equisetum*

All *Equisetum* species are herbaceous perennials. The plants consist of upright aerial stems which arise from a very extensive underground rhizome system (Hauke 1963). Morphologically, the genus *Equisetum* is characterized by jointed aerial stems and jointed rhizomes. The stems of horsetails are "anatomically [...] unique among plants" (Niklas 1997) although they have an external appearance somewhat reminiscent

of bamboo. The upright aerial stems branch monopodially, having one main axis of growth. This is the pattern which is also found in most gymnosperms and many angiosperms (Scagel et. al. 1984). *Equisetum* species also have small microphyllous leaves that are arranged in true whorls (Rustishauser 1999) and the leaves of each whorl are fused together to form a cylindrical sheath around each node (Hauke 1993). Some, but not all, species form whorls of lateral branches at the nodes of the aerial stems (Hauke 1993). Unlike many other vascular plants (such as gymnosperms, angiosperms and some ferns), which produce branches in the axils of leaves, the leaves of *Equisetum* alternate with branches at each node (Scagel et. al. 1984). Like other vascular plants, *Equisetum* produces new branches and leaves from the apical meristem. The aerial stems, but not the rhizomes, of some species die back seasonally, whereas other species are evergreen. The rhizomes have the same general morphology as the upright stems, although the rhizomes bear adventitious roots (i.e., roots arising from the stem rather than from other roots) at their joints in addition to leaf sheaths and branches (Hauke 1963).

Stem lengthening is produced by intercalary meristems above each node and this growth pattern produces a relatively rapid lengthening of the stem (Stewart and Rothwell 1993). The stem lengthening process is similar to that in bamboo, which also have stems that lengthen primarily via intercalary meristem growth (Judziewicz et al. 1999). The nature of stem elongation in *Equisetum* is easy to observe. In developing stems, the region of the internode close above a node is noticeably lighter green than the internode further away from the node. This color pattern appears because the internode tissue nearer the node is more recently generated by the intercalary meristem and is therefore less mature than tissue farther away. Two types of elongation meristems are found in

Equisetum rhizomes. French (1984) found that the three subgenus *Equisetum* species he studied had uninterrupted meristems "characterized by acropetal internode maturation". In contrast, the four species of subgenus *Hippochaete* that he studied had intercalary meristems in their rhizomes.

Unique characteristics of the genus *Equisetum* among vascular plants

Lateral branch origin:

Lateral branches of *Equisetum* stems arise between leaves (i.e., branches alternate with leaves). In other plants with lateral (as distinguished from terminal) branching, branches originate in leaf axils (i.e., in the vertex of the upper angle between a leaf and the stem from which it arises)

Stomatal structure:

Dayanandan (1977) observed that *Equisetum* species "possess perhaps the most structurally complex stomata in the entire plant kingdom." The stomata of equisetum are so unique that "a single well-preserved stomatal apparatus is all that is needed to identify the genus *Equisetum* (even the two subgenera) from among all other living plants" (Dayanandan, 1977). The uniqueness of *Equisetum* stomata is the result of two characteristics (Dayanandan, 1977):

- 1.) The two subsidiary cells overlie the guard cells completely, whereas in other plants the guard cells are the superficial cells.
- 2.) "The inner tangential wall of each subsidiary cell develops 7 to 24 ridge-like thickenings, a feature not found in any other genus." (Dayanandan 1977)

Elaters on spores:

Each *Equisetum* spore has four strap-like structures called elaters that are attached to the spore surface at a common point.

Silicon a required nutrient:

Among terrestrial plants, only the horsetails definitively have been shown to require silicon as an essential, not simply beneficial, mineral nutrient (Epstein 1999). Silicon is a requisite for *Equisetum arvense* (Chen and Lewin, 1969) and for *E. hyemale* (Hoffman and Hillson, 1979), so this requirement appears to hold for members of both subgenera within *Equisetum*.

Rhizome architecture:

Golub and Whetmore (1948) excavated the rhizome system of a colony of *Equisetum arvense* to a depth of 2 m and found five successive horizontal layers of rhizomes connected by vertical rhizomes. The rhizome system extended below 2 m, but the investigators did not excavate further. Such a "tiered" rhizome architecture may be unique in the plant kingdom. Indeed, other rhizomatous plants generally have but a single horizontal rhizome system layer (Bell and Tomlinson 1980).

Fossil history of the Sphenopsids

Equisetum is an ancient genus and comprises the sole surviving representatives of the class Sphenopsida (the only class of the subdivision Sphenophytina) (Scagel et al. 1984). Sphenopsids first appeared in the fossil record of the late Devonian. The earliest unequivocal sphenopsid that has been discovered is *Pseudobornia ursina*, a monopodial arborescent clonal plant of the upper Devonian which grew up to 20 m tall with stems up

to 60 cm thick (Stewart and Rothwell 1993, Scagel et al.1984). *Pseudobornia* dominated clastic streamside habitats during this time (Behrensmeyer 1992). Later, during the early Carboniferous, a greater diversity of distinctly sphenopsid plants became prominent. These Carboniferous sphenopsids are currently classified into two orders, the Sphenophyllales and the Equisetales (Stewart and Rothwell 1993). The Sphenophyllales, consisting of a single genus *Sphenophyllum*, were herbaceous plants with whorls of wedge-shaped leaves on a jointed stem. *Sphenophyllum* species increased in abundance until the Upper Carboniferous, but vanished by the end of the Permian. The Equisetales include the major families Archaeocalamitaceae, Calamitaceae, and Equisetaceae. Archaeocalamitaceae were arborescent sphenopsids which persisted from the Upper Devonian through the Lower Permian and were similar to the much more numerous Calamitaceae (Stewart and Rothwell 1993). Calamitaceae, which has a single genus, *Calamites*, encompasses the now extinct arborescent woody sphenopsids, some of which attained heights of up to 30 m and diameters of up to 30 cm (Scagel et al. 1984). Finally, Equisetaceae consists of the living genus *Equisetum* as well as other extinct herbaceous sphenopsids resembling *Equisetum*. Calamitaceae closely resembled the Equisetaceae in having rhizomatous growth, fused leaf sheaths at the nodes, and in many other respects. The chief differences between the two families lie in cone morphology and in the lack of secondary (woody) growth in the Equisetaceae in contrast to the presence of secondary growth in the Calamitaceae (Stewart and Rothwell 1993).

The Carboniferous, when 75% of the world's coal was formed (Pearson 1995), represented the peak of pteridophyte diversity and abundance (Rothwell 1996). Hence, there is rich fossil evidence for the ecology and biogeography of this period. The great

Carboniferous coal swamps were warm and humid and occupied the wet tropical low-lying areas (Pearson 1995). These swamps were dominated by giant arborescent lycophods in genera such as *Lepidodendron* (Stewart and Rothwell 1993). Sphenopsids, especially in the genera *Calamites* and *Sphenophyllum*, were common members of the Carboniferous flora. The Pennsylvanian plant assemblages are probably the best known plant assemblages of the Paleozoic, and possibly the entire pre-Cretaceous. From palynological and coal-ball analysis of Pennsylvanian floras, it is possible to gain insight into the ecology of Carboniferous sphenopsids. *Sphenophyllum* species were ground-cover plants which occurred in nearly all lowland habitats (Behrensmeyer et al. 1992). *Calamites* were hydrophytes, like *Equisetum*, and grew on loosely consolidated substrates such as sand bars, lake and stream margins, and other unstable moist substrates (Tiffney 1985). Therefore, it is probable that *Calamites* were centered outside the comparatively stable coal swamps. *Calamites* were the only Carboniferous lowland arborescent plants that had the capability for extensive vegetative propagation (Tiffney 1985). The rhizomatous growth of *Calamites*, like that of modern *Equisetum*, allowed them to form extensive colonies on disturbed wetland areas. However, *Calamites* and *Sphenophyllum* were relatively minor components of the vegetation in terms of overall biomass (Behrensmeyer et al. 1992, Tiffney 1985). Aerial stems of *Calamites* were of determinate growth, like those of modern horsetails, despite their capacity for secondary xylem formation (Eggert 1962).

During the Carboniferous, Laurasia and Gondwanaland collided and thus began the formation of the supercontinent Pangea. In the Late Carboniferous, there was widespread peat formation in the moist equatorial coal forests in what is now Europe and

central and eastern North America. Climate changes in the late Pennsylvanian and early Permian began to cause the demise of the great coal swamps. During this time, the equatorial regions of Pangea became drier and rainfall became more seasonal (Parrish 1993). The climate also became cooler with extensive glaciation in the southern hemisphere. This trend continued through the Triassic when arid to semiarid climates prevailed (Stewart and Rothwell 1993). The result was a worldwide change from hydric conditions to mesic conditions which are less favorable to sphenopsid growth. In addition, the inability of sphenopsids to grow in the increasingly dry sites probably reduced their ability to compete with the increasingly successful ferns, cycads, and conifers (Koske et al. 1985). These changes probably led to the extinction of *Calamites* during the Lower Permian and the extinction of the Sphenophyllales by the end of the Permian. These extinctions left the remaining members of the Equisetales as the only representatives of the Sphenophytina (Stewart and Rothwell 1993).

By the Mesozoic, all sphenopsids had the same basic structure as present day *Equisetum* (Behrensmeyer et al. 1992). The remaining Equisetales included the widespread *Schizoneura*, an upright herbaceous genus, with stems up to two meters tall and two cm wide (Behrensmeyer et al. 1992). It first appeared in the Carboniferous and continued into the Jurassic (Stewart and Rothwell, 1993). The large flat leaves of *Schizoneura* were a distinctive feature of this genus not commonly found in the Equisetales (Scagel et al. 1984). Another herbaceous sphenopsid that survived from the Carboniferous to the Lower Cretaceous was the genus *Phyllothea* (Stewart and Rothwell, 1993). In addition, the genus *Neocalamites*, first appeared in the Upper Permian and survived until the Lower Jurassic. *Neocalamites* resembled small *Calamites*

in gross morphology (Stewart and Rothwell 1993) with stems 10 to 30 cm thick and possibly 10 m high (Behrensmeyer et al. 1992). It was widely distributed during the later Triassic (Seward 1959). *Equisetites*, a genus which first appeared in the Carboniferous, was the other major surviving genus of sphenopsids. *Equisetites* were very similar to present day *Equisetum* and there is some controversy as to whether they may actually have been congeneric. If *Equisetites* actually were *Equisetum*, then *Equisetum* has existed since the Paleozoic and may indeed be the oldest extant vascular plant genus (Hauke 1963). However, some Triassic and Jurassic *Equisetites* were significantly larger than present day *Equisetum*, reaching 8 to 14 cm in diameter (Stewart and Rothwell 1993). Perhaps the largest *Equisetites* species, *E. arenaceus*, lived during the Upper Triassic period (Kelber and van Konijnenburg-van Cittert 1998). *Equisetites arenaceus* was a remarkable species had stems that averaged 20 cm in diameter and may have reached 4-5 m in height (Taylor et al. 2009). Stewart and Rothwell (1993) hypothesized that large *Equisetites* may have had secondary growth due to their size, but mention that there is no direct evidence for this. Seward (1898) cites indirect evidence that *E. arenaceus* had secondary growth. Some bamboos have stems approaching the diameter of *E. arenaceus*, yet lack secondary growth (Judziewicz et al. 1999). Bamboo stems are supported by extensive lignification (Judziewicz et al. 1999) and it seems possible that the large *Equisetites* likewise had lignified support tissues. Spatz et al. (1998) did not find lignification in the supporting tissues of giant *Equisetum* (likely *E. myriochaetum*) stems and Gierlinger et al. (2008) found *E. hyemale* stems free of lignin, but Speck et al. (1998) reported slight lignification in supporting tissues of the latter species. However, stable

lignin may be difficult to detect with conventional staining (J. Mauseth 2008, personal communication).

The distribution and anatomy of Mesozoic sphenopsids was consistent with primary colonization of open or disturbed moist habitats. The sphenopsids as a whole became less diverse and increasingly limited to herbaceous forms during the Triassic (Behrensmeyer et al. 1992). The trend towards an herbaceous habit was probably a result of increasingly arid conditions during the Triassic. However, the surviving order Equisetales was widely distributed and diverse during the Mesozoic. During the Jurassic, large *Equisetites* were present in nearly all parts of the world. From the Jurassic, however, *Equisetales* become smaller and less numerous (Schaffner 1930). By the beginning of the Cenozoic, only small species of *Equisetum* were extant (Stewart and Rothwell 1993). The decrease in size and abundance during the Cretaceous was probably also related to the rapid rise of angiosperms to dominance, and the resulting general decline in the prominence of pteridophytes and conifers (Schaffner 1930). However, despite this decline, during the Quaternary, *Equisetum* species were found to be widely distributed in the temperate zone (Seward 1959).

Distribution and taxonomy of *Equisetum*

Present day *Equisetum* species are naturally distributed throughout much of the world, although they are notably absent in the native flora of Australia and New Zealand (Scagel et al. 1984) and from the islands of the central Pacific, Indian Ocean, and South Atlantic islands (Schaffner 1930). However, horsetails have been introduced to Australia (Parsons and Cuthbertson 2001) and New Zealand (Roy et al. 2004) by humans. The

diversity of species increases from the equator to the temperate zone in the northern hemisphere, whereas there are only four species in the Southern Hemisphere (Hauke 1963, Hauke 1978). Such a pattern contrasts with the general trend of increasing plant species diversity nearer the equator for most taxa (Eo et al. 2008)

Extant *Equisetum* species are divided into two subgenera: *Equisetum*, with eight species and *Hippochaete* with seven species. There are several primary differences between the two subgenera. Species in subgenus *Equisetum* have stomata that are flush with the epidermal surface, whereas members of the subgenus *Hippochaete* have stomata that are sunken below the epidermal surface. The stems of the subgenus *Equisetum* are short-lived, relatively soft, and tend to be regularly branched, whereas the stems of the subgenus *Hippochaete*, with few exceptions, tend to be long-lived, hard, fibrous, and unbranched or irregularly branched (Hauke 1963, Hauke 1969a). In addition, four of the species of the subgenus *Equisetum* demonstrate stem dimorphism between non-photosynthetic, unbranched, coniferous stems and photosynthetic, branched, vegetative stems (Hauke 1978). No such dimorphism occurs in the subgenus *Hippochaete* (Hauke 1963). Although the chromosome number ($n=108$) is the same for all *Equisetum* species, the subgenus *Hippochaete* has larger chromosomes than those of subgenus *Equisetum* (Hauke 1978).

The subgenus *Hippochaete* includes the *Equisetum* species often called "scouring rushes" (although also known generally as horsetails) because of their rough, silica-impregnated epidermis. The rough siliceous stems of plants of this subgenus were used by American pioneer settlers for scouring dirty cookware and polishing wood (Scagel et al. 1984). The seven species in this group are *E. giganteum*, *E. myriochaetum*, *E.*

ramosissimum, *E. laevigatum*, *E. hyemale*, *E. variegatum* and *E. scirpoides*. The *Hippochaete* contain the two largest *Equisetum* species, *E. giganteum* and *E. myriochaetum*. With the exception of *E. laevigatum*, and some varieties of *E. ramosissimum*, all of the species in this subgenus have evergreen stems (Hauke 1963). *Hippochaete* are very widespread with species distributed over large areas of every continent, except for Australia and New Zealand. The Old World species *E. ramosissimum*, which ranges from 60° North latitude to 30° South latitude, has the widest latitudinal range of any *Equisetum* species (Schaffner 1930). The subgenus *Hippochaete*, as a whole, ranges as far north as Ellesmere Island (greater than 80° latitude) and as far south as Argentina (approximately 40° latitude) (Hauke 1963). *Equisetum giganteum* was long thought to be the most basal member of the genus, due to its size, having stomata in several lines within main stem grooves and having persistent sheath teeth, characters it shares with ancient fossil members of the genus (Hauke 1963). However, recent analyses of molecular data suggest that *E. giganteum* is not basal but is a more derived member of the *Hippochaete* (Des Marais et al. 2003, Guillon 2007).

The subgenus *Equisetum* contains the species commonly known as "horsetails." The eight species of this group are *E. arvense*, *E. pratense*, *E. sylvaticum*, *E. fluviatile*, *E. palustre*, *E. bogotense*, *E. diffusum*, and *E. telmateia*. The species in this group tend to be regularly branched and hence can resemble bushy horse-tails. Members of this subgenus have a similar latitudinal range to the *Hippochaete*. Only one species of this subgenus, the diminutive *E. bogotense* of Central and South America, has a range that extends to the Southern Hemisphere. The other seven species of this group are found in the Northern Hemisphere (Hauke 1963). Most species of subgenus *Equisetum* are temperate,

with a few extending their ranges into the subtropics and only *E. bogotense* ranging into the tropics. The aerial stems of all of these species, except for *E. bogotense* and *E. diffusum*, (the species from the warmest climates), are annual (Hauke 1978).

Ecology, physiology and reproductive biology of *Equisetum*

Equisetum species grow in wet places such as moist woods, ditches, wetlands, and in road fill where sufficient groundwater is available. Rhizomatous clonal growth is a universal feature of the genus and is very important in its ecology and its ability to utilize ground water. A single rhizome system may cover many square meters (Hauke 1963). The rhizomes can penetrate to soil depths of four meters in some circumstances (Page 1997). Deep rhizome growth gives the plants the ability to survive environmental disturbances such as plowing, burial, fire and drought. The extensive rhizome system also allows the *Equisetum* plants to supply themselves with water and mineral nutrients from deep underground and hence allows them to grow in habitats, such as road fill, which appear dry on the surface (Hauke 1966).

Sexual reproduction

As in other pteridophytes, sexual dispersal in *Equisetum* occurs by means of spores and gametophytes. *Equisetum* spores are green, spherical, and have thin spore walls (Hauke 1963). Each *Equisetum* spore has four unique strap-like structures called elaters attached to the spore surface at a common point. These elaters are hygroscopic (i.e., they expand and contract with changes in humidity) and probably function to help disperse the spores (Hauke 1963). *Equisetum* spores are short-lived and can germinate within 24 hours of release from the cone. After 5-17 days, depending on humidity, they

are no longer viable (Hauke 1963). In non-tropical species (the majority of *Equisetum*), the spores are produced over a short period of time during the growing season (Duckett 1985). *Equisetum* gametophytes appear to require a substrate of recently exposed bare mud to become established (Duckett and Duckett 1980). Like pioneer species, they rapidly attain sexual maturity and are adversely affected by competition from bryophytes and vascular plants (Duckett and Duckett 1980, Duckett 1985). The resulting inefficiency of spore germination and gametophyte reproduction in non-pioneer situations probably limits gene flow and leads to the high degree of genetic divergence researchers have found among *Equisetum* populations (Korpelainen and Kolkkala 1996). Therefore, sexual reproduction in *Equisetum* is limited to rather narrow ecological conditions and this limits the establishment of *Equisetum* via spores.

The uniform chromosome number throughout the genus facilitates hybridization between *Equisetum* species (Scagel et al. 1984). Hybridization is also favored by the relatively narrow ecological requirements of gametophytes which encourages the formation of mixed populations of gametophytes on suitable sites (Hauke 1978). These mixed populations increase the probability of cross fertilization between gametophytes of different, but compatible, species. In areas where environmental conditions are especially conducive to spore germination and gametophyte establishment, *Equisetum* hybrids are particularly frequent and widespread. In Britain and Ireland, for example, *Equisetum* hybrids are particularly successful (Page 1985). This success appears to be due primarily to the moist temperate oceanic climate and relatively low competition from other plants, conditions which favor both gametophyte and sporophyte generations of *Equisetum* (Page 1985). *Equisetum* hybridization is especially frequent within the

subgenus *Hippochaete* where five common hybrids are known. Within the subgenus *Equisetum*, there is only one common hybrid (Hauke 1978). There are many more known hybrids within each subgenus, but these hybrids tend to be much less common (Hauke 1978). DNA fingerprinting has recently proven to be a reliable method for identifying *Equisetum* hybrids and for determining the degree of sexual versus asexual reproduction in parental species populations (Brune et al. 2008). No hybrids between the two subgenera have yet been reported and this adds further evidence that the two subgenera are naturally distinct (Krahulec et al. 1996). Recent molecular phylogenetic studies have provided further evidence for the distinctness of the subgenera, although the position of *Equisetum bogotense* within the genus remains ambiguous (Guillon 2007, Des Marais et al. 2003).

Vegetative reproduction

Equisetum species have a remarkable ability to reproduce vegetatively. Vegetative reproduction helps to compensate for the inefficiency of spore reproduction. An extensive rhizome system allows *Equisetum* species to rapidly colonize disturbed areas (Hauke 1963). The ability to colonize gives *Equisetum* a distinct advantage over species requiring seed establishment or which have slow-growing rhizomes (Hauke 1969a). For instance, the widespread creation of roadside ditches in America has created significant new habitat for some *Equisetum* species. Roadsides constitute new habitats because the soil in ditches tends to be moist and the rhizomatous growth of *Equisetum* species allows them to survive and thrive under the conditions of sediment accumulation that are characteristic of ditches (Rutz and Farrar 1984). The remarkable ability of *Equisetum* to

survive and spread in areas of heavy sediment accumulation was also demonstrated after the 1912 eruption of Katmai Volcano in Alaska. In studies of vegetational recovery from the volcanic tephra (ash and silt) deposited by this eruption, *E. arvense* was found to be the most successful herb. It was able to penetrate as much as one meter of tephra, more than any other herbaceous species, and colonize large areas via rapid rhizomatous growth (Bilderback 1987). The remarkable ability of *Equisetum* to prosper under disturbed conditions was also demonstrated after the eruption of Mount St. Helens in 1980 when *Equisetum* formed almost monotypic stands in the newly deposited tephra (Rothwell 1996). The deep rhizome system of *Equisetum* also allows the plants to survive fire and rapidly recolonize burned-over sites (Beasleigh and Yarranton 1974). It is probable that the vigorous and extensive rhizomatous habit of *Equisetum* has been very important to the long term survival and spread of the genus (Hauke 1969a).

Fragmentation of rhizomes and stems allows *Equisetum* to disperse readily in suitable habitats where there is sufficient moisture. Even the aerial stem fragments can sprout and form new colonies (Wagner and Hammitt 1970, Schaffner 1931, Praeger 1934). Some members of the subgenus *Equisetum* (e.g. *E. arvense* and *E. palustre*) also reproduce vegetatively via tubers produced on the rhizomes (Hauke 1978). These tubers can contribute substantially to vegetative spread through soil disturbance (Marshall 1986, Sakamaki and Ino 2006). Hence, vegetative reproduction allows *Equisetum* clones to persist and spread even in the absence of sexual reproduction (Hauke 1963).

Vegetative reproduction most likely accounts for the widespread occurrence and persistence of common *Equisetum* hybrids (most of which are sterile) even where one or both of the parents are absent. The rhizome system of a vigorous hybrid clone

theoretically has the ability to maintain dense colonies within limited areas for long periods. Fragmentation and transport of rhizomes and stems then has the potential to disperse the clone from the site of the original hybridization, accounting for the abundance of *Equisetum* hybrids even if hybridization is a relatively uncommon occurrence (Hauke 1963).

Adventitious rooting as an adaptation to disturbance

All equiseta have preformed bud and root primordia at each node of both the aerial stems and underground rhizomes (Gifford and Foster 1988). *Equisetum* stems can quickly put forth new roots and shoots on aerial stems when the stems are partly or wholly buried in sediment. Hence, even if the deeper parts of a stem or rhizome become crushed or smothered by sediment, the upper parts may be able to survive and reestablish the clone (Gastaldo 1992). Clonal reproduction is clearly advantageous for enhancing survival of *Equisetum* species in the wake of disturbance events in riparian and other wetland habitats. The preformed primordia can also facilitate vegetative propagation and dispersal via stem pieces (Wagner and Hammitt 1970, Hauke 1963). Schaffner (1931) and Praeger (1934) utilized the adventitious rooting capabilities of *Equisetum* stems to successfully propagate many species from aerial stem cuttings.

Gastaldo (1992) gives evidence for similar stem regeneration abilities in the large extinct *Equisetum* relative *Calamites* and discusses the ecological importance of these abilities. Similarly, Kelber et al. (1998) found that the extinct close relative of extant horsetails, *Equisetites arenaceus*, could propagate vegetatively via the adventitious rooting of shed branches.

Adaptations to waterlogged soil and anaerobic conditions

Like nearly all organisms, plants require oxygen (O₂) for efficient cellular respiration. Plants that grow in water-saturated soil often have to cope with anoxic conditions around their underground organs (Blom and Voesenek 1995). O₂ diffuses 10,000 times more slowly through liquid water than through air, creating anoxia (Grable 1966). Under waterlogged conditions, cellular respiration by plant roots and soil microorganisms often quickly depletes the available O₂, leading to anoxic soil conditions (Drew and Lynch 1980, Kludze and DeLaune 1995). Anoxic conditions lead to a large decrease in plant nutrient availability (Ernst 1990) and to buildup of phytotoxins produced by anaerobic soil microbes or by anaerobic respiration in plant roots (Koch and Mendelssohn 1989).

To deal with anoxic conditions, wetland plants have several morphological and physiological adaptations to maintain aerobic respiration by facilitating transport of O₂ from the atmosphere to underground and underwater organs (Allen 1997). In many wetland plants, gas spaces (lacunae) in specialized tissue (called aerenchyma) provide pathways for O₂ and carbon dioxide (CO₂) to move from one part of the plant to another much more quickly than would be possible through tissue without lacunae (Allen 1977). Rhizomes and stems of wetland *Equisetum* species, including the giant horsetails, have large canals that are thought to function like aerenchyma tissue in facilitating O₂ transport (Hauke 1963, Hyvonen et al. 1998). Oxygen movement through aerenchyma occurs either by diffusion alone or by diffusion combined with convection (Allen 1997). These mechanisms and their relative effectiveness have many important implications both for wetland ecology and for the growth and productivity of crop plants, such as rice (*Oryza*

sativa L.), that typically grow in waterlogged soils (Wassmann and Aulakh 2000, Allen 1997). The most efficient known mechanism for oxygen transport to submerged plant parts is via pressurized convection (Allen 1997).

Up to this time, studies of pressurized O₂ transport in wetland plants have focused exclusively on angiosperms, with the exception of one study that investigated the gymnosperm *Taxodium distichum* L. (Grosse et al. 1992). However, the rhizomes of *Equisetum* species often concentrate more deeply than the roots and rhizomes of accompanying vegetation (Borg 1971). Marsh et al. (2000) found that, in an Alaska wetland, *Equisetum* rhizomes occurred in the deeper C soil horizon whereas the roots and rhizomes of other species were concentrated in the surface O horizon. The especially deep penetration of waterlogged sediments by *Equisetum* rhizomes suggests the existence of efficient mechanisms for rhizome aeration.

The only studies of gas transport in *Equisetum* to date have dealt with *E. fluviatile*, a species that frequently grows as an emergent aquatic plant (Hauke 1978) and one of the most anaerobiosis tolerant *Equisetum* species (at least in the temperate zone) (Page 2002). Barber (1961) earlier found a diffusion gradient from high concentrations of O₂ and low concentrations of CO₂ in aerial stems to the reverse condition in submerged rhizomes. In addition, diffusion along excised aerial stems was relatively efficient. However, Barber's study did not provide information that would indicate whether or not a pressurized ventilation mechanism might be active in *E. fluviatile*. A study by Hyvonen et al. (1998) of methane release from an *E. fluviatile* stand suggested that this species does not have a pressurized ventilation flow system because there was not a discernable diurnal pattern of methane efflux. Similarly, Strand (2002) found that

E. fluviatile had a "low or non-detectable" air flow rate in its stems, yet was found in "unexpectedly deep water".

Page (2002) mentioned that *Equisetum* species in the British Isles vary in their tolerance of anaerobic soil water conditions. *Equisetum fluviatile* appears able to tolerate the greatest degree of hypoxia in soil water whereas *E. telmateia* is least tolerant of anaerobic soil water and occupies sites with continually flowing groundwater.

Soil preferences and nutrient cycling

In Cuba *Equisetum giganteum* is associated with mineral rich, acidic, alluvial soils (Alvarez de Zayas 1982). Correspondingly, recent experience with *Equisetum* in cultivation suggests that they have a high requirement for some micronutrients (C. Husby, unpublished observation). *Equisetum* tissue is frequently observed to be rich in the minerals phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and silicon (Si) (Auclair 1979, Saint Paul 1979, Thomas and Prevett 1982, Pulliainen and Tunkkari 1991, Marsh et al. 2000). Andersson (1999a, 1999b) showed experimentally that *E. arvense* has a high K demand under high light conditions and was able to tolerate lower N levels (although its inability to respond much to increased N availability renders it vulnerable to being outcompeted by faster growing plants). Furthermore, certain *Equisetum* species have been shown to be highly nutritious for wildlife. For example, young stems of *E. fluviatile* can contain more than 20% protein along with sufficiently high levels of P, K, Ca and Mg to meet the mineral needs of breeding geese and their young (Thomas and Prevett 1982). In some areas, *Equisetum* species are an important part of the diet of black bears (Machutchon 1989), voles (Jean and Bergeron 1986,

Holisova 1976), rock ptarmigan (Emison and White 1988), young trumpeter swans (Grant et al. 1994) and fish (Brabrand 1985). Hauke (1969a) observed that cattle in Costa Rica appear to relish giant horsetails and at least one rancher Hauke met believed that his cattle benefited from eating it.

Members of the genus *Equisetum* have the ability to extend their rhizomes deeply into saturated soil (Marsh et al. 2000, Borg 1971). The rhizome system has generally been found to comprise most plant biomass (Borg 1971, Marshall 1986). The ability of *Equisetum* rhizomes to penetrate deeply into wetland soils plays an important role in their recently discovered role as nutrient pumps. In an Alaskan shrub wetland, Marsh et al. (2000) found that *Equisetum* species can acquire and accumulate substantial amounts of P, K, and Ca from lower soil layers and transport these nutrients to the surface where they are available to other plants. Remarkably, these investigators found that although *Equisetum* species made up only 5% of the total biomass of the wetland community, the *Equisetum* tissues had 16% of the total phosphorus and 24% of the total potassium (Marsh et al. 2000). Furthermore, *Equisetum* species contributed disproportionately to soil nutrient inputs in the shrub wetland. During the two-year study period, *Equisetum* litter provided 75% of the calcium, 55% of the phosphorus, and 41% of the K input to the soil (Marsh et al. 2000). The nutrient pumping of *Equisetum* species in the shrub wetland probably contributed to the unusually high primary productivity of the ecosystem (Marsh et al. 2000). Nutrient pumping function of the shrub wetland *Equisetum* species appears to be at least partly the result of the ability of *Equisetum* rhizomes and roots to penetrate more deeply into the soil than roots and rhizomes of other wetland plants.

Silicon requirement

A remarkable characteristic of *Equisetum* species is their ability to take up and accumulate silicon in their tissues. Silica accumulates on the epidermis of the plants, giving the epidermis a rough texture (Sapei et al. 2007, Parsons and Cuthbertson 2001). Research on the protective value of silica seems to indicate that silica solutions when applied to plants can provide effective protection from fungal diseases and from insect attack (Epstein 1999). The protective effect of silica would explain why gardeners have long used horsetail extract to protect plants against pathogens and predators (Quarles 1995). The outer layer of silica on *Equisetum* stems may help explain why horsetails appear to be little bothered by insect feeding or fungal diseases (Hauke 1969a, Kaufman et al. 1971). The outer layer may also help reduce water loss through the epidermis (Kaufman et al. 1971).

Timell (1964, cited in Kaufman et al. 1973) found that silicic acid content of *E. palustre* could reach 25.3% of dry weight. An important function of cell wall silica in *Equisetum* is in maintenance of shoot erectness (Kaufman et al. 1971) as an alternative to lignin (Siegel 1968, cited in Kaufman et al. 1973). Furthermore, silica content of stems appears to be directly associated with stem longevity (Srinivasan et al. 1979). Horsetails incorporate much silicon into their stem tissues and external ridges, knobs, and rosettes of silicon give the stems of many species their rough and abrasive character (Gifford and Foster 1989, Hauke 1963). People have taken advantage of this abrasive quality by using *Equisetum* stems for washing dishes (hence the common names 'scouring rush' and 'limpiaplata'), polishing woodwind reeds, and polishing silver (hence the name 'yerba del platero' in Argentina).

Mycorrhizae and root hairs

To date, there has been no clear evidence that *Equisetum* species are mycorrhizal (either in the gametophyte or the sporophyte stage) and most studies have found essentially no mycorrhizal colonization of horsetails (Read et al. 2000). Although Koske et al. (1985) found fungal structures in roots of *Equisetum* species growing in a sand dune habitat, the close association of the *Equisetum* roots with roots of characteristically mycotrophic plants raises the possibility that the observed fungal structures represented "simply the penetration [of *Equisetum* roots by] a 'non-host'" (Read et al. 2000). Hence, the role of mycorrhizae in *Equisetum* ecology remains controversial. Overall, however, *Equisetum* species clearly appear to do quite well in many situations without mycorrhizal associations (Read et al. 2000). For example, Marsh et al. (2000) found no mycorrhizal colonization of *Equisetum* roots in the Alaskan shrub wetland they studied. Although enhanced phosphorus acquisition is often a major contribution of mycorrhizal associations to plant nutrition (Orcutt and Nilsen 2000), the mycorrhizae-free *Equisetum* species studied by Marsh et al. (2000) absorbed soil nutrients, including phosphorus, very effectively. Recently Fernández et al. (2008) found the basal-most horsetail, *E. bogotense*, to be facultatively mycorrhizal in Argentina.

Schaffner (1938) and Page (2002) have observed that *Equisetum* species have exceptionally long root hairs and Page (2002) has noted that these hairs are 'unusually persistent,' at least in water culture. Page (2000) hypothesized that these root hairs may enhance the absorptive capacity of *Equisetum* roots in a manner similar to mycorrhizae. Marsh et al. (2000) noted the presence of root hairs on *Equisetum* roots in the O horizon but not on roots in the C horizon of the Alaskan shrub wetland they studied. The

investigators hypothesized that nutrient concentrations were lower in the O horizon, necessitating greater roots surface area for absorption, whereas nutrient concentrations were high enough in the C horizon to inhibit formation of root hairs.

Nitrogen fixation

Uchino et al. (1984) found evidence for high rates of nitrogen fixation activity (attributed to several strains of Enterobacteriaceae) in association with rhizomes and roots of several temperate *Equisetum* species (two from each subgenus) through measurements of acetylene reduction activity. These investigators hypothesized that association of *Equisetum* species with nitrogen-fixing bacteria may help horsetails survive in the nitrogen-limited habitats where they frequently grow.

Allelopathy

Equisetum species, like many angiosperms, appear to exhibit allelopathy. Milton and Duckett (1985) found that sporophytes of *E. sylvaticum* inhibit gametophyte development of that species. Furthermore, the same investigators found that water extracts from several *Equisetum* species reduced the germination of grass seedlings. Two of the three species studied were members of the subgenus *Equisetum* (*E. arvense* and *E. palustre*) and one was a member of the subgenus *Hippochaete* (*E. variegatum*). The inhibitory effects of the members of the subgenus *Equisetum* were greater than that of *E. variegatum*. Thus, members of the subgenus *Hippochaete*, and hence the giant equiseta, may be less allelopathic than members of the subgenus *Equisetum*.

Hydathodes and guttation

Equisetum species, like many other plants, have hydathodes (Johnson 1936). In *Equisetum*, these are structures that are associated with veins on the leaf or sheath (Johnson 1936) and serve as exit routes for xylem water when there is positive hydrostatic pressure (called root pressure) in the xylem (Nobel 1991). The exit of this xylem water, termed guttation, results in the formation of small droplets in the vicinity of the hydathodes. Guttation occurs when transpiration is nil, such as under very high relative humidity conditions or at night (Nobel 1999). Guttation may serve to prevent flooding of mesophyll tissue in leaves (Johnson 1936). Johnson (1936) studied the anatomy of hydathodes in many *Equisetum* species and noted that the hydathodes of *E. giganteum* are "confined to the leaf and sheath bases."

The giant horsetails

Like all equiseta, the giant horsetails spread vegetatively via extensive rhizome systems, often forming large clones. The rhizomes give rise to erect, determinate, aerial stems that have regular whorls of side branches, giving the stems a remarkably precise radial symmetry. Colonies of such stems have an ancient appearance, as the 19th century botanist Richard Spruce (1908) remarked upon seeing a grove of giant horsetails for the first time:

"But the most remarkable plant in the forest of Canelos is a gigantic *Equisetum*, 20 feet high, and the stem nearly as thick as the wrist!...It extends for a distance of a mile on a plain bordering the Pastasa, but elevated some 200 feet above it, where at every few steps one sinks over

the knees in black, white, and red mud. A wood of young larches may give you an idea of its appearance. I have never seen anything which so much astonished me. I could almost fancy myself in some primeval forest of *Calamites*, and if some gigantic Saurian had suddenly appeared, crushing its way among the succulent stems, my surprise could hardly have been increased. I could find no fruit, so that whether it be terminal, as in *E. giganteum*, or radical, as in *E. fluviatile*, is still doubtful, and for this reason I took no specimens at the time, though I shall make a point of gathering it in any state." (Spruce 1908)

Giant horsetails inhabit elevations between 150 and 3000 meters and their distributions tend to follow mountain ranges in the tropics (but not at the southern end of the range of *E. giganteum*, which reaches warm temperate southern latitudes). Like other *Equisetum* species, giant horsetails grow in areas with ample groundwater supply, often along rivers and in wetlands (Hauke 1963, 1969a).

Although giant horsetails are of considerable botanical interest, relatively little is known about these unusual plants beyond their taxonomy and anatomy. Indeed, the only two published papers that have dealt specifically with giant horsetail ecology were limited to some qualitative (but intriguing) observations on their natural history (Hauke 1969a, Álvarez de Zayas 1982). One study has investigated the biomechanics of aerial stems in *E. giganteum* (Spatz et al. 1998).

The most extensively studied aspect of the giant horsetails has been their medicinal properties. The medicinal use of giant horsetails has a history that reaches

back to the pre-conquest Inca of Peru (Tryon 1959) and the plants are currently used in medicine (often as diuretics, but also for many other medicinal purposes) throughout Latin America (Morton 1981, Hauke 1967, Murillo 1983, Gorzalczany et al.1999). Investigators using animal models have found that giant horsetail extracts have diuretic (Gutiérrez et al. 1985) and hypoglycemic (Cetto et al. 2000) effects and have "nerve growth factor (NGF)-potentiating activity" (Li et al. 1999). Furthermore, a controlled study by Revilla et al. (2002) showed that traditionally prepared *Equisetum myriochaetum* extract had significant hypoglycemic effect (not resulting from increased insulin secretion) in type 2 diabetics. Téllez et al. (2007) found that a phytotherapeutic extract of *E. myriochaetum* is not genotoxic.

Other horsetails also exhibit medicinal properties, so this group as a whole appears to have considerable bioactivity, which likely suggests that giant horsetails also have additional medicinal properties yet to be discovered. For example, anti-tumor peptides have been found in *E. arvense* (Tepkeeva et al 2008), antiulcerogenic compounds in *E. palustre* (Gurbuz et al. 2009) and antioxidant activity in several species (Stanjer et al. 2008).

Taxonomy of the giant horsetails

Plant taxonomists currently recognize two species of giant horsetail and a hybrid between them: *Equisetum giganteum* L., *E. myriochaetum* Schlecht. and Cham., and *E. x schaffneri* Milde (*E. giganteum* x *E. myriochaetum*) (Hauke 1963). Prior to Hauke's (1963) work, taxonomists recognized a relatively large number of species and varieties of giant horsetails. For example, Milde, in his 1867 monograph of *Equisetum*, recognized

seven species of tropical giant horsetails. Personal observations of morphology during this study (Husby, unpublished) suggest that at least one of the additional taxa of Milde (1867) should be recognized.

The giant horsetails are among the least studied of the 15 species in the genus. All of the giant horsetails are members of the subgenus *Hippochaete* within the genus *Equisetum*. The subgenus *Hippochaete* is characterized by species having sunken stomata (in contrast to the raised stomata of subg. *Equisetum*). Most of the seven members of the subgenus *Hippochaete*, including the giant horsetails and the familiar temperate "scouring rush" *E. hyemale*, have tough evergreen stems. However, the giant horsetails are the only *Equisetum* species that have stems that are both evergreen and regularly (i.e., radially symmetrically) branched. Furthermore, the regular branching habit of the giant horsetails is unique in the subgenus *Hippochaete* (Hauke 1963, 1978).

Because the three giant horsetails appear similar in overall habit, and because *Equisetum* species exhibit considerable morphological plasticity (Hauke 1963, Schaffner 1928), more stable anatomical characters are used to distinguish between the species (Table 2.1). The most important diagnostic characters (branch ridge patterns, stomatal patterns, and endodermal patterns) can only be observed under high magnification and many of characters of *E. x schaffneri* overlap with its parent species (Hauke 1963). Therefore, accurate identification of giant horsetails can be problematic. As a result, both dried specimens in herbaria (Stolze 1983, Husby, personal observation) and living specimens in botanical gardens (Moyroud 1991, Husby, personal observation) are often misidentified.

Misidentification of herbarium specimens and living plants

Historically, there appears to have been a tendency to label most large, regularly branched horsetails from Latin America as *Equisetum giganteum*. I have seen many giant horsetail herbarium specimens, originally identified as *E. giganteum*, that were later annotated as *E. myriochaetum* (and sometimes *E. x shaffneri*). An example of this is an herbarium specimen of *E. myriochaetum* from Guatemala that was labeled *E. giganteum* until Dr. Richard Hauke determined the specimen to be *E. myriochaetum*. Stolze (1983) also mentioned the frequent mislabeling of giant *Equisetum* herbarium specimens. The problem of mislabeling is largely due to the fact that the giant horsetails look quite similar in overall habit and the diagnostic characters that distinguish them are mostly microscopic (see discussion of taxonomy) (Hauke 1963).

A similar tendency to identify all giant horsetails as *E. giganteum* appears to hold sway for living accessions in many botanical gardens. For example, the two clones labeled *Equisetum giganteum* that Moyroud (1991) obtained from botanical gardens (one in garden the U.S. and one in Europe) were both later determined to be *E. myriochaetum* (R. Moyroud, 2001, personal communication). *Equisetum myriochaetum* appears to be by far the most common species in cultivation in the temperate northern hemisphere. Many of the plants labeled *E. giganteum* in U.S. botanical gardens appear to be divisions of a clone of *E. myriochaetum* that was collected in Ecuador in 1987 and brought to the University of California Botanic Garden. Until recently, this clone was labeled as *E. giganteum*. In early 2001, Dr. Alan R. Smith determined that the clone is *E. myriochaetum* (Holly Forbes, 2002, personal communication). Furthermore, after receiving a division of a clone labeled *E. giganteum* (originally collected in Peru) from

the Royal Botanic Gardens Edinburgh I determined that this clone is most likely *E. x schaffneri* (the plant has stomata in bands of 1-2 and irregular branch ridge tubercles). Confusion regarding the identities of cultivated plants has led to uncertainty about the implications of the results of scientific studies utilizing those plants. For example, Duckett and Pang's (1984) study of sex determination in *E. giganteum* gametophytes utilized spores from a mislabeled specimen of *E. myriochaetum* (Hauke 1985) calling into question the conclusions of the former study.

Ecology of the giant horsetails

Until the current study, there has been little direct knowledge of the ecology and physiology of the giant horsetails. Giant horsetails are pioneer (early-successional) obligate wetland plants and are poor competitors (Hauke 1969a, B. Øllgaard 2000, personal communication). Abundance of groundwater supply and lack of competition are key habitat requirements of these plants. Hence, they are often associated with rivers and alluvial soils (Hauke 1969a, Álvarez de Zayas 1982). The limitation of giant horsetails to higher altitudes in the tropics is probably the result of their poor competitive abilities and their inability to tolerate shade. Hauke (1969a) observed that the giant horsetails stop producing cones when shaded by other vegetation and are subsequently displaced by other plants. Hence, giant horsetails do not tend to persist in a given site unless the disturbance regime or other factor prevents shading-out of colonies. The lower competitive pressure in the cooler high altitudes combined with increased light intensity may allow the horsetails to "hold their own" against other vegetation (Hauke 1969a). Competition pressure may explain why the genus is absent from the lowland Amazon

basin where temperatures are warm and plant competition is especially intense. However, *E. giganteum* grows down to nearly sea level in northern and central Chile where competition is much less (C. Husby, personal observation). Hauke (1969a) described several cases wherein giant horsetail colonies in Costa Rica had disappeared, presumably due to the process of succession at once-suitable sites. Dr. Benjamin Øllgaard (2000, personal communication) has observed that giant horsetails are frequent pioneers on land and mud slides in the valleys of the Río Pilaton and Río Pastaza in Ecuador. They are also common on seepage slopes along Andean roadcuts (Bennett, personal communication 2009). Professor Øllgaard suspects that these pioneer stands can probably persist until sufficient forest regeneration occurs to shade out the horsetails (after ~25-50 years).

The giant horsetails, like other *Equisetum* species, develop extensive underground rhizome systems. Unfortunately, there has been no study of the rhizome architecture of the giant horsetails. However, it is known that the rhizomes of *E. telmateia* (the largest member of the subgenus *Equisetum*) can extend more than 4 m deep into wet clay soil (Page 1997). Anthony Huxley reported in the book "Plant and Planet" (1975, p. 243) that "field bindweed is recorded at a depth of 7 meters and horsetails in light soil two or three times as deep again", suggesting that *Equisetum* rhizomes may penetrate to the extraordinary depth of 21 m in certain situations! Unfortunately, Huxley did not mention his source for this report or the species and location involved, so it would be proper to remain skeptical of this claim.

Rhizome segments that are exposed by erosion and broken-off can be carried downstream to establish new clones (Hauke 1969a). *Equisetum* species generally invest

a large proportion of resources in rhizome growth. Borg (1971) found that *E. palustre* may produce more than 100 times more rhizome biomass than aerial stem biomass. *Equisetum arvense* also allocates the larger proportion of its dry matter to rhizomes and tubers (though not to such an extreme extent as *E. palustre*) (Marshall 1986). The large pool of resources stored in *Equisetum* rhizomes facilitates aerial shoot regeneration if a disturbance destroys the aboveground stems. Hence, this growth strategy is adaptive for the types of disturbance-prone habitats favored by many equiseta, including giant equiseta. There have been no studies of the ratio of above to below ground biomass allocation in giant horsetails. It would also be interesting to know whether the two subgenera of the genus *Equisetum* differ overall in their biomass allocation patterns.

The distribution and ecology of the giant *Equisetum* species of the American tropics, *E. giganteum* and *E. myriochaetum*, and *E. x schaffneri* provides an interesting case study in the importance of vegetative persistence of hybrids in the genus. These three species are largely confined to the upper elevations between 150 and 3000 meters. It is the most widespread horsetail in Latin America, ranging from Guatemala to Brazil, Argentina and Chile as well as Hispaniola, Jamaica and Cuba (Hauke 1969a, Hauke 1963). *Equisetum myriochaetum* has a more limited range and is distributed from southern Mexico to Peru (Hauke 1963). There is also a widespread hybrid, *E. x schaffneri*, between these two giant horsetails which ranges from Mexico to Peru (Hauke 1963). Although *E. x schaffneri* is sterile, it persists via vegetative reproduction and may form large colonies (Hauke 1967). The hybrid is found throughout the region of overlap between its parent species, but it is also found in Mexico, where *E. giganteum* is not known to occur, and in Venezuela, where *E. myriochaetum* is not known to occur. The

unexpectedly extensive distribution may be the result of vegetative dispersal or to the production of an occasional, rare, viable spore (Hauke 1963). Viable spores have been observed for other *Equisetum* subgenus *Hippochaete* hybrids (Krahulec et al. 1996), so the hypothesis appears plausible. *Equisetum x schaffneri* once again demonstrates the remarkable frequency and persistence of *Equisetum* hybrids.

Tables

	<i>E. giganteum</i>	<i>E. myriochaetum</i>	<i>E. x schaffneri</i>
Type locality	Jamaica (or Hispaniola ¹)	Vera Cruz, Mexico	Orizaba, Mexico
Distribution ²	Cuba, Jamaica, Hispaniola, Central American (Guatemala, El Salvador, Honduras, Nicaragua (Luis Diego Gómez, 1985), Costa Rica, Panama), and South America (Venezuela, Colombia, Ecuador, Peru, Bolivia, Brazil, Paraguay, Uruguay, Chile, and Argentina)	Mexico, Central America, Colombia, Ecuador, and Peru	Mexico ³ , Central America, Colombia, Venezuela ³ , Ecuador, Peru
Habitat	"...along rivers or in swampy places, usually shaded"	"In swampy places or along rivers and streams, usually at the edge of or within forested areas..."	"...in springy, marshy places, or along rivers and streams."
Altitudinal range	150 - 2600 m	200 - 3000 m	500 - 3000 m

Maximum stem height ⁴	5 m	8 m	4.5 m
Maximum stem diameter ⁴	2.4 cm	1.8 cm	2.2 cm
Branch ridge pattern	"...square or flattened in profile, in Brazil, Peru, and southern South America tending to be irregular."	sawtooth pattern oriented apically	"...sawtooth to irregular."
Main stem stomatal pattern	"...in bands of 3-4 (rarely 2-3 or 4-5)"	"Stomata in one line on each side of the groove."	"...in bands of 1-2 (occasionally 2-3)"
Endodermal arrangement	"Cross section with separate endodermis around each vascular bundle (individual endodermises)"	"...double common..."	individual endodermises

Sheath teeth persistence	"mostly persistent" (Tryon, R. M. and Tryon, A. F., 1982)	"...usually thin and brown to white, drying and breaking off to produce a clipped appearance at the top of the sheath, or (especially in South America), the bases or much of the teeth persisting"	"...mostly smoothly shed."
Cones	have a "short (about 0.5 mm) but distinct apiculum"	"blunt, or the branch cones frequently with a slight apiculum"	"acute or with slight apiculum"

Table 2.1 Morphological characters distinguishing giant *Equisetum* species. Compiled from Dr. Richard L. Hauke's (1963) monograph of *Equisetum* subgenus *Hippochaete*, unless otherwise referenced. Dr. Hauke's work was based upon intensive study and analysis of a large number of herbarium specimens.

¹Proctor (1985) and Lellinger (1989) gave the type locality as "presumably...Hispaniola", whereas Hauke (1963) gave the locality as Jamaica.

²Only two other *Equisetum* species reach the tropics in Latin America, *E. hyemale* (which reaches Guatemala) and *E. bogotense* (From Costa Rica to Chile and Argentina) (Moran and Riba, 1995 ; Stolze, 1983).

³Interestingly, this hybrid is found in Mexico, where its parent, *E. giganetum*, is not known to be present, and in Venezuela, where its other parent *E. myriochaetum* is not

known to be present. Hauke (1963) hypothesized that this unexpected phenomenon may be due to occasional viable spores being produced by *E. x schaffneri* and the resulting plants persisting vegetatively. Viable spores have been observed for other *Equisetum* subg. *Hippochaete* hybrids (Krahulec et al., 1996), so this hypothesis appears plausible. Rolla M. Tryon and Alice F. Tryon (1982) have suggested that *E. x schaffneri* may not be a hybrid at all, but rather an intermediate form of a single polymorphic horsetail species *E. giganteum* (with the currently recognized *E. giganteum* and *E. myriochaetum* representing extremes of this species). However, Hauke (1963) notes in support of *E. x schaffneri*'s hybrid status that its spores are not viable in contrast to those of the other two species. The status of *E. x schaffneri* could probably be settled by attempting to synthesize *E. x schaffneri* from a cross of *E. giganteum* and *E. myriochaetum* in the laboratory, as has been done for other subgenus *Hippochaete* hybrids (Duckett 1979). Molecular taxonomic analysis would likely shed additional light on this question.

⁴These numbers are based on collector's notes and measurements of herbarium specimens

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3. BIOMECHANICS OF *EQUISETUM GIGANTEUM* L. STEMS FROM
CONTRASTING ENVIRONMENTS IN SOUTH AMERICA

Abstract

Equisetum giganteum L., a giant horsetail, is one of the largest living members of an ancient group of non-flowering plants. Its hollow upright stems, reinforced by nodal thickenings and septa, can grow to over 5 m in height. However, the relatively small diameters of *E. giganteum* stems (less than 4 cm) pose a challenge to their mechanical stability against buckling. I measured the bending properties of *E. giganteum* stems growing under contrasting environmental conditions in South America, isolated river valleys in extremely arid Atacama Desert in Chile and humid environments in northwestern Argentina, and in greenhouse cultivation in Miami, Florida. Despite very different growth environments and different outer stem morphology, the average Young's moduli of elasticity (E) of plants in Chile and Argentina were similar (4.29 and 3.96 GPa, respectively). The log-log linear relationships between the second moment of area (I) and flexural stiffness (EI) are consistent with an earlier characterization of *E. giganteum* as having semi-self-supporting habit, relying on synergistic support among neighboring stems to prevent buckling. I also found a lack of relationship between I and E that is consistent with this growth habit. The similar scaling relationships between stem diameter and stem wall thickness for the Chilean and Argentinian plants suggests that spatial distribution of stem tissue is little affected by the different environments of these regions. However, the values of E found in this study were nearly three times higher than the values measured in a prior study of a greenhouse-grown giant horsetail clone,

suggesting that highly sheltered growing conditions can reduce the E of *E. giganteum* stems. In the current study, average E for greenhouse cultivated *E. giganteum* from northwest Argentina was not significantly lower (3.12 GPa) than that for field grown plants from the same area, suggesting that thigmomorphogenesis may not play a substantial role in determining stem mechanical properties in this species. Calculations of theoretical maximum stem height were correspondingly much larger than those in the previously published study, and more consistent with reported maximum stem heights in the field. The contribution of nodal septa to stem bending properties was low and within the error of the measurement techniques.

Introduction

Equisetum giganteum is one of the largest living members of an ancient pteridophyte group, the sphenopsids, whose precise phylogenetic position remains controversial, but appears to form a sister clade to the Marattiaceae within the ferns (Pryer et al. 2001, Smith et al. 2006, Rothwell and Nixon 2006, Qiu et al. 2007). In contrast to their Carboniferous Period forebears, the tree-sized *Calamites*, all living *Equisetum* (horsetails) lack secondary growth (Gifford and Foster 1989) and thus must rely on other means to support height growth. The horsetails range in size from the diminutive *E. scirpoides* (stems averaging 12.9 cm tall and 0.5 - 1.0 mm diameter) to the giant horsetails, *E. giganteum* and *E. myriochaetum*, reaching heights of greater than 5 m (Hauke 1963). The hollow upright stems of *E. giganteum* are reinforced by nodal thickenings and septa. However, the relatively small diameters of *E. giganteum* stems (not known to attain more than ~4 cm) pose a challenge for stem mechanical stability

against local buckling. The biomechanical challenge must be met by a combination of stem biomechanical properties and clonal growth habit, wherein neighboring stems provide mutual support (Spatz et al. 1998a).

Among the most remarkable habitats in which *E. giganteum* grows are the river valleys of the Atacama Desert in northern Chile. *Equisetum giganteum* is a widespread species associated with wetlands and other moist habitats, usually at elevations above 500 m in the American tropics and extending to temperate South America (Hauke 1963), although a some populations are known from lower altitudes. Coastal northern Chile (~18-19° S latitude) appears to be one of the few localities where this species grows near sea level (3-90 m). The Atacama Desert, which extends along the western part of Tarapacá, is the “driest terrestrial region on this planet” (Ehleringer *et al.* 1998). The only sources of water in the area are surface and ground water from high Andean precipitation (Margaritz *et al.* 1990) and coastal fog from the Pacific Ocean (Goudie *et al.* 2002). *Equisetum giganteum* may have arrived in the northern Chilean valleys during a time when the region had a wetter climate and, unlike competing vegetation, adapted to the drying atmosphere and increasing salinity in the valleys as the Atacama region became highly desertified during the past 1000 years (Graf 1994).

In the Atacama valleys, where there is little woody vegetation to provide stem support, *Equisetum giganteum* is often exposed to significant wind stress (Figure 3.1). Nevertheless, *E. giganteum* stems in these habitats often reach heights in excess of 5 m with little support from other vegetation besides neighboring conspecific stems. In contrast, *E. giganteum* in mesic habitats in northwestern Argentina usually reaches significant heights only in relatively sheltered areas where nearby vegetation provides

support for the tall stems. Although biomechanical properties of *E. giganteum* stems have been studied in a greenhouse clone with relatively slender stems (~1 cm diameter, Spatz et al. 1998a), the present study is the first to investigate how *Equisetum* stem biomechanical properties vary among different environments over a range of stem diameters.

The structure of a plant stem determines the extent to which it can support its own weight and resist additional mechanical stresses such as wind. Therefore, the biomechanical properties of stems are closely linked to the growth form and habitat of a plant (Niklas 1992). Three important quantities used to describe the bending characteristics plant stems are Young's modulus of elasticity (E), the axial second moment of area (I), and the product of these two quantities, flexural stiffness (EI). Young's modulus of elasticity is the ratio of stress to strain and describes average bending properties of stem material. The second moment of area is a geometric parameter describing the size and shape of a stem's cross sectional area. Flexural stiffness describes the overall ability of a stem to resist bending and incorporates both the bending properties of stem material (E) and the arrangement of this material (I).

The study of biomechanical properties of extant plants can shed significant light on the growth habits of extinct fossilized plants (Speck 1994). Indeed, tissue properties of extant horsetails, including the putative *E. giganteum* studied by Spatz et al. (1998a) have been used to estimate tissue mechanical properties of extinct arborescent *Equisetum* relatives in the genus *Calamites*, a prominent vegetation component of the coal swamps of the Carboniferous period (Spatz et al. 1998b). Like modern horsetails, *Calamites* had hollow jointed stems, yet unlike modern *Equisetum*, these tree-sized predecessors

produced secondary wood (Kenrick and Davis 2004). Nevertheless, the overall biomechanical strategy was the same, to concentrate reinforcement towards the outside of the stem, where bending stresses are greatest, a strategy different from other arborescent plants of the Paleozoic, the lycopods, tree ferns and conifers (Kenrick and Davis 2004). Since biomechanical properties, in addition to stem hydraulic factors, are a crucial limiting factor in arborescent plants (Niklas 2007), obtaining an estimate of the mechanical properties of the most robust extant sphenopsid stems, which appear to be those of *E. giganteum* in the Atacama Desert, would provide valuable insight into the properties of *Calamites* stems.

Objectives of this study

Objective 1

Compare the average elastic modulus of *Equisetum giganteum* stems among plants growing in the Atacama Desert, in wetter habitats in northwestern Argentina, and in greenhouse cultivation (Argentine provenance). In addition, compare the elastic modulus values measured in this study with those measured in the study of Spatz et al. (1998a).

Objective 2

Compare scaling of stem bending properties (E and EI) with second moment of area for *E. giganteum* in the Atacama Desert and northwestern Argentina.

Objective 3

Assess whether the scaling relationships described in objective 2 are consistent with the semi-self-supporting growth habit proposed by Spatz et al. (1998a) for *E. giganteum*.

Objective 4

Compare scaling of stem diameter and wall thickness among *E. giganteum* in the Atacama Desert and northwestern Argentina.

Objective 5

Determine the theoretical maximum height that a self-supporting stem of *E. giganteum* can attain.

Objective 6

Determine the contribution of nodal septa to stem bending properties and compare this with the contributions found by Niklas (1989) for the related species *E. hyemale*.

Objective 7

Determine the relationship between stem diameter and stem height for mature, completely intact *E. giganteum* stems growing in a habitat where stems are not stunted by exposure.

Methods

Study sites

Northern Chile (Tarapacá region)

Lluta (Figure 3.1, elev. ~200 m) and Tarapacá elev. (~1200 m) valleys. In this region virtually no rain falls in most places where *E. giganteum* grows. However, several river valleys carry moisture from the Andes to the desert and this moisture supports vegetation on the valley floors, including *E. giganteum*.

Northwestern Argentina

1) Salta Province, roadside population (elev. ~700 m). 2) Tucumán Province roadside population and wild population in Lules valley. These sites, each ~400 m in elevation, were in areas receiving sufficient rainfall to support lush vegetation.

Cultivation in Miami, Florida

Container grown plants of northwest Argentina provenance (Salta Province) were cultivated in the research greenhouse at Florida International University, Miami, Florida under ambient light.

Biomechanical properties

At each study site, mature straight stems were selected for straightness across of stem diameters. Each stem was cut near the base and the cut end immersed in a bottle of water to maintain stem hydration during measurements. Lateral branches, if present, were removed prior to measurements. A 3-point bending apparatus was used to measure flexural stiffness. A length of the lower-middle portion of each stem (~0.4 to 1 m long), which receives the greatest bending moment for an intact stem, was suspended between two U-bolt vertical supports mounted on tripods (Figure 3.2). Bending force was applied by addition of weights to a container hung from the center of the suspended stem length. As weights were added to the container, the corresponding deflections of the stem were measured using a meter stick or electronic caliper. For each stem, five to seven different weights were applied. The range of weights was chosen to produce readily measurable deflections while attempting to avoid slippage of the stem at the supports. Each stem was marked at its initial position on the supports to facilitate detection of slippage. After

completion of each bending test, the supported stem length was cut transversely at the supports and in the center. At each cut, the outer stem diameter and wall thickness were measured with a digital caliper for calculation of approximate I . Photographs of stem cross sections were also taken for more precise calculation of I , taking into account the vallicular canals within the stems (Figure 3.3).

The following formulas were used to compute the quantities used in the analysis (Niklas 1992):

$$I = \int_A \rho^2 dA \approx \frac{\pi}{4} [\bar{r}_{outer}^4 - \bar{r}_{inner}^4] \quad \text{Eq. (1)}$$

$$I_{apparent} \approx \frac{\pi}{4} [\bar{r}_{outer}^4] \quad \text{Eq. (2)}$$

$$E = \frac{Stress}{Strain} = \left(\frac{F}{y} \right) \left(\frac{l^3}{48I} \right) \quad \text{Eq. (3)}$$

where ρ = perpendicular distance from axis of stem cross section to a given point of stem tissue, A = area of stem tissue in cross section, F = force exerted on stem by weights, y = (stem deflection from the vertical), l = (length of stem between supports), \bar{r}_{outer} = (mean outer radius of supported stem) and \bar{r}_{inner} = (mean inner radius of supported stem). For computation of E , the initial slope of the force/deflection relationship was estimated via ordinary least-squares regression. Digital image analysis was utilized to find the relationship between cylindrical approximation to I above and actual I for correction of values used in the analysis. There was a very strong correlation between digitized image calculation of I and the cylindrical approximation based on inner and outer stem

diameter measurements (Figure 3.4). Thus, the cylindrical approximations measured in the field were corrected accordingly to account for the fine structure of I .

For calculation of the theoretical maximum height that a stem of *E. giganteum* can attain, Greenhill's (1881) formula was used:

$$l_{crit} = c \left(\frac{E \times r_0^2}{\gamma} \right)^{1/3} \quad \text{Eq. (4)}$$

Where, l_{crit} is the maximum height a vertical slender tapering pole (with a circular cross section) can attain and r_0 is the basal stem radius and γ is the specific weight (N/m^3). The shape factor, c , accounts for the tapering mode of the stem and E is the structural Young's modulus averaged over the stem. A shape factor of $c = 1.7$ was used for typical giant horsetail stem tapering, following Spatz et al. (1998a).

For the Greenhill calculation measurements, two straight fully intact *E. giganteum* stems were selected in northern Chile. The branches were removed and the specific weight of the stem was calculated by weighing it, then cutting it into several sections, sealing the ends with ParafilmTM, immersing the stem in water, and measuring the volume of displaced water. Bending properties of each stem section were measured using the 3-point apparatus described above to determine the average E of the stem.

To assess contributions of nodal septa to stem bending properties, flexural stiffness was measured before and after septa were destroyed by passing a rod through the measured stem section.

To assess the scaling relationship between stem diameter and height, a group of fully mature stems were measured in the Tarapacá valley of northern Chile. Straight

stems with fully intact tops in an area without obvious stunting (which occurs in more exposed areas) were selected and their midpoint diameter and height were measured. An allometric power function was then fit to the resulting data using Reduced Major Axis Regression (Niklas 1994).

Statistical Analyses

Statistical analyses were carried out using JMP-IN (5.1.2, SAS Institute Inc., 2004) software. Analysis of variance was used for mean comparisons and multiple regression for slope comparisons.

Results

Mean E values were not significantly different among plants from northern Chile (4.29 GPa, $n=11$), northwestern Argentina (3.96 GPa, $n=16$) or greenhouse-grown *E. giganteum* from northwestern Argentina (3.12 GPa, $n=10$, ANOVA, $p = 0.098$) (Figure 3.5). The maximum E value was measured in Northern Chile, followed by northwest Argentina and the greenhouse plants (Figure 3.5). The minimum and mean values followed the same pattern, but with smaller differences.

Significant linear relationships were found between $\log(I_{app})$ and $\log(EI)$ for both the Chilean and Argentinean plants ($p < 0.001$, Figure 3.6). However, the slopes of these relationships were not significantly different among regions. There were significant ($p < 0.001$) linear relationships between mean stem outer diameter and wall thickness (Figure 3.7) but no difference in slope was detected between regions

The \log_{10} of I was uncorrelated with the \log_{10} of E for plants from northern Chile, northwestern Argentina and for greenhouse-grown Argentinean plants (Figure 3.8).

A standard power function provided a significant fit to the stem allometry data (Figure 3.9, $p = 0.01$) (Niklas 1994). A 95% confidence interval for the exponent is (0.12, 0.68). When this is converted to a Reduced Major Axis Regression exponent, useful for testing analytical scaling hypotheses (Niklas 1994), the resulting exponent is 0.47 and the corresponding 95% confidence interval is (0.16, 0.89).

The mean E value and specific weight value for the two stems measured in northern Chile were larger than those of the greenhouse plants measured by Spatz et al. (1998a) (Table 3.1). Average theoretical maximum heights were calculated for *E. giganteum* stems in the Atacama Desert and for the greenhouse clone studied by Spatz et al. (1998a), using Greenhill's (1881) formula (adjusting for branch weight according to Spatz et al. (1998a)). This predicts a maximum height of 10.6 m for a 4 cm diameter stem (Figure 3.10), slightly larger than the largest stem diameter measured in the Atacama (C. Husby, unpubl. data). The allometric fit (Figure 3.9) predicted a maximum height of 4.4 m for a 4 cm stem under field, whereas Spatz et al. (1998a)'s fit to Greenhill's formula predicts a maximum height of 5.5 m under northern greenhouse conditions (Figure 3.10). The average ratio between maximum stem height according to the Greenhill calculation and maximum stem height according to the allometric function was 1.96, ranging from 1.28 for a 0.5 cm diameter stem to 2.4 for a 4 cm diameter stem.

The contribution of nodal septa to stem bending properties was small and within the measurement error of the methods used in this study; flexural stiffness after septum destruction was slightly below or above the value measured before septum destruction, depending on slight changes in the experimental setup (e.g. slight rotation of the stem, data not shown).

Discussion

Objective 1

Although *Equisetum giganteum* plants in northern Chile and northwestern Argentina are different in external morphology (C. Husby, personal observation) and habitat, their average tissue bending properties (E) are similar, as are the properties of Argentinean plants cultivated in a greenhouse in Florida. Thus, tissue bending properties in *E. giganteum* appear relatively insensitive to environmental factors (but see point 2 below).

Objective 2

The mean values of E (4.29 GPa in Chile, 3.96 GPa in Argentina, and 3.12 GPa for greenhouse cultivation in Florida) measured in this study are far larger than those previously measured for this species using stems from a greenhouse clone in Germany (< 1.4 GPa, Spatz *et al.* 1998a), and actually fall near the bottom range measured for wood samples (Niklas 1992). Perhaps the E of *E. giganteum* stems becomes less under the highly sheltered conditions of a greenhouse, especially at the high latitudes of northern Europe, where light is limiting for much of the year, or that the provenance used in the study of Spatz *et al.* (1998a) was not actually *E. giganteum*. The latter hypothesis is quite plausible because two taxa with a similar appearance (*E. x schaffneri* and *E. myriochaetum*) are often misidentified as *E. giganteum* (C. Husby, personal observation). In fact, the plant in the photograph at the Royal Botanical Garden Edinburgh in Spatz *et al.* (1998a) was determined by Hauke (1985) to be *E. myriochaetum*. Unfortunately,

Spatz *et al.* (1998a) did not indicate whether the plant they studied is a clone of the Edinburgh plant, or a different clone.

Objective 3

The log-log linear relationships found between I_{apparent} and EI (Figure 3.6) are consistent with Spatz *et al.*'s (1998a) characterization of *E. giganteum* as a species with a semi-self-supporting habit. In fact, the slopes of the I_{apparent} and EI relationships found in this study (0.92, 1.0 and 0.97) are similar to the slope of 0.94 found by Spatz *et al.* (1998a).

The lack of relationship between I and E (Figure 38) is also typical of semi-self-supporting plants (Speck and Rowe 1999). *Equisetum giganteum* stems often grow close together and thereby provide mutual synergistic support via interlocking side branches. As a result, tall stems can remain upright even when they would not be mechanically stable alone.

Objective 4

The similar scaling relationships between stem diameter and stem wall thickness for the Chilean and Argentinean plants in the field or in cultivation suggests that spatial distribution of stem tissue is not significantly affected by the different growing environments of the plants. Together with the similarities in E between plants from the two regions (see 1. above), the consistent stem proportions explain why EI scales similarly with I_{apparent} in both northern Chile and northwestern Argentina.

Objective 5

The theoretical maximum heights attainable by *E. giganteum* stems from northern Chile correspond well with maximum stem heights observed in the field such as Andé's (1881) report of 5 m high stems in Ecuador and Spruce's (1908) report of 6.1 m high stems in the same country. Stems as tall as 5 m high are present in northern Chile (C. Husby, unpublished data). The theoretical maximum heights predicted by Spatz *et al.* (1998a) for a stem of 1.5 cm diameter stem in a European greenhouse plant is much lower (2.89 m) than that predicted for such a stem of a northern Chilean plant (5.23 m) reflecting the much lower *E* values and smaller diameters of the cultivated giant horsetail stems they measured (Figure 3.8). The Greenhill calculations for northern Chilean plants with a diameter of 3.8 cm (the maximum stem diameter measured in northern Chile) predict a maximum stem height of 9.71 m (in contrast to the predicted maximum 5.36 m based on the biomechanical properties of the greenhouse clone of Spatz *et al.* (1998a)). Furthermore, maximum stem heights measured by the author (C. Husby, unpublished data) as well as other botanists observing *E. giganteum* in situ (Hauke 1963) were in the range of 5 m. Thus, *E. giganteum* stems in northern Chile are “overbuilt” (i.e. incorporate a substantial “safety factor”), which allows the stems to better withstand mechanical stresses, such as wind, without buckling (Niklas 1992). This safety factor averages nearly 2 over a range of stem diameters. The fact that these stems are “overbuilt” is made clear by the fact that they can support the weight of a modest-sized bird on the tip of the stem without buckling (Figure 3.11).

The considerably greater overall stem tissue *E* measured in this study in comparison to that measured by Spatz *et al.* (1998a) suggests that the values used by

Spatz et al. (1998b) to analyze the biomechanical properties of *Calamites* were likely underestimates, probably leading to underestimates of the E of the trunks and branches of these ancient horsetail relatives.

Objective 6

The contribution of nodal septa to stem bending properties appeared to be low, because it could not be detected in the current study. Thus, in comparison to the large contribution of nodal septa to the Young's modulus of *E. hyemale* stems (17-32%, Niklas 1989), the septa of *E. giganteum* in northern Chile appear to contribute little.

Objective 7

The upper range of the 95% confidence interval for the Reduced Major Axis Regression scaling exponent of *E. giganteum*, places it within the range known for trees, and considerably lower than that of typical nonwoody species (Niklas 1994). The scaling behavior of *E. giganteum* stems coincides with the relatively large values of E for the species stems (see 2 above). However, the broad confidence interval prevents assessment of which allometric model may best account for the scaling relationship between diameter and height in this species (Niklas 1994).

Acknowledgments

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Tables

Location	Mean E for stem (MPa)	Mean specific weight of stem (N/m³)	Mean specific weight of stem + branches (N/m³)
Field (Atacama Desert, Chile)	5113	4164	8329
Greenhouse (Friburg, Germany, Spatz et al. 1998a)	600	3450	6900

Table 3.1 Parameters used for Greenhill's (1881) formula calculations in Figure 3.10.

Figures



Figure 3.1 *Equisetum giganteum* colony, Lluta Valley, Atacama Desert, northern Chile.



Figure 3.2 Three point bending apparatus for measuring flexural stiffness of giant *Equisetum* stems.

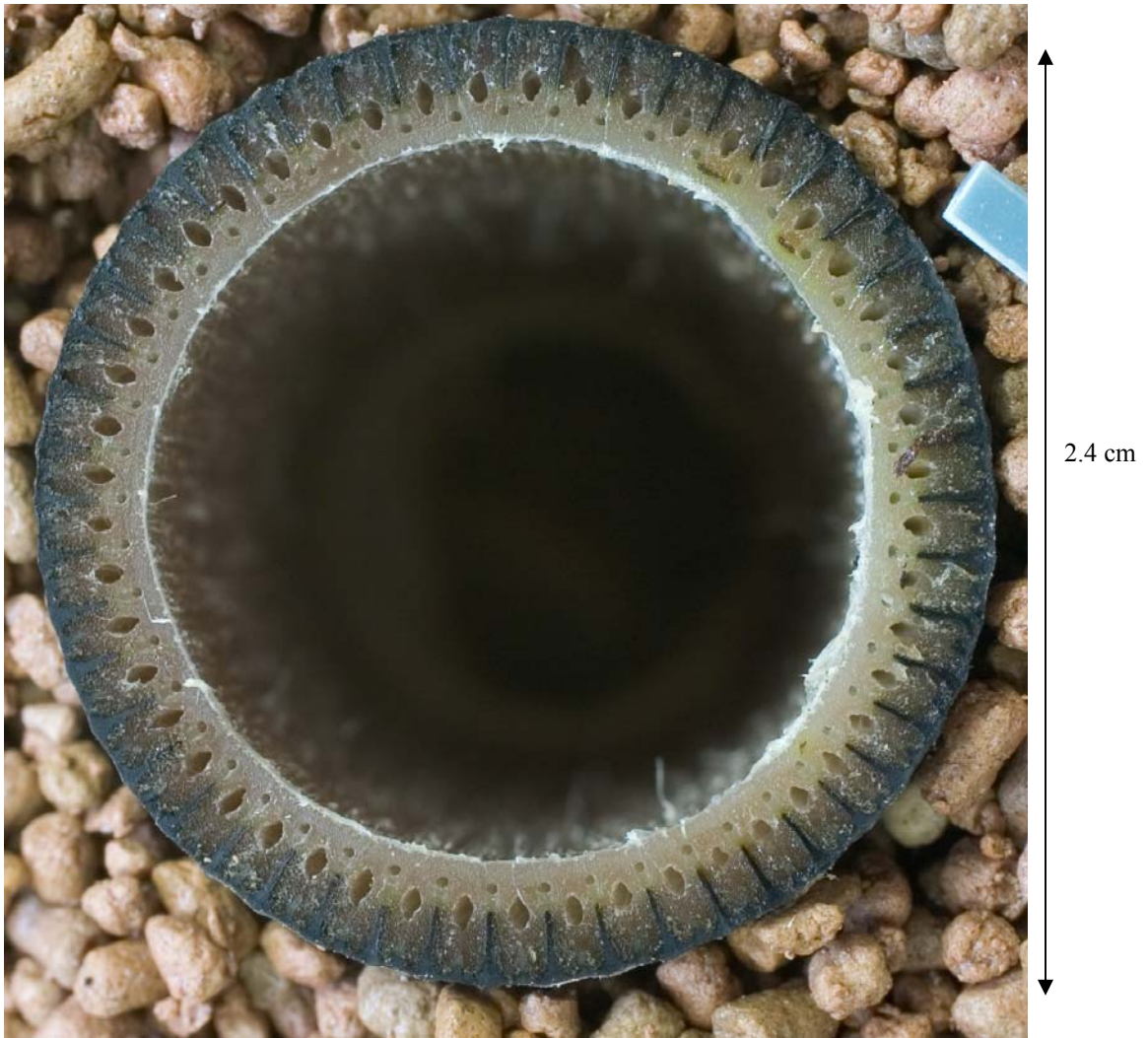


Figure 3.3 Cross section of *Equisetum giganteum* stem from northern Chile. Large channels are vallicular canals and smaller channels are carinal canals.

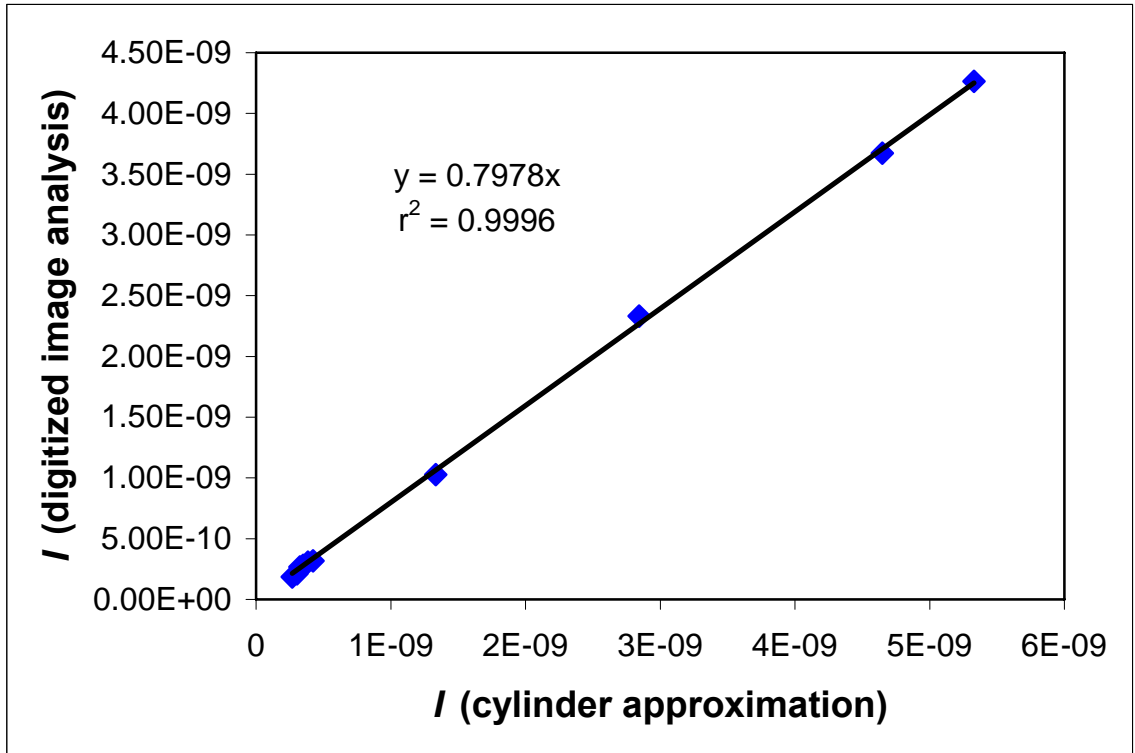


Figure 3.4 Relationship between cylindrical approximation for second moment of area (I) in *Equisetum giganteum* stems (northwestern Argentina) and actual second moment of area calculated by digitized image analysis.

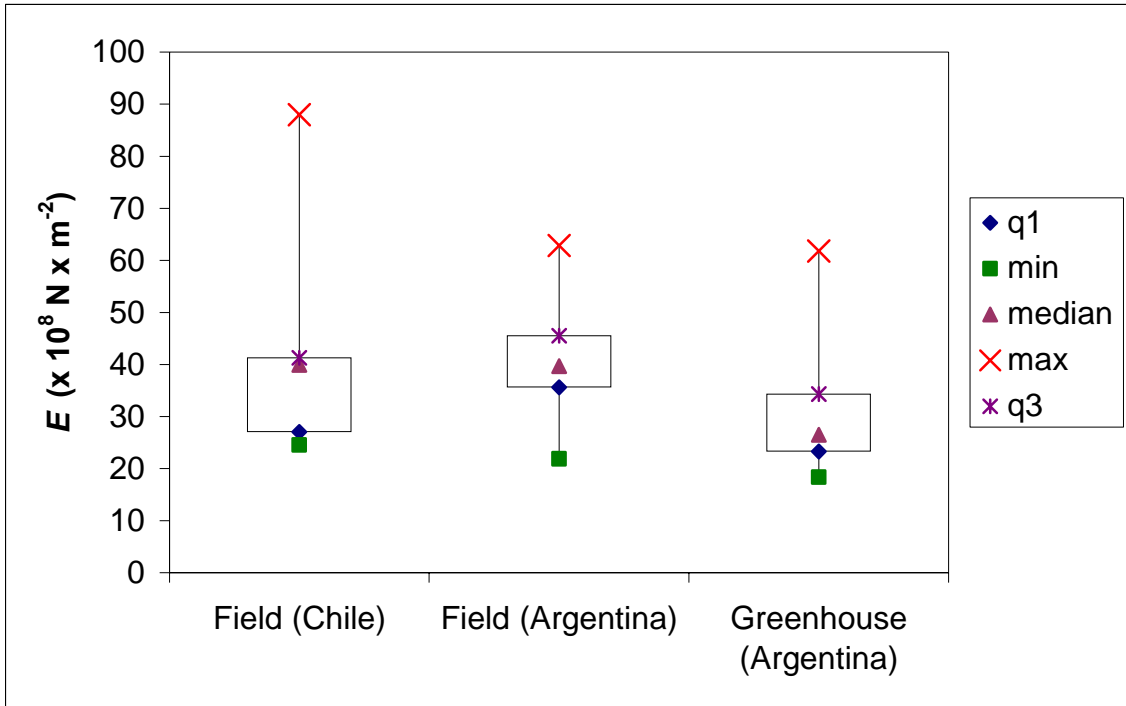


Figure 3.5 Box plots showing Young's modulus of elasticity (E) for *Equisetum giganteum* stems in the field in northern Chile and northwest Argentina and for greenhouse-grown stems of clones from northwest Argentina.

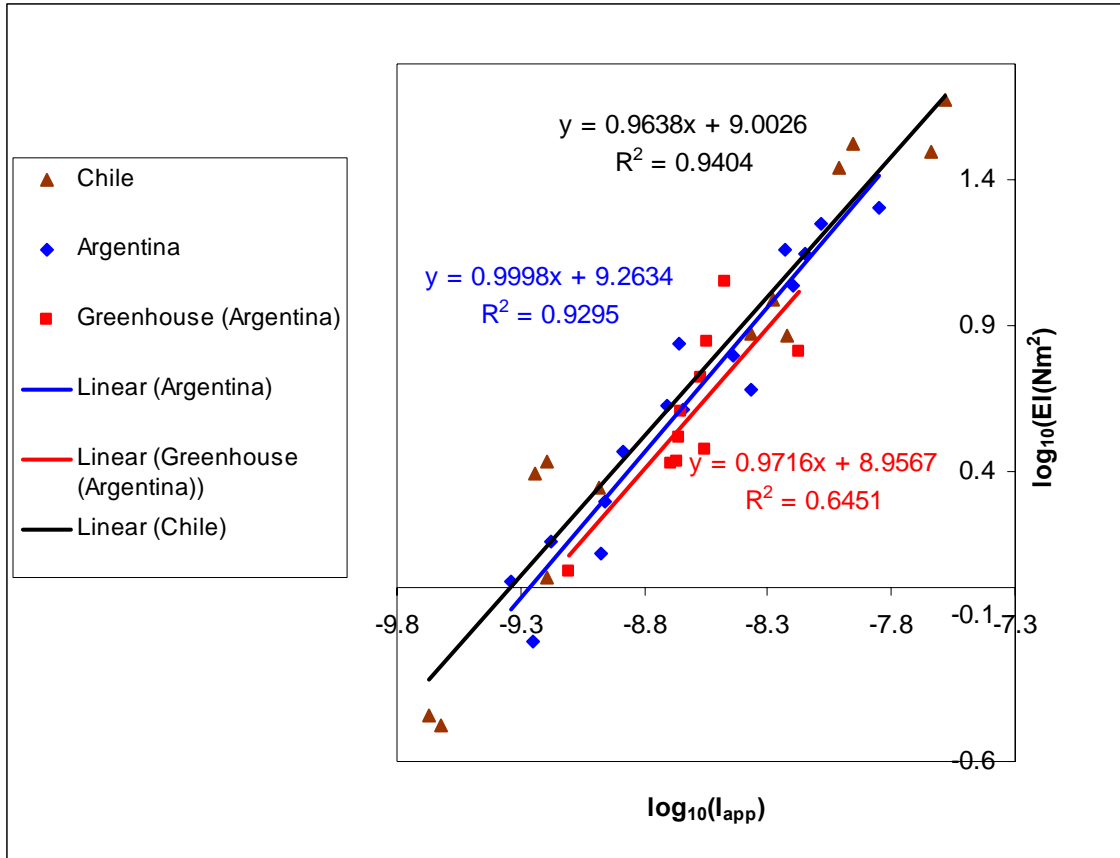


Figure 3.6 Apparent second moment of area vs. flexural stiffness, *Equisetum giganteum*.

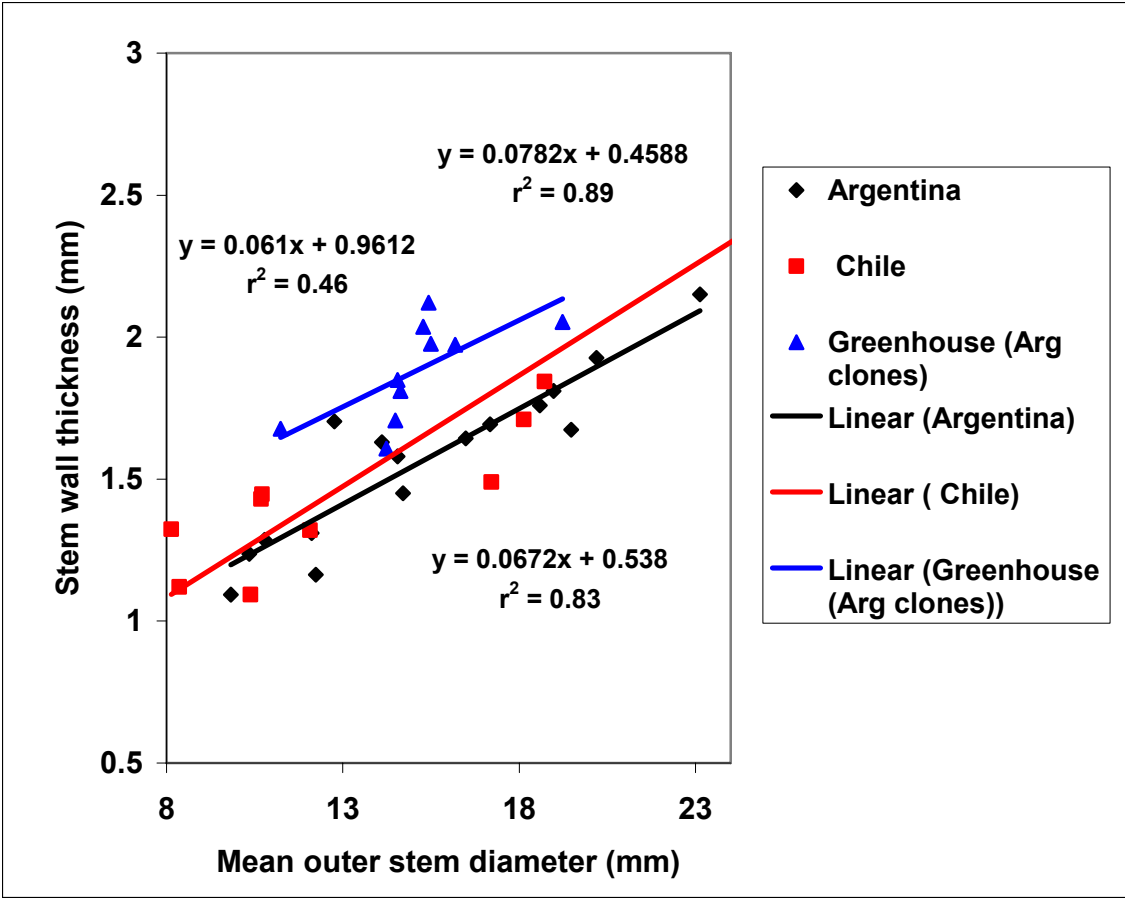


Figure 3.7 Stem diameter vs. wall thickness, *Equisetum giganteum*

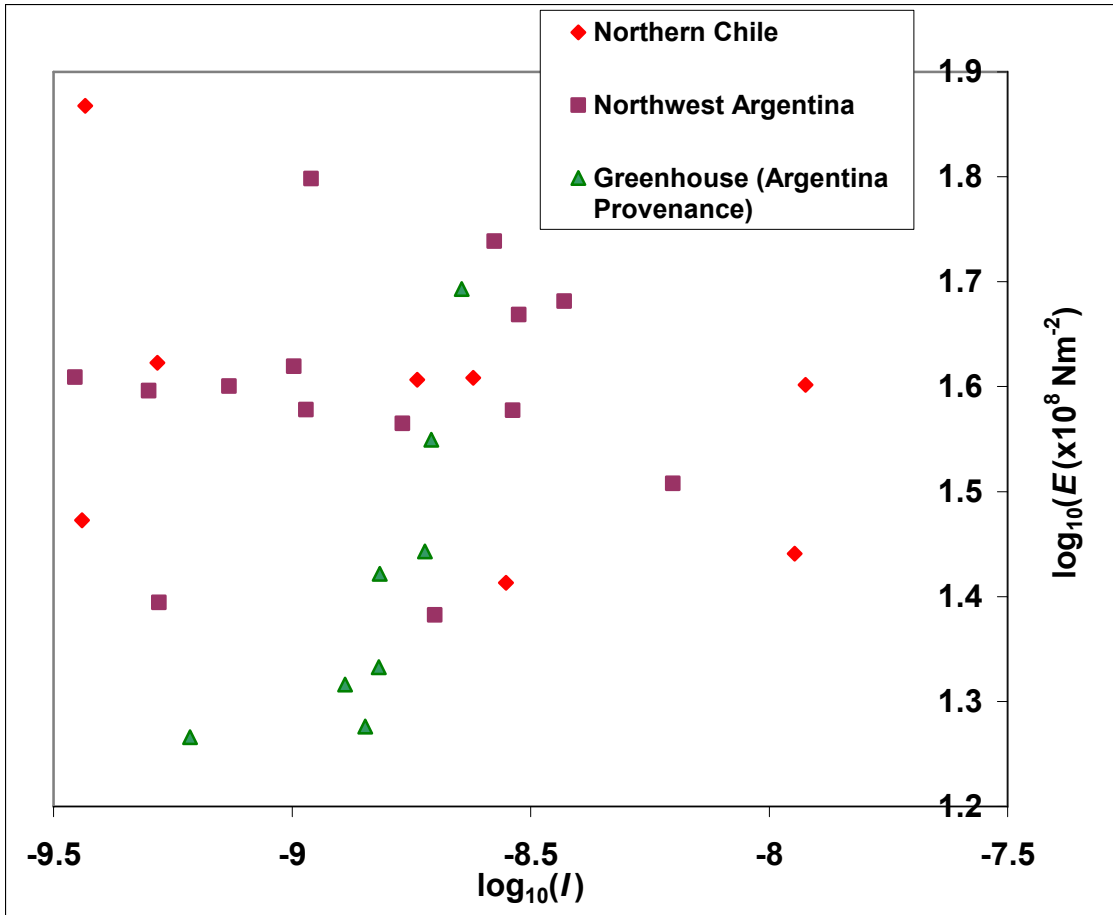


Figure 3.8 Second moment of area vs. Young's modulus of elasticity, *Equisetum giganteum*, in the wild (northern Chile and northwest Argentina) and in cultivation (greenhouse grown clone from northwest Argentina)

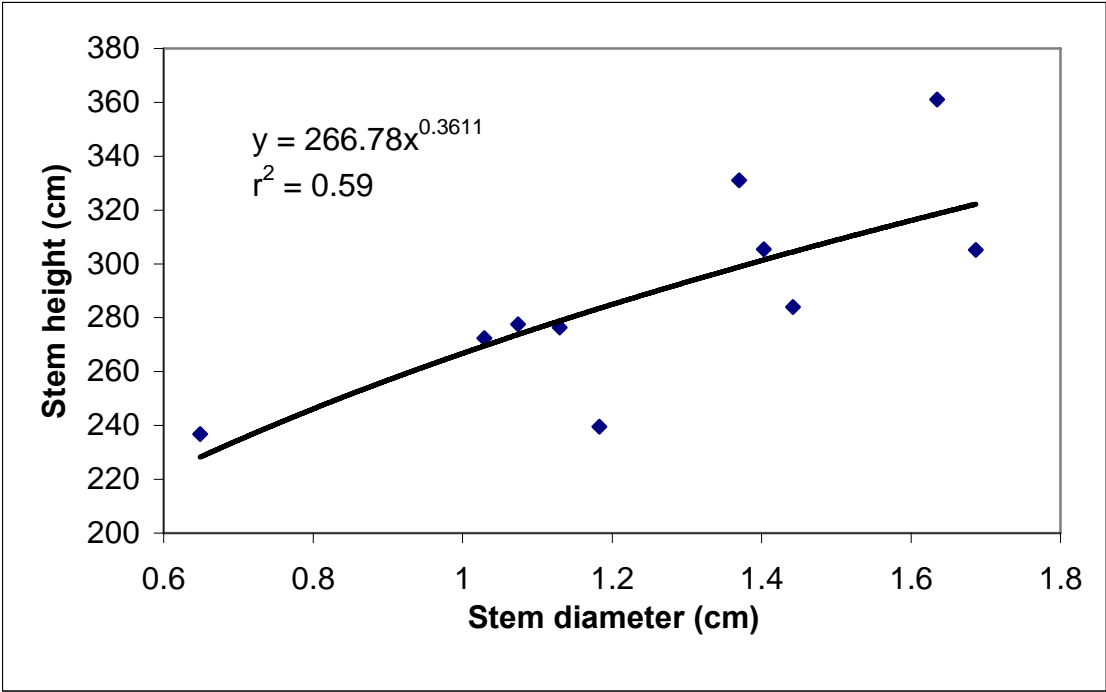


Figure 3.9 Stem diameter vs. stem height for intact *Equisetum giganteum* stems, northern Chile.

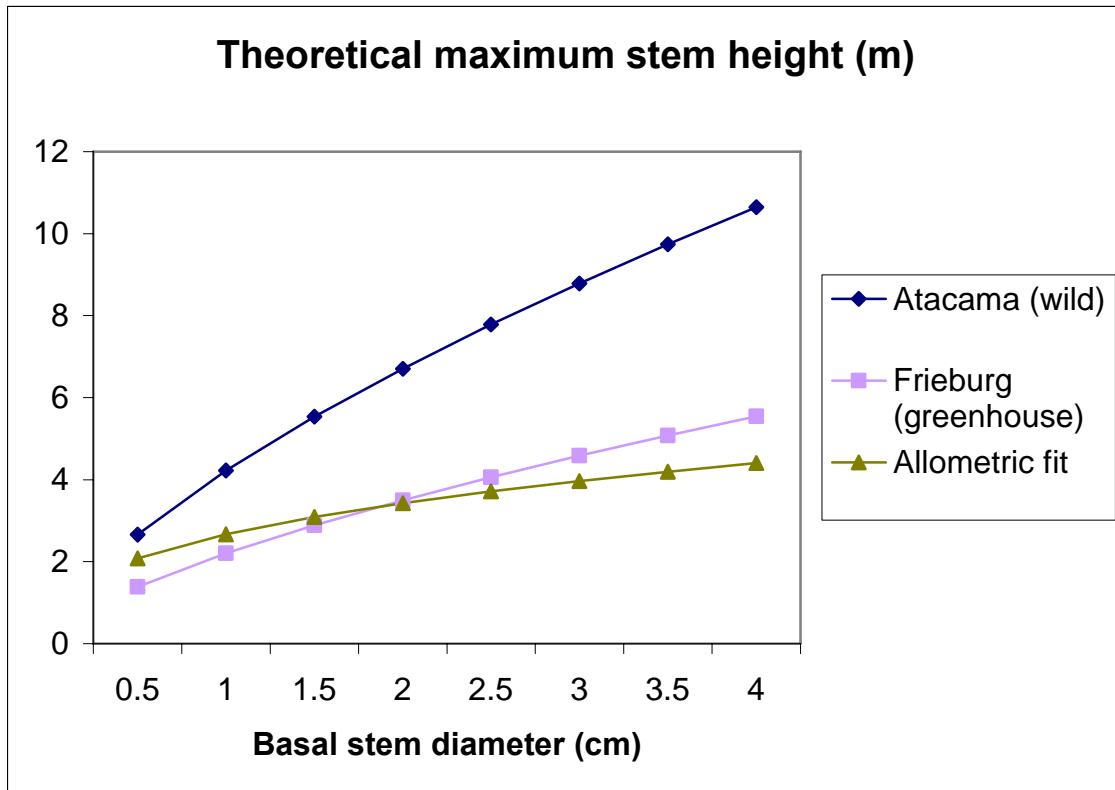


Figure 3.10 Theoretical maximum stem heights of *Equisetum giganteum* in the Atacama Desert and in a greenhouse in Frieburg (Spatz et al. 1998a), calculated for a range of basal stem diameters using Greenhill's (1881) formula with adjustments according to Spatz et al. (1998a). The allometric fit function was calibrated using data on height versus diameter of actual stems (Figure 3.9).



Figure 3.11 Dove perched on an *Equisetum giganteum* stem, Lluta valley, Tarapacá province, northern Chile.

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4. STOMATAL CONDUCTANCE PATTERNS OF *EQUISETUM GIGANTEUM*
STEMS IN SOUTH AMERICA

Abstract

The genus *Equisetum* has a stomatal apparatus that is unique among plants. However, the behavior of *Equisetum* stomata has largely been a matter of speculation, with investigators suggesting that stomata may remain tightly shut at maturity, only capable of movement while young, or permanently open while young. However, because *Equisetum* stems have morphological features indicative of water conservation (reduced leaves, stomata in deep grooves, and, in subgenus *Hippochaete*, sunken in the epidermis) suggest that active stomatal regulation of transpiration is likely to occur. In the study, patterns of stomatal diffusive conductance (g_w) were measured in the giant horsetail, *Equisetum giganteum*, central stem internodes in arid river valleys of the Atacama Desert in northern Chile and in a mesic environment in northwest Argentina. Maximum g_w values ($<200 \text{ mmol m}^{-2} \text{ s}^{-1}$) were low in comparison to typical angiosperm leaves, but were in the range measured in other pteridophytes. The range of measured g_w was similar in the contrasting environments of the Atacama Desert and a mesic valley in northwest Argentina. Stomatal conductance exhibited a diurnal pattern typical of C3 plants. Stems in shade had a significantly lower g_w than those in light, with a greater light response in mature stems than in developing stems. Developing stems had higher average g_w than mature stems. Stomatal conductance was higher for upper stem internodes than for middle internodes. Late-morning g_w was primarily related to stem diameter, stem surface

temperature and interactions among these factors and vapor pressure deficit, light, elevation, and groundwater salinity.

Introduction

Stomata play a central role in regulating gas fluxes in vascular land plants, responding to a plant's requirement to assimilate CO₂ while avoiding excessive transpirational water loss (Raven 2002). Opening and closing of stomata are influenced by a number of external and internal factors, including light, air humidity, CO₂ concentration and water stress (Lambers et al. 1998). However, the partial pressure of CO₂ within the stomatal cavity and the internal water status of the plant are the main influences on stomatal movements (Larcher 2003). Stomatal conductance of mesic C₃ plants frequently exhibits a distinct diurnal pattern with stomata being closed at night, opening at dawn, conductance reaching a peak in mid-late morning and falling off in the afternoon (Kramer and Boyer 1995; Lambers et al. 1998). However, different patterns may appear in plant growing in arid environments (Gao et al. 2005; Lambers et al. 1998) and the specific stomatal response often varies with growth form and native habitat of a plant (Franks and Farquhar 1999). Plant hydraulic properties also influence stomatal behavior (Zweifel et al. 2007).

Although stomatal behavior of flowering plants has been studied a great deal, less is known about stomatal behavior in pteridophytes and almost all of these studies have dealt exclusively with ferns. The relative lack of knowledge is surprising given the key phylogenetic position of pteridophytes as the most basal vascular plants to exhibit stomatal regulation of gas exchange (Brodribb and Holbrook 2004, Bell and Hemsley

2000). The stomatal behavior of ferns appears to be highly influenced by the environment in which the fern typically grows (Nobel et al. 1984). Ferns from environments that remain moist and shaded tend to respond minimally or not at all to changes in vapor pressure deficit (VPD) (Hollinger 1987; Hietz and Briones 1998), but ferns from dry habitats may exhibit reduced stomatal response to light and vapor pressure deficit when grown under well-watered conditions (Nobel 1978). On the other hand, ferns from drier and more exposed habitats tend to exhibit strong stomatal response to increasing VPD (Losch et al. 2007, Nobel et al. 1984, Hietz and Briones 1998), although this response may be modified in ferns with leaf desiccation tolerance that close their stomata more slowly (Hietz and Briones 1998). The tree fern *Dicksonia antarctica*, a long-lived species that is normally exposed to a wide range of environmental conditions, exhibits high stomatal sensitivity to VPD (Hunt et al. 2002). Brodribb and Holbrook (2004) found that stomata of tropical dry forest pteridophytes were considerably more sensitive to decreased leaf water potential than associated angiosperms, demonstrating a more conservative response to water stress. Brodribb and Holbrook (2004) was the only report of a stomatal response in a lycophyte, *Selaginella pallescens*. The species exhibited a response similar to the ferns studied.

Stomatal behavior in *Equisetum* has been very little studied and only in temperate species. The stomata of horsetails are structurally unique among plants, consisting of two cell pairs, wherein the guard cell pair is overlain by a pair of subsidiary cells with ridge-like thickenings on the inner walls (Dayanandan and Kaufman 1972; Hauke 1957). Dodsall (1919) was the first to investigate stomatal behavior of *Equisetum*. Her visual observations of stomata of cultivated plants indicated that the stomata of *Equisetum*

hyemale and *E. fluviatile* remained fixed open at all times, even at night. Kaufman et al. (1973) and Dayanandan and Kaufman (1973) observed that subsidiary cells of substomatal pores of *Equisetum hyemale* are always shut tightly in mature stems, whereas the young stomata remain open. The only published study with quantitative study of stomatal behavior in *Equisetum* was by Andersson and Lundegardh (1999) who measured g_w in *Equisetum arvense* branches grown with different potassium (K) amounts under a range of light intensities. These investigators found that g_w declined with increasing light intensity, but this effect was less when plants were grown with higher K supplementation. The observed g_w values were very low, however, ranging from 12-25 $\text{mmol m}^{-2}\text{s}^{-1}$. Dayanandan (1977) studied stomatal function in cultivated *E. hyemale* plants under controlled conditions and found that leaf sheaths of young stems transpired more water than internodes and that sheath transpiration was little affected by light intensity, suggesting that sheath stomata are fixed open. However, general shoot transpiration of young and old shoots responded to light/dark treatments, suggesting stomatal movement. Furthermore, diffusion resistance of mature internodes increased with decreasing light, indicating stomatal movement. Overall, the older shoots transpired far less water, partly due to the death and drying out of the leaf sheaths, as also happens in *E. giganteum*. However, both of these studies were on small laboratory grown plants, whereas field conditions are likely to produce somewhat different behavior (Dayanandan 1977). Furthermore, the large tall stems of *E. giganteum* present an additional challenge for regulation of water loss, since airflow rates increase with height from the surface, thinning the boundary layer around the shoots and thus increasing potential

evapotranspiration (Niklas 1994). The current study is the first to report measurements of g_w of large *in situ Equisetum* plants.

Because horsetails are very sensitive to soil moisture deficits (Hauke 1963, Dossdall 1919), lack vessels, have complex stomata and, in the subgenus *Hippochaete*, have sunken stomata, one would expect horsetails to exhibit stomatal control of transpiration. However, the studies discussed above give little indication of this.

Of the living horsetails, the giant horsetails (*Equisetum giganteum* L., *E. myrichaetum* and *E. x schaffneri*) lend themselves most readily to field measurement of g_w patterns because the stems are large (reaching 3.8 cm in diameter, C.E. Husby personal observation), tall (reaching 5+ m in height, Hauke (1963)) and their range includes areas of the tropics with high evaporative demand. One would expect to find stomatal regulation in such large exposed stems. These large stems also lend themselves to conventional steady-state porometry because it is relatively easy to create a seal between the stem and the steady-state chamber aperture and to calculate the area of stem exposed to the chamber.

The giant horsetail, *Equisetum giganteum*, is one of the few surviving members of a highly distinctive and ancient group of pteridophytes. *Equisetum giganteum* has a remarkably wide latitudinal range, extending from the Greater Antilles to central Chile and Argentina. *Equisetum giganteum* also grows in a wide variety of habitats including tropical montane forests and temperate wetlands. Among the most remarkable habitats in which *E. giganteum* grows are the river valleys of the Atacama Desert in northern Chile (Figure 4.1). Along with intense solar radiation and a highly desiccating atmosphere, soil salinity greatly restricts native and cultivated plant diversity in these valleys (Sotomayor

et al. 1994). Of the very few pteridophytes that live in these habitats, *E. giganteum* is the only species that constitutes a major vegetation component, often forming dense stands of stems reaching heights of more than 4 m (Figure 4.2).

Objectives of this study

1. To determine whether *Equisetum giganteum* stems exhibit diurnal changes in g_w in the Atacama Desert.
2. To determine whether g_w differs between the middle nodes and top nodes of a stem.
3. To determine whether developing and mature *E. giganteum* stems differ in their stomatal behavior, including different light responses, and, if so, how this may be linked to the growth dynamics of the young stems.
4. To determine whether the range of g_w values of *E. giganteum* stems in the Atacama Desert are similar to those of the same species growing in a more mesic habitat typical over most of the range of *E. giganteum*.
5. To determine which environmental factors influence maximum g_w of *E. giganteum*.

Materials and Methods

Data were collected during fieldwork in northern Chile and northwest Argentina from March 15 – May 4, 2004. Stomatal conductances of *Equisetum giganteum* stems were measured using a steady-state porometer (LI-1600, Li-Cor, Inc, Lincoln, NE) with a narrow leaf aperture on the chamber. Adjustment was made for the curvature of the central stem by measuring the diameter of the internode to which the porometer head was applied and using a cylindrical approximation for the area covered by the aperture. The adjustment is appropriate because *Equisetum* internodes are approximately uniformly

cylindrical although the overall stem shape is usually conical due to changes in diameter from node to node (Bierhorst 1959). Except for the measurements made on developing stems, all other stems measured were mature with fully expanded side branches and no evidence of continued central stem expansion (i.e. no lighter green area above the node, indicating freshly expanded tissue from the intercalary meristem). Stems were selected that were growing exposed to sunlight and not shaded, except when shaded stems were explicitly studied (objective 3). The chamber was sealed against the side of the stem with light continuous pressure (Figure 4.3).

For each objective, the methods were as follows:

1. Diurnal patterns of conductance

A healthy streamside colony of *E. giganteum* was studied growing in full sun (not shaded by the valley wall during the day) at Calatambo, Camiña Valley, Tarapacá Province, Northern Chile. Because temperatures drop considerably in the Atacama Desert valleys at night and due to the presence of surface water and vegetation, condensation can occur on stems. Furthermore, *E. giganteum* stems often exhibit guttation at night. Therefore, early morning conductance could not be measured due to surface moisture on the stems. Sunrise at this time of year was 0644 18:39, although actual sunrise and sunset in the valley were later and earlier, respectively, due to the shadows cast by the steep valley walls. Stems were dry by 0830, so measurements commenced at that time and proceeded every 3 hours thereafter until 2030. Seventeen mature healthy stems were selected within the colony for measurement. These stems were labeled with aluminum tags and g_w measured repeatedly during the course of one day. Data was analyzed using a repeated-measures model.

2. *Comparison of conductance on middle and top nodes of main stems*

A vigorous streamside colony of *E. giganteum* was studied growing at Tana Bridge, Camiña Valley, Tarapacá Province, Northern Chile. Ten mature, healthy, readily accessible stems were selected for measurement. At mid morning, g_w was measured on a middle node and an upper node (highest node that was large and robust enough to readily form a seal with the porometer chamber). A paired t-test was used to analyze the data.

3. *Comparison of stomatal behavior of developing and mature main stems*

A vigorous streamside colony of *E. giganteum* was studied growing at Tana Bridge, Camiña Valley, Tarapacá Province, Northern Chile. Mature (fully expanded main stems and fully expanded branches) and developing (actively elongating main stem and branches at early stage of elongation) stems were selected for comparison. The Tana bridge population near the valley edge was selected because it provided both shaded and sun-exposed stems in the morning for comparison of response of stomata to light. Eight stems were selected for each treatment combination. Data were analyzed using a two-way (factors were light and stem age) ANOVA model. In addition, to assess the growth activity of developing main stems, stem elongation rates were measured at two sites of contrasting exposure, but similar elevations: the Chiza valley with high wind velocity and exposure and the Camiña valley, near the Tana bridge, where wind velocity and groundwater salinity is less. Developing stems (in early stages of expansion, before side branches emerge) were tagged, and initial height and midpoint diameter were measured. Stem height was re-measured 49 days later (Tana site) and 33 days later (Chiza site). Growth rate measurements were normalized by initial stem height, using the standard

relative growth rate calculation, $r_{ser} = \frac{[\ln(h_2) - \ln(h_1)]}{t_2 - t_1}$, where r_{ser} = relative stem

elongation rate, h_1 and h_2 are the initial and final heights, respectively, and $t_2 - t_1$ is the total number of days elapsed between stem length measurements.

*4. Comparison of range of *E. giganteum* g_w values in the Atacama Desert and at a more mesic site in Northwest Argentina*

In addition to the measurements for the objectives above, g_w of mature stems in two populations (one in a river valley and one beside an agricultural field) was measured near the Lules Valley, Tucumán Province, Northwest Argentina.

*5. Determination of environmental factors influence maximum g_w of *E. giganteum* stems*

After determining the range of time of day of maximum g_w (Objective 1), g_w 's were measured during this time period at a variety of sites where *E. giganteum* grows in northern Chile and northwestern Argentina. These sites varied in altitude, exposure, groundwater salinity, humidity and rainfall. Stomatal conductances of mature stems at each site were measured in the late morning, along with photosynthetic photon flux density (PPFD), air temperature, stem surface temperature, relative humidity, groundwater electrical conductivity (EC) and altitude. The influences of these variables and their interactions on g_w were analyzed via multiple regression, using centered interaction variables to reduce effects of multicollinearity between the interaction terms and the predictors from which they are formed (Aiken and West 1991). A mixed-direction stepwise selection procedure was used to obtain a parsimonious model of stomatal behavior from among the potential regression terms.

Statistical analysis

Statistical analyses were carried out using SAS (version 9.1, SAS Institute, Cary, North Carolina) proc MIXED and JMP (version 7, SAS Institute Inc., Cary, North Carolina).

Results

Objective 1

Average g_w varied significantly over the course of the day ($p = 0.0022$), was highest in the morning, and declined in the afternoon (Figure 4.4). The decline began to occur as vapor pressure deficit (VPD), temperature and PPFD reached their peak (Figures 4.4-4.6). The best fit trendline was a 3rd order polynomial (Figure 4.5). Diurnal conductance patterns varied significantly among stems ($p = 0.0017$, Figure 4.7). Mean g_w at midday morning was $66.7 \text{ mmol m}^{-2} \text{ s}^{-1}$, and after sundown was $10.1 \text{ mmol m}^{-2} \text{ s}^{-1}$.

Objective 2

Stomatal conductance at the tops of main stems was significantly higher than at the middle of the stem ($p = 0.019$, one-tailed paired t-test, Figure 4.8). Mean g_w for stem tops was $129.7 \text{ mmol m}^{-2} \text{ s}^{-1}$ and for mid-stem was $115.4 \text{ mmol m}^{-2} \text{ s}^{-1}$.

Objective 3

Stem age and locality significantly affected g_w at midday, with developing stems having higher g_w at both sites and higher g_w at the Tana site than at the Calatambo site ($p < 0.0001$ for each effect). The effect of stem age was greater at the Calatambo site than at the Tana site ($p < 0.0001$, Figure 4.9). Stem age and light exposure each affected g_w at the Tana site ($p = 0.003$) (Figure 4.10). There was also a clear interaction between these factors with the effect of shade on reducing g_w being greater for mature stems than for

developing stems ($p = 0.0005$, Fig. 4.10). Rate of young stem elongation averaged 1.343 cm/day at the Chiza valley site and 0.83 cm/day at the Tana bridge site. When normalized by initial stem height, relative stem elongation rates were not significantly different between sites (Figure 4.11, $p = 0.0530$).

Objective 4

Stomatal conductances in the vicinity of the Lules Valley, a mesic environment very different from northern Chile, averaged $86.1 \text{ mmol m}^{-2} \text{ s}^{-1}$ (Figure 4.12). Stomatal conductances in northern Chile averaged $109.6 \text{ mmol m}^{-2} \text{ s}^{-1}$.

Objective 5

Because several of the environmental variables evaluated as predictors in this regression study tend to be correlated (e.g. VPD, PPFD, air temperature, relative humidity, and stem surface temperature), an initial attempt was made to reduce the dimensionality of the predictors using principle components analysis. However, the regression models using the composite variables resulting from this analysis produced poor fits to the data in comparison to models using the variables themselves. Therefore, the variables themselves were used in the analysis, retaining only those variables that had greatest predictive power among the highly correlated pairs of variables (e.g., stem surface temperature was retained, but not air temperature) to reduce multicollinearity problems.

A regression model involving stem diameter, VPD, PPFD, EC, elevation, stem surface temperature and several interactions explains about 57% (measured by r^2_{adjusted} to account for the number of terms in the model) of the variation in later morning g_w in *E. giganteum* across a wide range of sites (Table 4.1). The model was obtained through a

stepwise variable selection and the overall model was significant (Table 4.1, $p < 0.001$). Plotting of residuals revealed no problematic violations of the normality or homogeneity of variance assumptions. Because of the many terms included in the model, a Bonferroni correction was used to control the overall type I error rate at $\alpha = 0.05$.

Main stem diameter (Figure 4.13) and stem surface temperature (Figure 4.14) significantly affected late morning g_w , with larger diameter and higher surface temperature associated with lower g_w . These two terms also interacted significantly. The other positive interactions were between VPD and stem surface temperature, between PPFD and elevation and between EC and stem surface temperature.

Discussion

The absolute g_w s measured in this study were low ($<150 \text{ mmol m}^{-2} \text{ s}^{-1}$ for mature stems and $<200 \text{ mmol m}^{-2} \text{ s}^{-1}$ for developing stems) and would fall at the lowest end of a range of mesic woody and herbaceous spermatophyte species reported in Lambers et al. (1998). However, the conductance values observed for the tree fern *Dicksonia antarctica* (Hunt et al. 2002) are comparable to those of mature *E. giganteum* stems, suggesting that pteridophyte hydraulic conductivity limitations may necessitate lower stomatal conductivity to avoid losing water faster than it can be replaced. Hydraulic conductivity of an *E. giganteum* stem is likely to be greater than that of a tree fern because the pathway is much simpler in the former and leaves tend to represent a large proportion (25-80%) of the hydraulic resistance in a plant (Sack et al. 2003, Nardini 2001), and the higher end of this range seems plausible for highly compound tree fern lamina. However, David et al. (1990) observed a marked water potential depression in *Equisetum telmateia*

L. around noon even when VPD was relatively mild, deficit despite the presence of a high surface water table. This suggests that either water transport or root absorption were unable to keep up with evaporative demand during those periods. Brodribb and Jordan (2008) found a strong correlation between leaf hydraulic conductivity and maximum g_w across a range of taxa, including monocots, dicots and ferns. Thus, the low maximum g_w of *E. giganteum* is likely indicative to limitations in stem hydraulic conductivity if stems follow the same pattern as leaves.

The hydraulic architecture of *Equisetum* stems has yet to be elucidated, although the carinal canals in each internode may provide low resistance pathways through internodes, analogous to the role played by vessels in angiosperms (Xia et al. 1993). Since hydraulic conductivity is generally closely correlated with g_w (Franks and Brodribb 2005), it appears likely that there exist hydraulic “bottlenecks” at the nodes in *Equisetum* stems that limit water transport sufficiently to reduce g_w . The carinal canals are not continuous through the nodes (Gifford and Foster 1989), which likely increases resistance to water flow.

If hydraulic architecture does not limit stomatal conductivity in *Equisetum giganteum*, then this limitation may be due to the stomatal openings of *Equisetum* stems being partly occluded by the overlying subsidiary cells, as observed by Dayanandan and Kaufman (1973). In addition, the stomata of *Equisetum giganteum* are concentrated in deep grooves and are also sunken within the epidermis (Hauke 1963). These grooves may create a substantial boundary layer and if the conductance of this layer is low enough then it may limit the maximal g_w (Niklas 1994, Meinzer et al. 1997).

Equisetum giganteum stems exhibited marked variation in g_w , affected by time of day and shade, indicating that even in mature stems stomata actively regulate transpiration. In fact, the response of g_w to light was greater in mature stems than in developing stems (Fig. 10) suggesting that stomata of *E. giganteum* are not “fixed shut” at stem maturity, in contrast to the observations of Dayanandan and Kaufman (1973) in *E. hyemale*. Although Dayanandan (1977) found a smaller response in young shoots than in old shoots, he attributed this to stomata on leaf sheaths that remain permanently open. Since I did not measure sheath conductance in this study, the difference observed between stem developmental stages in *E. giganteum* must be due to either differences in stomatal behavior or differences in cuticular conductance. Developing *E. giganteum* shoots elongate rapidly over extended periods of time (up to the maximum of 49 days measured in this study), yet this relative stem elongation rate seems relatively insensitive to contrasting environments (Figure 4.11). The consistency of young stem elongation rates in different environments is consistent with the relative insensitivity of g_w developing stems to contrasting light and locality as observed in the Tana and Calatambo valleys, in contrast to large differences in g_w for mature stems in the same localities (Figure 4.9). Since the surfaces of developing stems are likely less silicified than those of mature stems, cuticular conductance may be considerably larger for the developing stems, reducing the detectability of stomatal movements via the porometer, which does not distinguish between cuticular and stomatal conductance.

The diurnal patterns of g_w observed are similar to those observed in mesic C3 plants (Kramer and Boyer 1995). Mean g_w was near peak at the beginning of measurement at 8:30 am and increased slightly by late morning, suggesting that

saturating PPFD for photosynthesis in *E. giganteum* stems is between 800 and 1600 $\mu\text{mol sec}^{-1} \text{m}^{-2}$ (Figure 4.6). Stem photosynthesizing desert legume species tend to reach saturating PPFD between 1200 and 1200 and 1600 $\mu\text{mol sec}^{-1} \text{m}^{-2}$ (Nilsen and Sharifi 1994). The correspondence in behavior is especially interesting given the “*Equisetum*-like shoot structure” of these plants and of many primarily stem photosynthesizing plants of dry habitats (Aschan and Pfanz 2003).

Variation from stem to stem was marked even though many of them were within the same small population and many were likely connected by rhizomes (Figure 4.7). Such extreme variation is puzzling and may suggest that stomatal regulation is primarily at the individual stem level, rather than the clone level, and that the internal water status of individual stems may vary substantially even in a small population, leading to stomatal closure. The variability be due to limitations imposed by rhizome hydraulic conductivity, making water transfer within a clone inefficient, thus subjecting individual stems to variation in local water availability to the roots at the base of the stem, even if abundant moisture is available to the rhizome elsewhere.

The slightly higher g_w measured near the tops of *E. giganteum* stems than at the middle of those stems suggest that internal stem water status is more favorable near the top. The structure of *Equisetum* stems likely leads them to behave hydraulically like “porous pipes” (Zwieniecki et al. 2002), with a marked water pressure drop towards the top (Zwieniecki et al. 2002). The increasingly narrow diameters of internodes near the stem tip and the correspondingly smaller evaporative stem surface area and smaller area of vascular tissue likely compensates for the water pressure drop, allowing sufficient water potential to maintain stomatal opening. The higher degree of surface suberization

on the lower part of the stem (Speck et al. 1998, C.E. Husby, personal observation) may also help maintain sufficient xylem pressure higher in the stem by limiting evaporative potential on the lower part of the stem (Zwieniecki et al. 2002). When *Equisetum* stems experience water stress, the apical internodes wilt first and progressive wilting proceeds lower on the stem as water stress increases (Doddall 1919, C. E. Husby, unpublished data), suggesting that there is a significant pressure drop from one internode to the next distally, apparently because of high hydraulic resistance at stem nodes. Alternatively, the tops of stems may receive more light, resulting in higher photosynthetic rates and thus higher g_w .

Maximum g_w in late morning was affected by a variety of variables and their interactions. Stem diameter exhibited a significant influence alone and in interaction with stem surface temperature (Table 4.1, Figures 4.13-4.14). Stem diameter may affect g_w through its effect on increasing boundary layer thickness of a cylinder (Niklas 1994). Thus, increasing stem diameter is likely to increase the boundary layer and thus potentially a decrease g_w (Meinzer et al. 1997). However, the steady state porometer used in this study disrupts the boundary layer through vigorous convection. Thus, the stem diameter effect is more likely due to changes in stomatal density with stem diameter. Alternatively, larger stems may have deeper stomatal grooves, creating a boundary layer that is not completely destroyed by the porometer.

Increasing stem surface temperature leads to a decrease in g_w , most likely to reduce evapotranspiration during hot weather or the high insolation part of the day when VPD is highest. This may also reflect a potential negative effect of higher temperatures on photosynthesis, leading to less need for stomatal opening due to higher intracellular

CO₂ concentrations (Fitter and Hay 2002). Alternatively, the stem surface temperature effect may be partly a result of stomatal closure causing reduction in evaporative cooling, and thus an effect rather than a cause. However, there is a significant interaction between diameter and stem temperature that moderates the negative influence each has on stomatal conductance. This may be due to the fact that an increase in stem diameter decreases the surface to volume ratio of the stem, reducing the relative desiccating effect of higher stem temperatures. In contrast, VPD and stem temperature interact negatively with regard to g_w , which may partly reflect the fact that air temperature and stem temperature are highly correlated and thus are correlated with VPD. In addition, a higher stem surface temperature may increase the VPD near the stem surface, promoting stomatal closure. Sensitivity of leaves to VPD is highly affected by the ratio of stem hydraulic conductivity to maximum g_w (Brodribb and Jordan 2008), so to gain a deeper understanding of the influence of VPD on *E. giganteum* stomatal behavior would likely require elucidation of stem hydraulic properties. Stem surface temperature also interacts negatively with groundwater salinity, suggesting that salinity compounds the stress of increasing stem temperature.

Photosynthetic photon flux density, by itself, did not influence g_w , possibly because late morning PPFD was saturating at all measured intensities. However, the interaction between PPFD and elevation has a negative influence on g_w , which may reflect the fact that partial pressure of CO₂ tends to decrease with increasing elevation and may lead to increased photorespiration (Nobel 1999) resulting in an increase in CO₂ in the stomatal cavity, resulting in reduced stomatal aperture. Conditions at higher elevations in this study, which include higher late morning VPD (probably due to

increased distance from the cooling effects of the Pacific Ocean), may exacerbate the effect by preventing greater initial stomatal opening to compensate for the lower compensation partial pressure of CO₂. The effect of VPD alone in this study remains ambiguous because there is not a consistent trend for this factor by alone (Figure 4.15), but it appears to mainly influence g_w through interaction with other factors (Table 4.1). Likewise, PPFD does not show a clear effect on late morning g_w (Figure 4.16) by itself, but does appear to interact with elevation.

The model only partially accounts for the observed patterns of g_w suggesting that other important factors remain to be studied. The very high degree of variability in diurnal g_w patterns, even within a single colony of stems growing in full sun (Figure 4.7), as discussed above, suggests that internal stem or clone factors, are likely to be key drivers of stomatal behavior in *E. giganteum*. Deeper understanding of water relations and stomatal behavior of *Equisetum* will require detailed study of hydraulic architecture of the whole plant, as well as further stomatal behavior under varying environmental conditions in the field and in cultivation under controlled conditions.

Acknowledgments

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fieldwork in Argentina. I am also very grateful to Dr. Thomas Schoepke for bringing to my attention the Atacama Desert populations of *E. giganteum*.

Tables

ANOVA Table				
	df	Sum of squares	Mean square	F ratio
Model	15	100582.1	6705	10.27
Error	88	57453.8	652.9	
Total	103	158035.9		Prob > F
				< 0.0001
Fit	r^2	0.636		
	r^2_{adj}	0.574		
Parameter estimates				
	Estimate	Standard error	Prob > t	
Intercept	730.5	180.80	0.0001139*	
Stem diameter	-3.954	1.090	0.0004806*	
VPD	64.79	27.35	0.02003	
PPFD	-0.2901	0.1256	0.02327	
EC	-15.70	6.864	0.02461	
Elevation	0.02807	0.01303	0.03393	
Stem surface temp	-9.389	2.417	0.0001986*	
(Diam-13.00)*(PPFD-1527)	-0.01273	0.005431	0.02132	
(Diam-13.00)*(Elev-673.8)	-0.005858	0.003776	0.1244	
(Diam-13.00)*(Stem surf temp-26.86)	1.828	0.5665	0.001750*	
(VPD-2.144)*(PPFD-1527)	-0.6736	0.3333	0.04632	
(VPD-2.144)*(Elev-673.8)	0.9715	0.4387	0.02938	
(VPD-2.144)*(Stem surf temp-26.86)	-15.66	4.850	0.001745*	
(PPFD-1527)*(Elev-673.8)	-0.0005835	0.0001388	6.276x10 ⁻⁵ *	
(PPFD-1527)*(Stem surf temp-26.86)	0.01869	0.009702	0.05731	
(EC-4.469)*(Stem surf temp-26.86)	-2.262	0.7159	0.002161*	

Table 4.1 Best fit model for late morning conductance, via stepwise variable selection procedure beginning with a full model with all possible 2-way interactions. Starred p-values are significant after Bonferroni correction (p cutoff for overall $\alpha = 0.04$ is 0.003125).

Figures



Figure 4.1 Valley of the Lluta River, Tarapacá Province, northern Chile.



Figure 4.2 *Equisetum giganteum*, valley of the Lluta River, Tarapacá Province, northern Chile.



Figure 4.3 Measurement of stomatal conductance (g_w) of *Equisetum giganteum* stems with a steady state porometer in the Calatambo, Camiña Valley, Tarapacá Province, Northern Chile, March-May 2004.

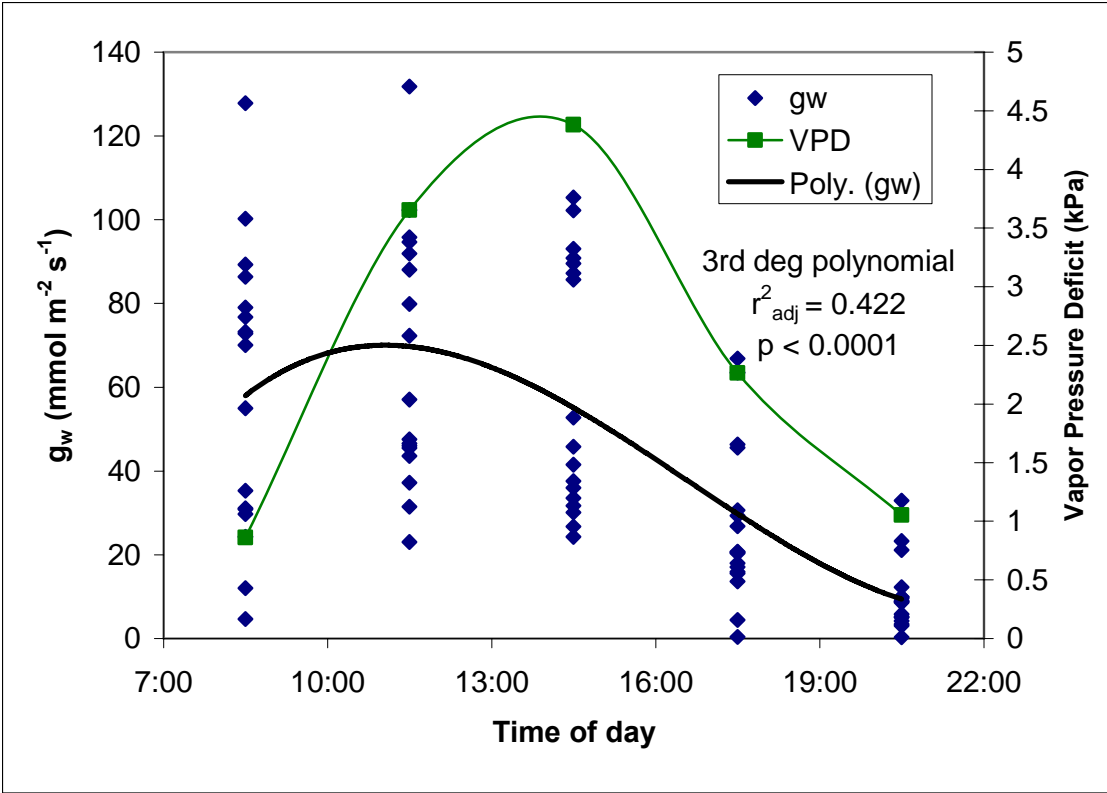


Figure 4.4 Best fit polynomial function to the diurnal pattern of mean stomatal conductance (g_w) and Vapor Pressure Deficit (VPD) in *Equisetum giganteum* stems, Calatambo, Camiña Valley, Tarapacá Province, Northern Chile.

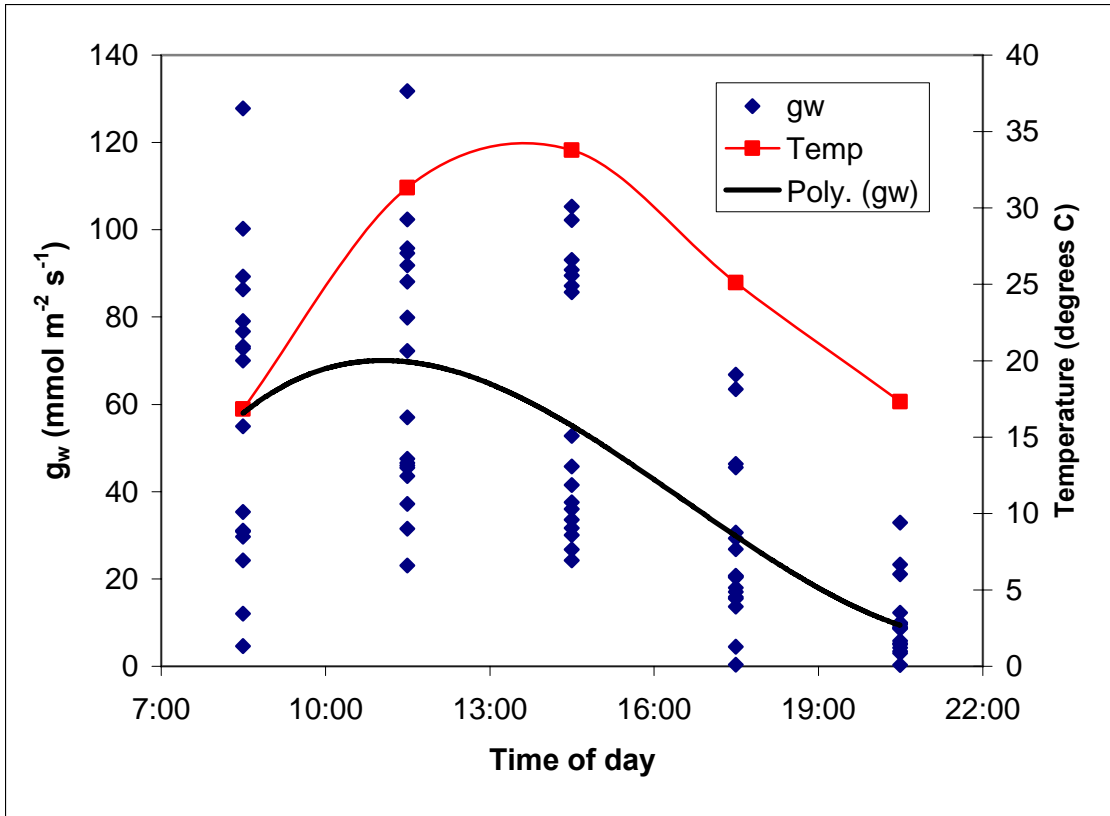


Figure 4.5 Best fit polynomial function to the diurnal pattern of mean stomatal conductance (g_w) and temperature in *Equisetum giganteum* stems, Calatambo, Camiña Valley, Tarapacá Province, Northern Chile.

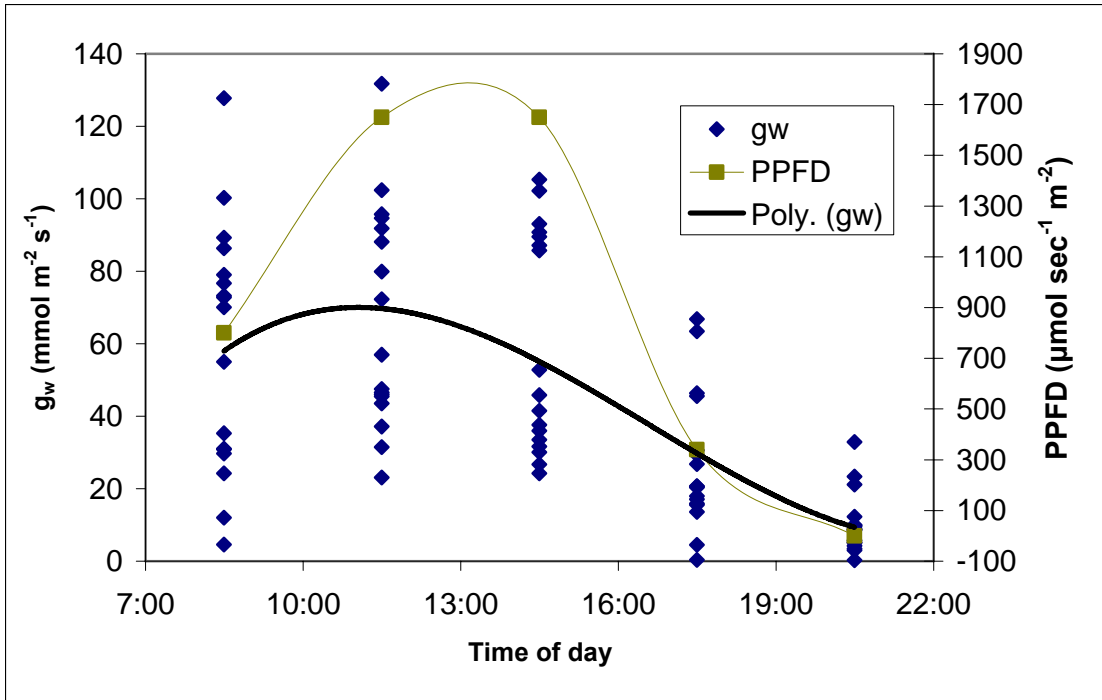


Figure 4.6 Best fit polynomial function to the diurnal pattern of mean stomatal conductance (g_w) and photosynthetic photon flux density (PPFD) in *Equisetum giganteum* stems, Calatambo, Camiña Valley, Tarapacá Province, Northern Chile.

Although the 11:30 PPFD was not measured due to technical difficulties, the 14:30 value was substituted as an approximation of maximum irradiance.

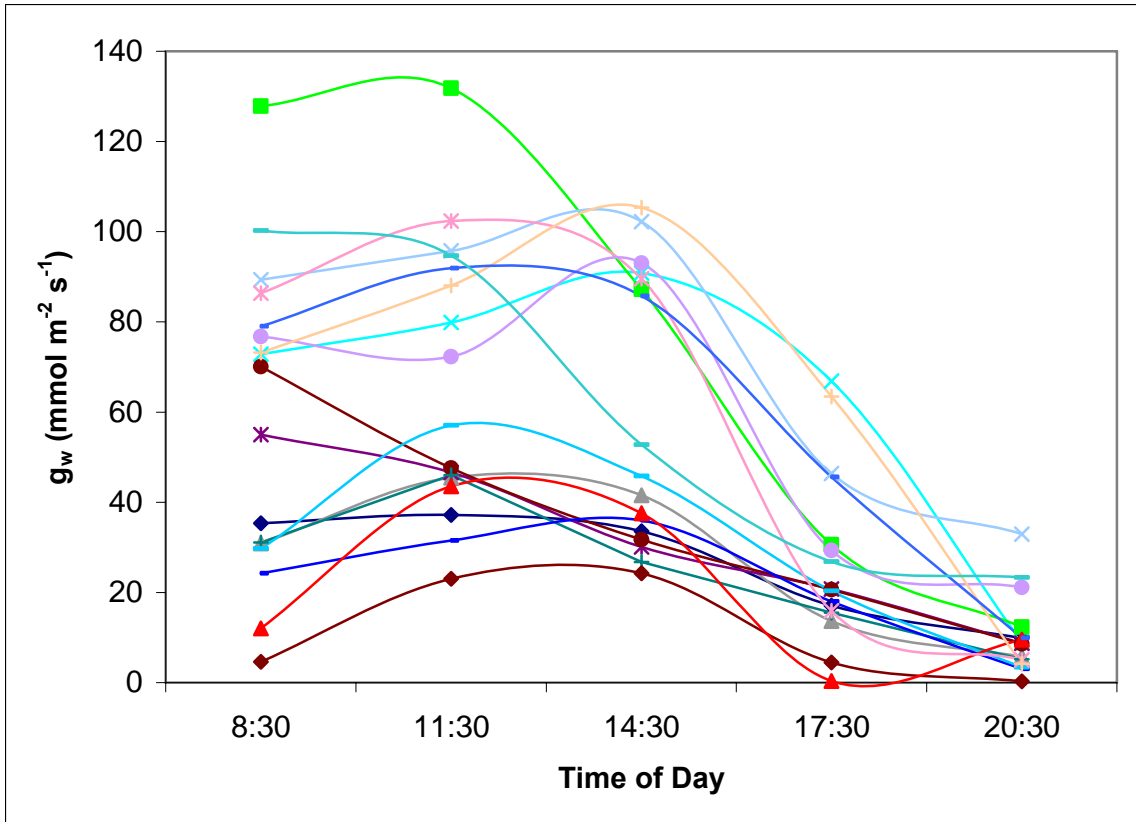


Figure 4.7 Diurnal pattern of g_w for individual stems in *Equisetum giganteum* stems, Calatambo, Camiña Valley, Tarapacá Province, Northern Chile. Connecting lines have been smoothed. Symbols represent different individual stems.

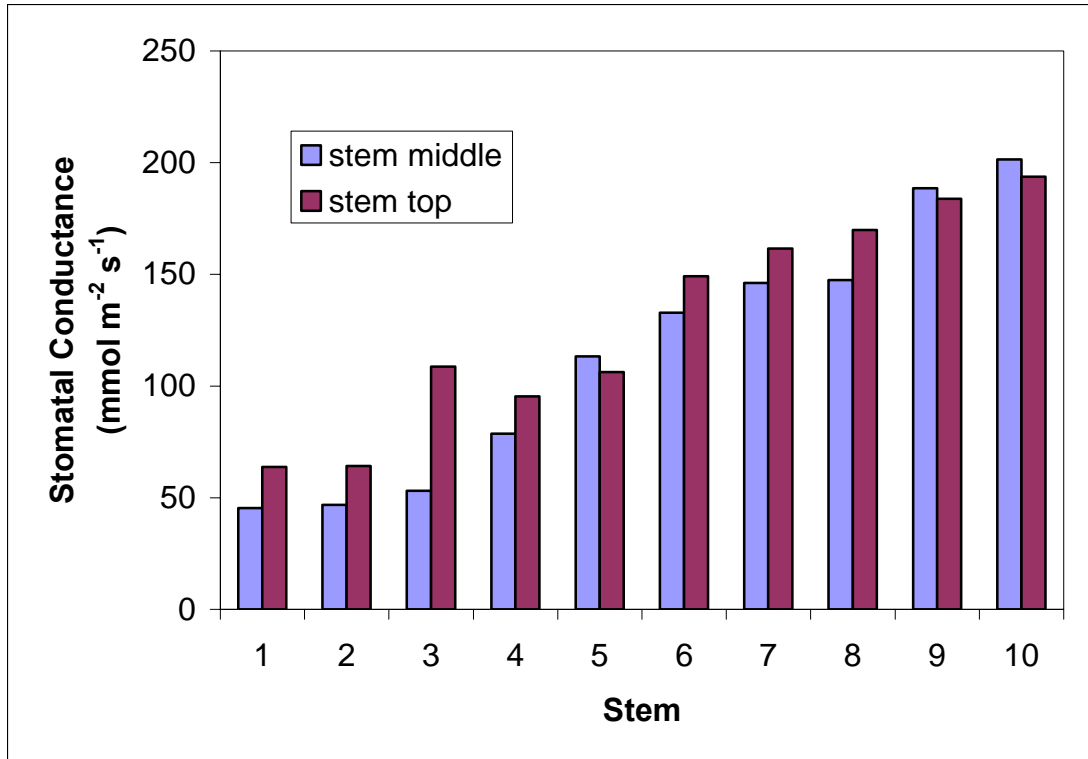


Figure 4.8 Stomatal conductance (g_w) measured at the midpoint and near the top of *Equisetum giganteum* main stems in the Camiña Valley, Tarapacá Province, Northern Chile. Stems in ascending order of mid-stem g_w .

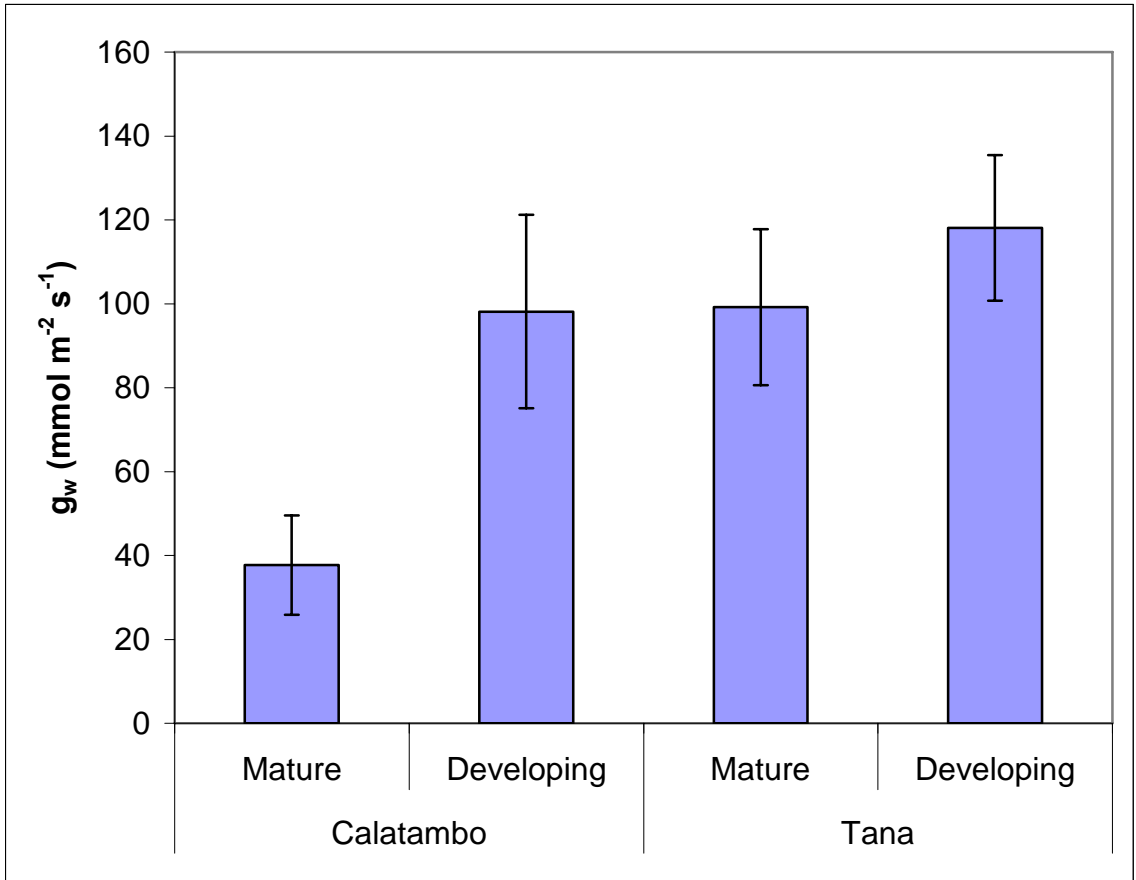


Figure 4.9 Mean stomatal conductance (g_w) of *Equisetum giganteum* stems at different developmental stages at two sites in the Camiña Valley, Tarapacá Province, Northern Chile. Error bars are standard deviations.

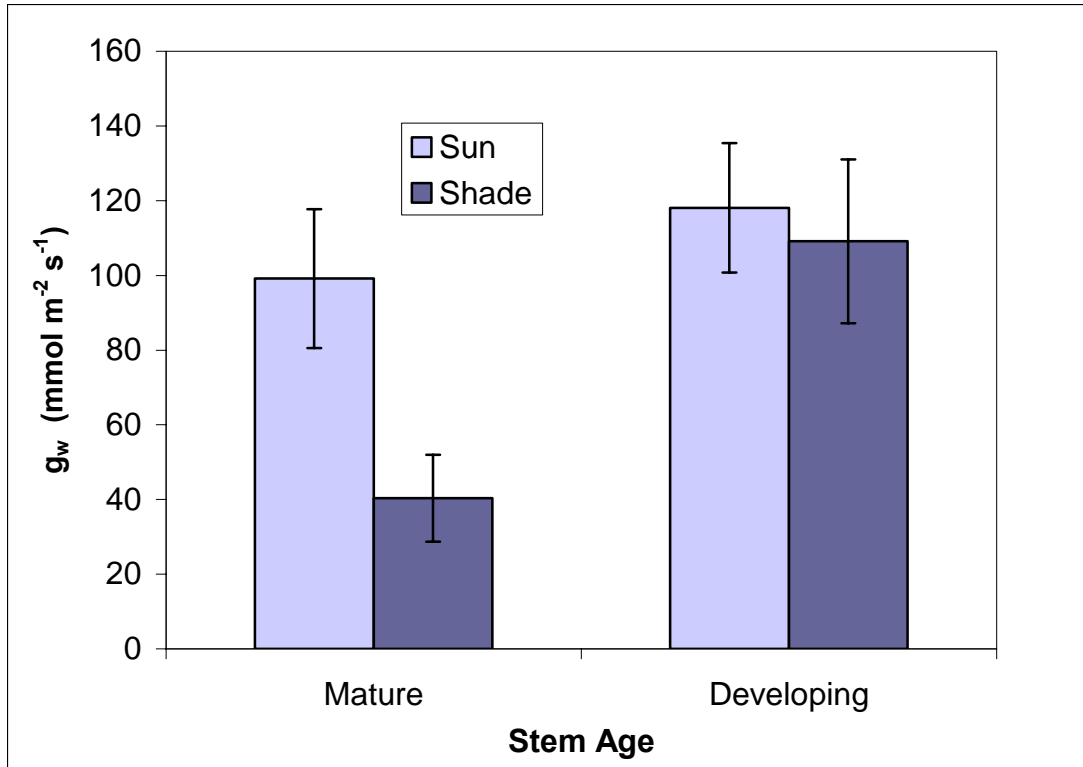


Figure 4.10 Mean stomatal conductances (g_w) of mature and developing *Equisetum giganteum* main stems in sun or shade at mid-morning at Puente Tana, Camiña Valley, Tarapacá Province, Northern Chile. Error bars are standard deviations

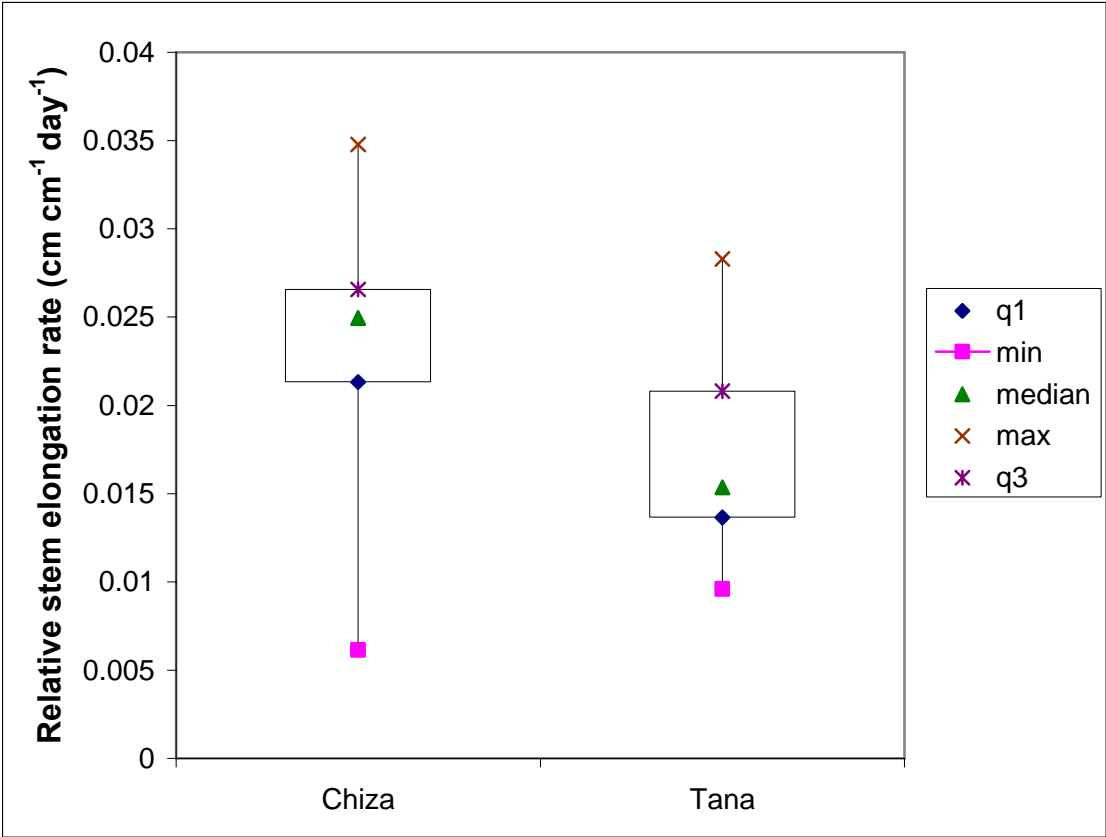


Figure 4.11 Relative stem elongation rates for young developing main stems (pre-branch emergence).

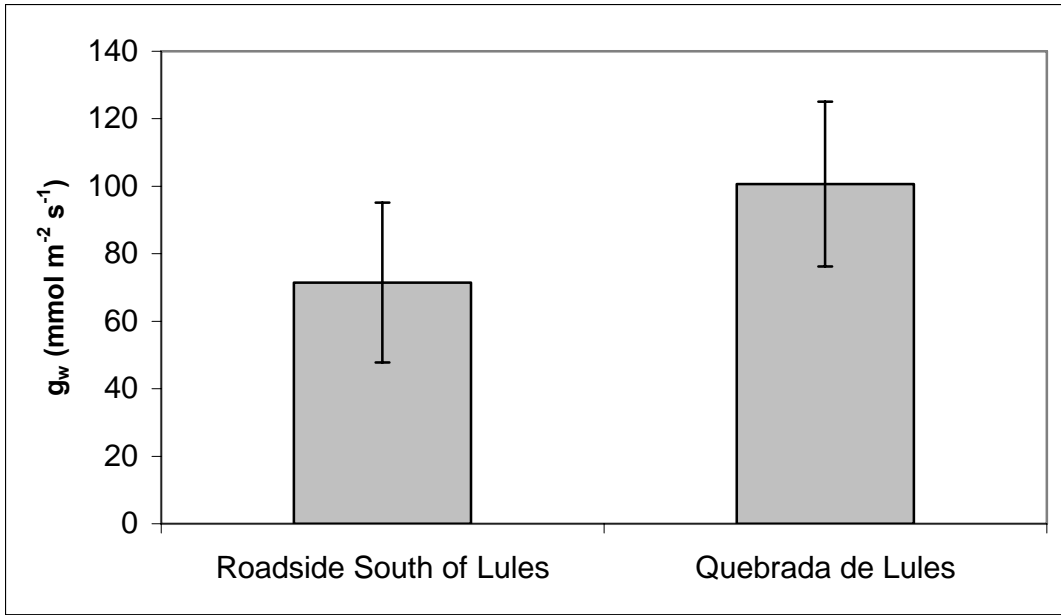


Figure 4.12 Mean stomatal conductances (g_w) of *Equisetum giganteum* stems in the vicinity of the Lules Valley, Tucumán Province, Northwest Argentina. Error bars are standard deviations.

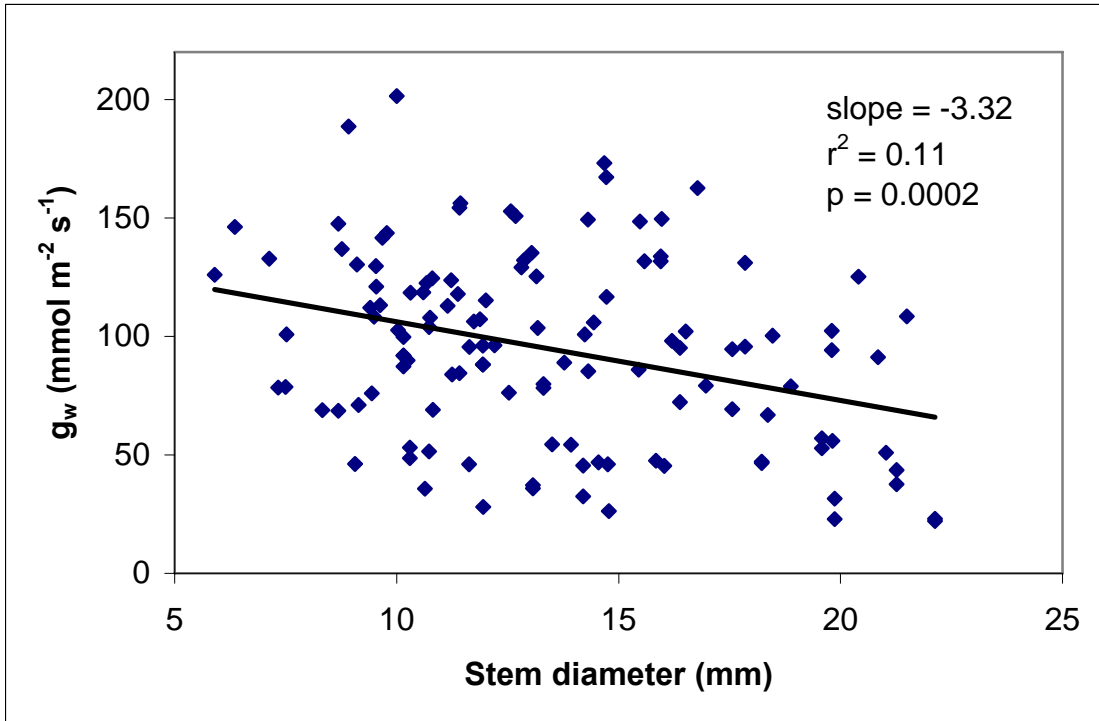


Figure 4.13 Relationship between stem diameter and stomatal conductance (g_w) of *Equisetum giganteum* across sites in northern Chile and northwest Argentina.

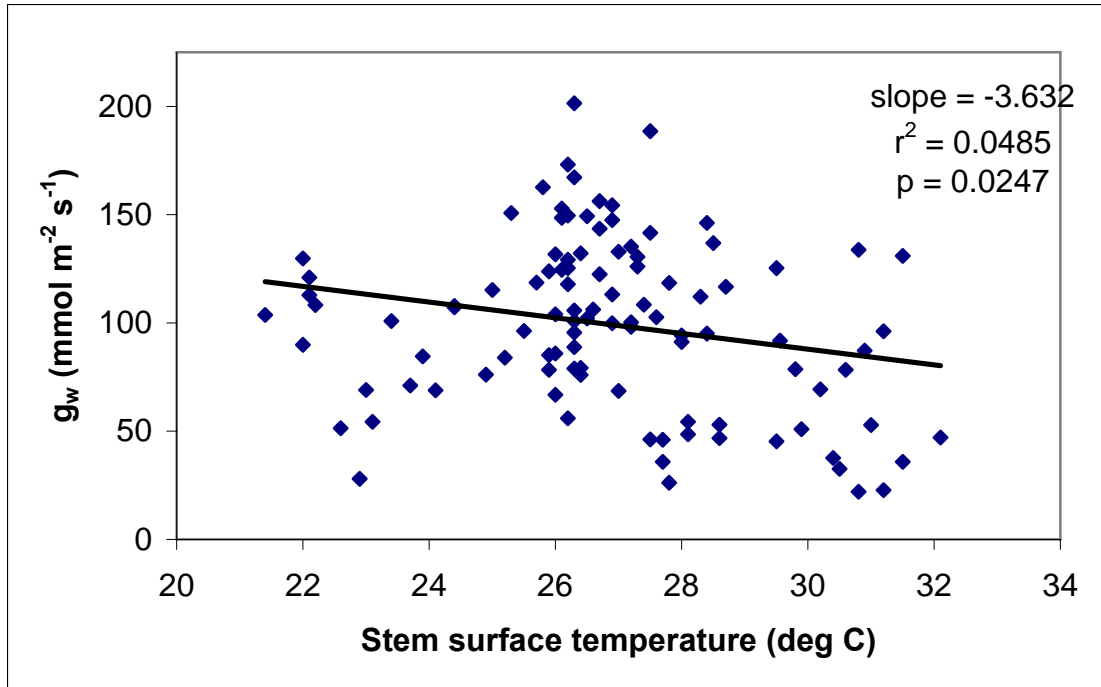


Figure 4.14 Relationship between stem surface temperature and stomatal conductance (g_w) of *Equisetum giganteum* across sites in northern Chile and northwest Argentina.

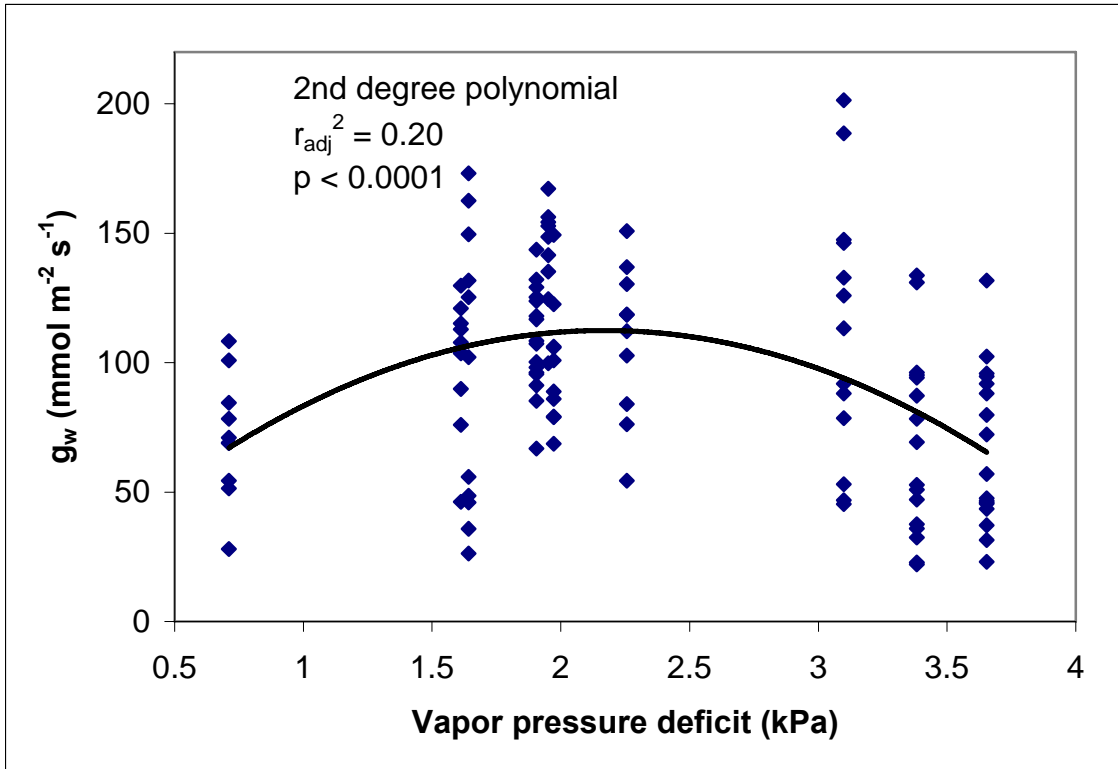


Figure 4.15 Relationship between vapor pressure deficit and stomatal conductance (g_w) for *Equisetum giganteum* stems across sites in northern Chile and northwest Argentina.

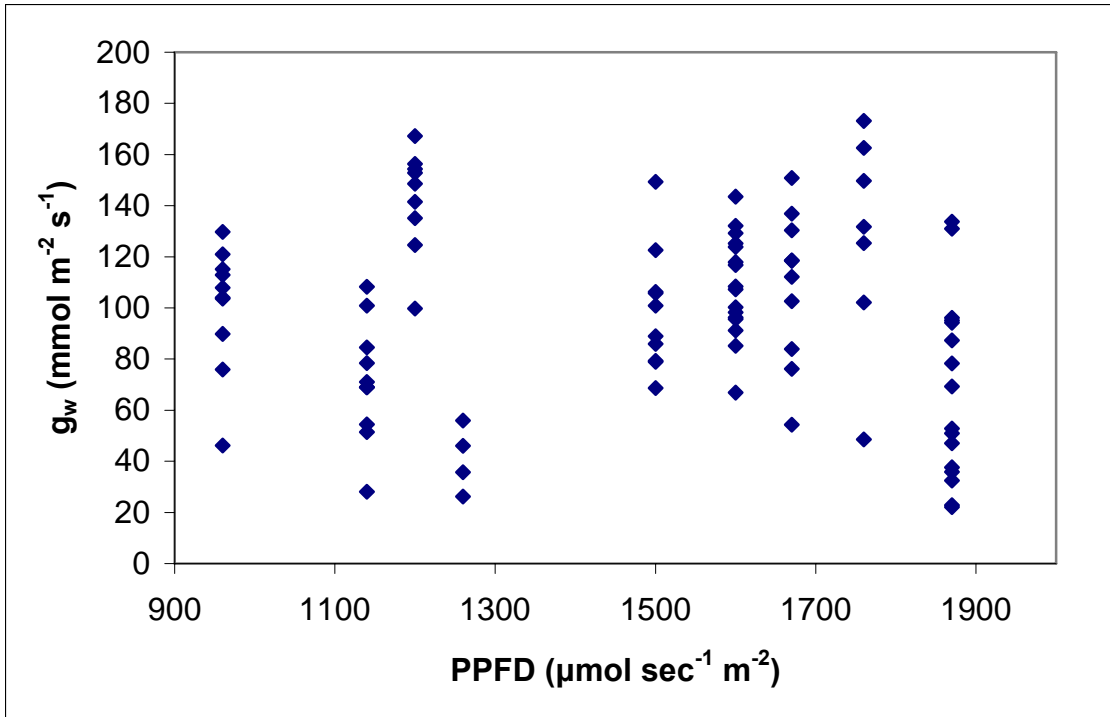


Figure 4.16 Relationship between photosynthetic photon flux density (PPFD) and stomatal conductance (g_w) for *Equisetum giganteum* stems across sites in northern Chile and northwest Argentina.

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5. ECOPHYSIOLOGY OF SALINITY TOLERANCE IN *EQUISETUM*
GIGANTEUM IN SOUTH AMERICA

Abstract

Soil salinity is a major environmental stress for wild and cultivated plants in many parts of the world. However, scientists have yet to determine a basic set of adaptations necessary for salinity tolerance in all vascular plants. Although a large body of research exists on plant responses to salinity stress, almost all such studies have dealt with spermatophytes. Therefore, studies of salinity tolerance mechanisms in pteridophytes hold promise for revealing the fundamental adaptations that all salt tolerant vascular plants may share. Perhaps the most basal pteridophytes to exhibit salinity tolerance are certain members of the genus *Equisetum*, including the giant horsetail, *Equisetum giganteum*. The most unusual environment in which *E. giganteum* occurs is the Atacama Desert in northern Chile. In the river valleys of the Atacama Desert, high soil salinity often greatly restricts native and cultivated plant diversity. *Equisetum giganteum* is the only pteridophyte that occurs in the high salinity regions of the Atacama valleys and often constitutes a significant vegetation component, forming dense stands of stems more than 4 m high with diameters reaching 3.8 cm. My field studies in the Atacama have revealed that *E. giganteum* exhibits similar stomatal conductances and photochemical efficiencies of photosystem II across a wide range of groundwater salinities. The focal species lowers the osmotic potential of its cytoplasm with increasing salinity and produces positive root pressure evidenced by guttation at the full range of salinities that it experiences in the Atacama Desert. *Equisetum giganteum* effectively maintains low Na

concentrations in its xylem fluid and cytoplasm when soil water Na is high. Furthermore, the species maintains very high K/Na ratios in its xylem fluid and cytoplasm when soil waters have very low K/Na ratios. These findings suggest that *E. giganteum* is well adapted to salinity stress and that efficient K uptake and Na exclusion may be important stress adaptations. This adaptation appears similar to that of the facultative halophyte fern *Acrostichum aureum*.

Introduction

Although there is much research on plant responses to salinity stress, almost all such studies of salinity tolerance have dealt with spermatophytes (especially angiosperms) because of their preeminent economic and ecological importance. Only a very few studies have addressed salinity tolerance in pteridophytes, partly because only a very few pteridophytes are known to exhibit salinity tolerance (Yensen 1999). Until now pteridophyte studies have exclusively involved the ferns (class *Pteropsida*) and usually only the gametophyte stage of the fern lifecycle. The current study helps to redress paucity of knowledge of pteridophyte salinity tolerance by investigating adaptations to this environmental stress in *Equisetum giganteum* L., a species belonging to a distinct pteridophyte class, the *Sphenopsida*.

The small (15 species), but widespread, genus *Equisetum* is the only surviving genus in a class that is morphologically and physiologically unique among living plants. Rothwell (1996) considers *Equisetum* the ‘most successful living genus of living vascular plants’ because of its wide distribution in physical and ecological space. However, the ecophysiology of *Equisetum* is poorly understood, especially for those species outside the

north temperate zone. In the current study, I investigated the comparative ecophysiology of *Equisetum giganteum* populations growing in salt-affected river valleys of extreme northern Chile, as well as freshwater areas of central Chile and northwest Argentina.

Equisetum giganteum is a widespread species associated with wetlands and other moist habitats at elevations above 500 m in the American tropics and extending to temperate South America (Hauke 1963). Coastal northern Chile (~18-20° S latitude) appears to be one of the few localities where this species grows in saline soil. Coastal Chile also includes some of the few low elevation (3-90 m) populations known for this species. *Equisetum giganteum* likely arrived in the northern Chilean valleys during a time when the region had a wetter climate and, unlike competing vegetation, adapted to increasing salinity in the valleys as the Atacama region became highly desertified during the past 1000 years (Graf 1994).

In a broad sense, saline soils and waters are those that have a high concentration of soluble ions, particularly sodium (Na^+), but also including calcium (Ca^{2+}), potassium (K^+), sulfate (SO_4^{2-}), chloride (Cl^-), and carbonate (CO_3^{2-}). In addition, boron (B) or selenium (Se^{2+}) may be present at toxic levels in saline soils (Orcutt and Nilsen 2000). Inland areas of high salinity usually occur in arid regions where evaporation is greater than precipitation and little leaching occurs (Fitter and Hay 2002), often combined with the presence of rocks high in sodium salts (Chapman 1975). Such conditions prevail in the Tarapacá province of extreme northern Chile. In the coastal Atacama Desert region of Tarapacá, annual precipitation averages less than 2.5 mm (González 1986) and the geology is rich in saline minerals (Ericksen 1981 cited in Goudie et al. 2002). The coastal area of this region, where populations of *E. giganteum* are found generally also

has saline river and irrigation waters, with high Na⁺, Cl⁻, and B concentrations (Ferreya et al. 1997).

Researchers have discovered a variety of direct and indirect harmful effects of salinity on plant physiology. The first salinity effect a plant experiences is the indirect effect of water uptake limitation due to reduction of soil water potential. Subsequent uptake of salts into plant tissues from the soil solution can result in direct harm to cellular metabolism through toxic effects of ions such as Na⁺ and Cl⁻ and to nutrient imbalances due to disruption of uptake or translocation mechanisms (Orcutt and Nilsen 2000).

Previous studies of salinity tolerance in pteridophytes:

Salinity tolerance occurs widely in the plant kingdom, but only a very few non-flowering plants and a handful of pteridophytes are known to be salinity tolerant (Yensen 2006, 1999). Until now, most studies of pteridophyte salinity tolerance have dealt with the gametophyte generation (Warne et al. 1996, Warne et al. 1999, Li and Ong 1997, 1998, Wendell et al. 1999, Lloyd and Buckley 1986). Auge et al. (1989) found that a salinity-tolerant mutant sporophyte of the aquatic fern, *Ceratopteris richardii* Brongn. grew better than the wild type under high salinity, but more poorly under low salinity. A few laboratory studies have been published on salinity tolerance of the sporophytes of floating aquatic ferns in the genera *Azolla* and *Salvinia* (Divarakan et al. 1980, Haller et al. 1974). A study of *Azolla pinnata* R. Brown showed that increased tolerance to higher salinity levels can be induced by first acclimating plants at intermediate salinity levels (Rai and Rai 1999). Masood et al. (2006) found that under salinity stress one species of *Azolla* increased antioxidant activity and more effectively maintained ion balance than did

another, although all *Azolla* species studied so far are relatively sensitive to salinity (Arora and Singh 2003).

Medina et al. (1990) undertook a field study of salinity tolerance of sporophytes of the mangrove fern *Acrostichum aureum* L. in Costa Rica. The investigators found that, with the exception of plants growing in shade, leaf cell sap osmotic concentration increased substantially with increasing soil salinity. The increase was largely due to increases in Na, Mg²⁺, and sucrose concentrations. The K/Na and Cl⁻/Na ratios of *A. aureum* were markedly higher than those found for typical (angiosperm) mangroves. Medina et al. (1995) consider the “mangrove fern”, *A. aureum* a facultative halophyte that maintains a significantly higher K/Na ratio than true mangroves.

Page (1997) discussed isolated colonies of the hybrid horsetail, *Equisetum x moorei*, on the southeast coast of Ireland. These colonies, which are the only known populations of this hybrid in the British Isles, are on dunes and grow quite near to the high tide line, suggesting considerable exposure to saline soil water and salt spray. Interestingly, only one parent, *E. hyemale*, of this hybrid is present in the British Isles. However, both the other parent, *E. ramossissimum*, and other populations of *E. x moorei* are present on the European mainland. These facts lead naturally to the hypothesis that sterile *E. x moorei* arrived in Ireland via vegetative propagules such as stem pieces stems or rhizomes of the hybrid may have floated to Ireland from the mainland. Page (1997) noted an experiment with this hybrid that involved floating cut stem pieces in seawater for various lengths of time and evaluating their ability to re-sprout. Remarkably, immersion for up to 10 days in seawater did not reduce the ability of stems to sprout roots and form new plants.

Objectives of this study:

1. Determine the range of groundwater salinities, as well as groundwater Na and K concentrations, in which *E. giganteum* grows in northern and central Chile and northwest Argentina.
2. Determine whether *E. giganteum* exhibits evidence of physiological stress, as measured by stomatal conductance and photosynthetic efficiency of photosystem II across salinity gradients.
3. Determine whether *E. giganteum* osmotically adjusts to changes in groundwater potential, indicating whether *E. giganteum* accesses saline groundwater or avoids salinity stress by accessing less saline sources of water.
4. Determine whether xylem fluid and cell sap composition of *E. giganteum* change with changes in groundwater salinity (EC).
5. Determine whether shoot cell sap composition of *E. giganteum* changes in different groundwater overall salinity (EC), Na and K concentrations.

Materials and Methods

Field setting

My study consists of fieldwork carried out in northern and central Chile and northwest Argentina from March-May 2004 and December 2005-February 2006. The Atacama Desert, which extends along the western part of Tarapacá, is the “driest terrestrial region on this planet” (Ehleringer et al. 1998). The only sources of water in the area are surface and ground water from high Andean precipitation (Margaritz et al. 1990) and coastal fog from the Pacific Ocean (Goudie et al. 2002). The Lluta River, which has highly acidic

water, and the Camarones River, which has alkaline water, are the major river systems in the area (Barr et al. 1993). The waters from the Andes become increasingly saline as they flow west towards the Pacific Ocean (Margaritz et al., 1990), as is also the case for the Lluta River (Escobar and Morales 1994) and presumably the Camarones River. In the Lluta river valley, groundwater salinity reaches nearly half that of ocean water near the mouth of the river (Husby, unpubl. data).

The environment in which these *E. giganteum* populations are found in northern Chile appears quite unusual for what is essentially a wetland species (Hauke 1963). There is virtually no precipitation and little natural vegetation in the region (Cortes 1976), although the major rivers do provide adequate moisture for the few crops growing in the irrigated alluvial soils of the river valleys (Ferreya et al. 1997). However, soils and irrigation water of the Lluta have high salinity levels and high Boron concentrations that limit agriculture to a few specially-adapted local cultivars of corn, onions, tomatoes, and alfalfa (Sotomayor et al. 1994). The Camarones and Tarapacá Rivers, where *E. giganteum* populations are also found, exhibit even higher salinity levels (J. Delatorre, unpubl. data, González 1986). In contrast, the environments in which *E. giganteum* grows in northwestern Argentina and in central Chile are much milder. Rainfall in these areas is either sufficient to support lush vegetation, prevent salinity buildup, or precipitation in the mountains is sufficient to prevent accumulation of excess salts.

Equipment

Overall salinity measurements

Salinity was measured using electrical conductivity (EC) meters. A Con 100 temperature compensating meter (Oakton Instruments, Vernon Hills, Illinois) was used for groundwater and riverwater measurements. A Cardy Twin Conductivity Meter B-173 (Horiba Ltd, Tokyo, Japan), also temperature compensating, was used for measuring guttation fluid EC. Estimation of ppm was done via an approximate conversion factor $700 \times \text{EC}$ (Bunt 1988). This conversion to TDS accounts for the high proportion of sulfate ions in many north Chilean waters (Sotomayor 1994, Escobar 1994).

Na and K measurements

Sodium ion concentrations were measured using a Cardy Na Compact Ion Meter C-122 (Horiba Ltd, Tokyo, Japan). Potassium ion concentrations were measured using a Cardy K Compact Ion Meter C-131 (Horiba Ltd, Tokyo, Japan).

Water and osmotic potential measurements

Water potentials were measured using an HR-33T Dew Point Microvoltmeter (Wescor Inc., Logan, Utah). C-30 chambers were used for total stem water potential measurements and a C-52 chamber was used for most sap osmotic potential measurements. Sap was expressed using either a Markhart Leaf Press (Wescor Inc., Logan, Utah) (Markhart 1985) for side branches or a heavy-duty garlic press for young main shoots. For larger samples of groundwater and expressed young shoot sap, an

Advanced Instruments Model 3300 Micro Osmometer (Advanced Instruments, Norwood, Massachusetts), which is based on the freezing point method, was used.

Chlorophyll fluorescence measurements

Maximum photochemical efficiency of photosystem II (Fv/Fm) was measured using an Opti-Sciences OS-1 Modulated Fluorometer (Opti-Sciences, Inc., Hudson, New Hampshire) with dark adaptation clips was used to measure chlorophyll fluorescence of side branches of young fertile *E. giganteum* stems that were of mature size, but had green cones. Selecting these stems allowed us to standardize the technique among sites and minimize the confounding effect of differences in stem and side branch age. Side branches appear to senesce with age, even when the central stem remains green and even produces new branches (Figure 5.1). I measured Fv/Fm of one or two side branches for each stem.

Stomatal conductance measurements

Maximum stomatal conductances to water vapor (g_w) and transpiration of main stems of giant horsetails (see Chapter 4) were measured using a Li-Cor LI-1600 steady state porometer (Li-Cor Biosciences, Lincoln, NE) by sealing the chamber vertically against an internode in the mid section of young but mature and healthy stems with green branches at each site. Health of side branches was used as a major criterion of main stem health because these branches senesce as stems age (Figure 5.1). Stems were selected such that the sheaths were turning brown (a sign of stem maturity in *E. giganteum*, (Hauke 1963)) and the branches had extended to a greater length than the internodes, but did not exhibit signs of senescence (such as browning of the branch tips). Stomatal

conductances were measured near the middle of the main vertical portion, since there was detectable variation in g_w between the middle and top portions of the stem (see Chapter 4). I also tracked stomatal conductance of a population of stems over the diurnal period (8:30 am to 8:00 pm) and found that the maximum stomatal conductance of stems occurred, on average, between 10:00 am and 12:00 pm for stems exposed to full sun (see Chapter 4). Therefore, I measured g_w between those times. Also, by mid-morning guttation fluid and dew had evaporated, leaving the stems dry, a necessary prerequisite for use of the porometer. At that time of year, sunrise was at 6:44 am and sunset was at 6:39 pm, although the actual time sunlight reached plants in the valley was generally later due to the shadows cast by the valley walls.

Statistical analyses

Curve fitting was carried out using JMP (version 7, SAS Institute Inc., Cary, North Carolina).

Specific methods addressing objectives

1. Determine the range of groundwater salinities in which *E. giganteum* grows in northern and central Chile and northwest Argentina

Populations of *E. giganteum* in Chile and Argentina were explored during three expeditions, in December 2002, March-May 2004 and December 2005 – February 2006. Sampling focused on three areas where this species grows extensively: the river valleys of Tarapacá province in extreme northern Chile, where surface and groundwaters become increasingly saline as they flow west towards the Pacific Ocean (Margaritz et al. 1990),

thus providing a natural gradient of salinity for study of *E. giganteum* physiological tolerance. In addition, groundwater was sampled in the vicinity of *E. giganteum* colonies in two areas not affected by salinity: The Elqui valley in central Chile and mesic portions of Salta and Tucumán provinces in northwest Argentina.

2. Determine whether *E. giganteum* exhibits evidence of physiological stress across salinity gradients

Because reduction in the efficiency of the photosynthetic apparatus and reduction in stomatal conductance are typical reactions to salinity stress (Larcher 2003), F_v/F_m and maximum stomatal conductance were measured in healthy mature stems across a range of sites with differing groundwater salinities in northern Chile and northwest Argentina from March-May 2004.

3. Determine whether *E. giganteum* osmotically adjusts to changes in groundwater potential

Total and osmotic water potentials of branches (ψ_t), as well as osmotic potential (ψ_s) of young shoots, were measured across a range of sites varying in groundwater salinity in northern and central Chile. Measurements of pre-dawn water potential allow inference about the water potentials of the water sources *E. giganteum* is utilizing in northern Chile in comparison to measured water potentials of ground and surface water sources (Berger and Heurteaux 1985). Branches were sampled in the pre-dawn hours and sealed in C-30 chambers in the field. Chambers were placed in a cooler (to prevent tissue degradation during equilibration) with ice for vapor pressure equilibration and total water potential was measured in a room of stable temperature 4-6 hours later. After ψ_t was measured,

branches were removed from the chambers, sap expressed to saturate a filter paper disk with a Markhart Leaf Press and osmotic potential measured with a C-52 chamber.

4. Determine whether xylem fluid composition of *E. giganteum* changes with changes in groundwater salinity

Healthy young *Equisetum* shoots readily guttate under conditions of high humidity and negligible transpiration often at night or early morning (Johnson 1936, Nobel 1999).

Equisetum species are able to guttate because they have hydathodes (Johnson 1936) that serve as exit routes for xylem water when there is positive hydrostatic pressure (called root pressure) in the xylem (Nobel 1999). The exit of this xylem water, termed guttation, results in the formation of small droplets in the vicinity of the hydathodes (Figure 5.2).

These droplets provide a non-disruptive way to measure xylem fluid properties in the field. Because solutes in xylem fluid accumulate over time on young stems (Figure 5.3a) it is necessary to rinse these deposits off before guttation occurs to avoid contamination of the xylem fluid with extra solutes. Thus, in the late afternoon before measurement, stems to be sampled were tagged and thoroughly rinsed with distilled water to remove surface deposits. Tops of stems were then enclosed with plastic garbage bags to prevent evaporation of guttation fluid. Guttation fluid was then sampled in the pre-dawn hours (3:00 – 5:00 am) and measured for EC, Na concentration and K concentration. Also, at the Tarapacá valley site, the white guttation fluid deposits (Figure 5.3a) were sampled by wiping water saturated Cardy meter sampling sheets across them and measuring the Na concentration and K concentrations to obtain a time integrated measure of guttation fluid composition.

5. Determine how shoot cell sap composition of *E. giganteum* is related to groundwater osmolality, overall salinity (EC), Na and K concentrations

The tops of young developing main stem shoots (Figure 5.3b) were sampled at sites of varying groundwater salinities. These succulent new shoots tend to be less physiologically sensitive to environmental factors than older mature shoots (see Chapter 4) and thus provided a more consistent comparison of differences in cell sap composition and osmotic potential among sites. Also, because these shoots are unlikely to be photosynthetically independent of the clone, they should provide an integrated measure of the cell sap composition of the overall clone. The shoots sampled across sites were broken off, sealed in plastic bags to prevent desiccation and transported in a cooler filled with ice back to the laboratory for measurement. Shoots were crushed in a sturdy garlic press and the expressed sap was measured for EC, Na, K and osmolality. Furthermore, groundwater samples from each site were measured for the same parameters. Contribution of cell sap K to osmotic adjustment was calculated using a temperature adjusted model of the chemical potentials of KCl solutions of various concentrations (Rawlins and Campbell 1986).

Results

Qualitative observations

Equisetum giganteum showed clear evidence of rhizomes and roots growing in a surface water, suggesting that it does not avoid surface water salinity. Rhizomes were observed in surface streams in the Tarapacá valley (Figure 5.4), the Camiña valley and in the Lluta valley. Furthermore, there was clear evidence of surface salt accumulation in areas

where *E. giganteum* was growing (Figure 5.5). In two valleys gametophytes and young sporophytes were found, indicating that sexual reproduction is occurring (Figure 5.6). Since the gametophytes and young sporophytes must access water sources near the surface, these must be able to cope with the higher salt levels there. Guttation was observed in the pre-dawn hours at all sites on developing young stems (Figure 5.2), but not on mature stems. Also, guttation was less frequent at the highest salinity sites than in others.

Objective 1

Equisetum giganteum was observed growing in habitats with groundwater electrical conductivities (EC) ranging from 0.44 (in northwest Argentina) to 25.7 mS/cm (near the mouth of the Lluta river, Chile, in 2004). The EC range corresponds to concentrations of 315 to 17,990 mg/L total dissolved solids (TDS). In comparison, full-strength seawater has a salinity of ~35,000 mg/L (EC ~44-50 mS/cm). At all sites, groundwater EC was measured in close proximity to an *E. giganteum* stand and the water table was never more than ~0.5 m below the surface. The highest EC was measured near the coast north of the city of Arica where Lluta river reaches the Pacific. In 2006, the groundwater EC at this site was considerably less, only 9.6 mS/cm, whereas EC at a much further inland site, along the road to Mamiña, was 14.5 mS/cm, the highest measured that year. Clearly there is variability in groundwater salinity, likely due to variation in the amount of precipitation in the Andes.

At some sites, groundwater reached the surface and was measured where *Equisetum* stems were growing out of the surface water. Since *Equisetum* generally draw

water from saturated soil near the water table (Hauke 1963), it seems likely that these salinities represent those of the actual water sources utilized by the plants. Furthermore, the soil above the water table in the Atacama valleys receives its moisture exclusively from the water table (via capillarity) and there is no rain to leach salts away. Hence, evaporation should cause the soil water above the water table to be even more saline than the groundwater (Figures 5.5, 5.6).

Groundwater Na concentration was highly correlated with EC (Figure 5.7), whereas K was not significantly correlated with EC (Figure 5.8). Although there was a significant linear association between Na and K concentrations, the correlation was weak (Figure 5.9).

Objective 2

There was no significant effect of groundwater salinity on stomatal conductance (g_w) (Figure 5.10). There was a significant negative association between increasing EC and F_v/F_m , but the correlation was very weak (Figure 5.11). Also, some of the highest F_v/F_m values were measured at a site of relatively high EC. Average F_v/F_m and average stomatal conductance showed no significant association (Figure 5.12).

Objective 3

Both ψ_t and ψ_s of side branches were consistently lower than groundwater potential at all sites. Furthermore, at the sites with the highest groundwater EC, branch ψ_t and ψ_s were lowest (Figure 5.13).

Objective 4

Guttation fluid EC was highly positively correlated with groundwater EC (Figure 5.14). There was a weak but significant correlation between groundwater Na and guttation fluid Na, but the significance of this relationship disappears when a natural log transformation is used to reduce variance heteroskedasticity (Figure 5.15). A stronger and more robust correlation was found between groundwater K and guttation fluid K (Figure 5.16), which remains significant when log transformed to ameliorate heteroskedasticity. The Na/K ratio in guttation fluid showed no significant correlation with this ratio in groundwater (Figure 5.17). Guttation fluid K was significantly correlated with guttation fluid Na, with K concentration increasing 4 fold for every unit increase in Na. (Figure 5.18). The whitish stem deposits had a low mean proportion of Na to K (0.12, 95% confidence interval from 0.04 to 0.21) as did the fresh guttation fluid (0.20, 95% confidence interval from 0.14 to 0.26) and there was a significant exponential increase in K concentration with increasing Na concentration (Figure 5.19). In contrast, the mean Na/K of groundwater was two orders of magnitude higher (95% confidence interval from 6.1 to 51.5).

Objective 5

Although shoot sap Na concentration increased initially with increasing groundwater Na, at the highest groundwater EC, the increase had stopped (Figure 5.20). A similar pattern was observed for K (Figure 5.21). There was no significant association between groundwater Na/K and shoot sap Na/K (Figure 5.22). Shoot sap EC exhibited a non-linear association with groundwater osmolality, showing an initial decrease and then an

increase at the highest osmolality levels (Figure 5.23). The pattern approximately paralleled that of shoot sap K, suggesting K as a major component of shoot sap EC as was corroborated by the very strong linear correlation between shoot sap K and shoot sap EC (Figure 5.24). In contrast, the association of shoot sap Na and shoot sap EC, although significant, was considerably weaker and showed a rapid leveling off (Figure 5.25). Shoot sap K increased initially with shoot sap Na, but then leveled off (Figure 5.26). Shoot sap EC and shoot sap osmolality were highly correlated (Figure 5.27). There was a significant linear relationship between shoot sap K and osmolality (Figure 5.28) and the mean contribution of K (as KCl) to shoot sap osmotic potential was 67.7 % with the contribution leveling off at more negative sap osmotic potentials (Figure 5.29).

Discussion

These results indicate that *Equisetum giganteum* maintains a high degree of homeostasis in stomatal conductance, photosynthetic physiology, and root function over a broad range of groundwater salinities in the Atacama desert and northwestern Argentina. Since root pressure “develops only when root systems are healthy” (Kramer and Boyer 1995), the observation that *E. giganteum* generates root pressure, even under highly saline conditions, suggests that its roots are coping well with groundwater salinity. These findings suggest that *E. giganteum* is best classified as a facultative halophyte, like *Acrostichum aureum*, because it adapts well to moderate salinities and maintains a low Na/K ratio in its cell sap (Medina et al. 1995). In contrast, if *E. giganteum* were, like nearly all pteridophytes (Yensen 1999), a typical glycophyte exhibiting salinity stress, I would expect to have seen evidence of water stress (reduced g_w) and photosynthetic stress

(reduced F_v/F_m) at these salinities (Netondo et al. 2004). A saturated soil paste EC of 4 mS/cm is considered saline, meaning that many of the soils where *E. giganteum* grows in northern Chile contains amounts of salts well in excess of levels that causes substantial yield reductions in crops (Munns and Tester 2008). *Equisetum* growing in such saline conditions is especially surprising because these species are almost never associated with saline habitats and the highest salinities experienced by *E. giganteum* in northern Chile are in the range of those tolerated only by halophytes (Brady and Weil 1996). These unexpected findings suggest that *E. giganteum* has very effective mechanisms for mitigating salinity stress.

The preferential accumulation of K by *E. giganteum* is not surprising given the high K requirement exhibited by the related species *E. arvense* (Andersson 1999). Shoot sap EC and shoot sap osmolality were highly correlated (Figure 5.27) suggesting that ionic solutes such as K are the major contributors to osmotic adjustment in *E. giganteum*, rather than nonpolar osmolytes such as cytoplasmically compatible sugars. The fact that the contribution of K to shoot sap ψ_s decreases with more negative ψ_s (Figure 5.29) coupled with the lack of leveling off of shoot sap osmolality with increasing EC (Figure 5.27), suggests that polar solutes besides K contribute increasingly to ψ_s as osmotic adjustment becomes greater.

Recent studies have shown that increasing concentrations of polar solutes can enhance hydraulic conductivity of angiosperm vessels by affecting the properties of pectins in vessel pit membranes (Trifilo et al. 2008, Gascó and Gortan 2008). K has been shown to be particularly effective in this regard (Gascó and Gortan 2008, Zwieniecki et al 2001) and there is evidence of possible active regulation of xylem conductivity through

interactions with the phloem (Zwieniecki et al. 2004). Sodium chloride may play a role in regulating xylem conductivity in mangroves (López-Portillo et al. 2005). Although this phenomenon has not yet been studied in pteridophytes or gymnosperms, it is possible that preferential accumulation of K by *E. giganteum* under saline conditions facilitates water uptake by enhancing hydraulic conductivity of the xylem, thus requiring less osmotic adjustment to extract water from the soil. However, important questions remain regarding the extent to which this phenomenon may function *in planta* (van Leperen 2007).

Plants exhibit three types of salinity tolerance: 1) Initial osmotic stress tolerance at the onset of salinity stress, involving osmotic adjustment and increased leaf area and stomatal conductance under greater stress to produce a more negative water potential to extract soil water, 2) Exclusion of Na at the roots and 3) Tolerance of plant tissues to Na accumulation (Munns and Tester 2008). The osmotic response tends to happen initially and later the response to toxic ions becomes manifest (Munns and Tester 2008). The fact that Na does not accumulate in either xylem fluid of older shoots or cell sap of young shoots suggests that *E. giganteum* effectively excludes this ion at the roots during water absorption. In addition, the fact that older main stems remain green and capable of producing new side branches for at least two years (Figure 5.1) suggests that *E. giganteum* does not tolerate salinity by allowing buildup of Na in older stems. The fact that lateral branches have a shorter lifespan than central stems allows for the possibility that Na may accumulate in the branches leading to their senescence. However, Na contents of old and young branches were not measured in this study. Also, because Na content of roots was not measured in this study, it is not possible to rule out Na

accumulation in roots, as occurs, for example, in a corn variety selected to grow in high salinity areas of the Atacama river valleys (Bastias et al. 2004).

In another study, I found that g_w of mature stems is more sensitive to environmental factors (light and other site differences) than is g_w of young developing stems (Chapter 4). Along with the guttation frequently observed in developing stems (Figure 5.2), these observations suggest that *E. giganteum* has mechanisms for maintaining higher water potentials in developing stems than in mature stems. Guttation of developing stems clearly shows that *E. giganteum* is able to generate substantial root pressure, even when its water source is moderately saline, and can selectively channel this pressure to developing stems (Figure 5.2). Such a strategy would allow young stems to elongate even when salinity substantially lowers the osmotic potential of the groundwater.

Theoretical and experimental studies of root pressure by Pickard (2003a, 2003b) provide compelling arguments that active osmolyte uptake mechanisms in root symplast play a crucial role in generating positive xylem pressures during conditions of low transpiration. Because *E. giganteum*, like other *Equisetum* species (Johnson 1936, Schaffner 1933), exhibits extensive guttation it would appear to be an excellent subject for testing the generality of Pickard's (2003a) root pressure model and exploring its implications. Equiseta have the advantage of consistently guttating without requiring cutting off of the stem as in the typical herbaceous plants used for such studies. Use of *Equisetum* as experimental plants would allow for likely greater precision in studying whole plant water relations without the need for extreme intervention to observe root pressure.

Further experimental studies are necessary to elucidate the nature of salinity tolerance in *E. giganteum*. Unfortunately, the northern Chilean provenance of this species has proven recalcitrant to attempted cultivation in a variety of settings in the US. By contrast, plants of the northwest Argentinian provenance, which grow in non-saline areas, adapt much more readily to cultivation. Experimental studies of the Argentinian clones may provide insight into how much of the salinity tolerance of the Atacama form of *E. giganteum* is because of local selection and how much is simply because of physiological mechanisms already present in this species of very ancient lineage. The occurrence of salinity tolerance in a sterile *Equisetum* hybrid in Ireland (Page 1997) suggests that a significant level of “pre-adaptation” to this stress may be present in the genus. In any case, the fact that a plant from such an ancient fern lineage (Qiu et al. 2007, Hauke 1963) exhibits the ability to thrive in an environment where levels of salinity, irradiance and vapor pressure deficit are high, is quite unexpected. Since low humidity tends to exacerbate salinity stress in plants that exhibit little salinity tolerance (An et al. 2001), the fact that *E. giganteum* thrives in the Atacama valleys appears all the more remarkable, and provides further evidence that the Atacama form of *E. giganteum* exhibits a considerable degree of stress tolerance. As Rothwell (1996) has observed, the remarkable ecological range occupied by horsetails makes it “extremely difficult to regard *Equisetum* as a relict”.

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Figures



Figure 5.1 Approximately two year old stem, first labeled as a young mature stem for stomatal conductance measurement in spring 2004 and re-located in January 2006, Chiza valley, Tarapacá Province, northern Chile.

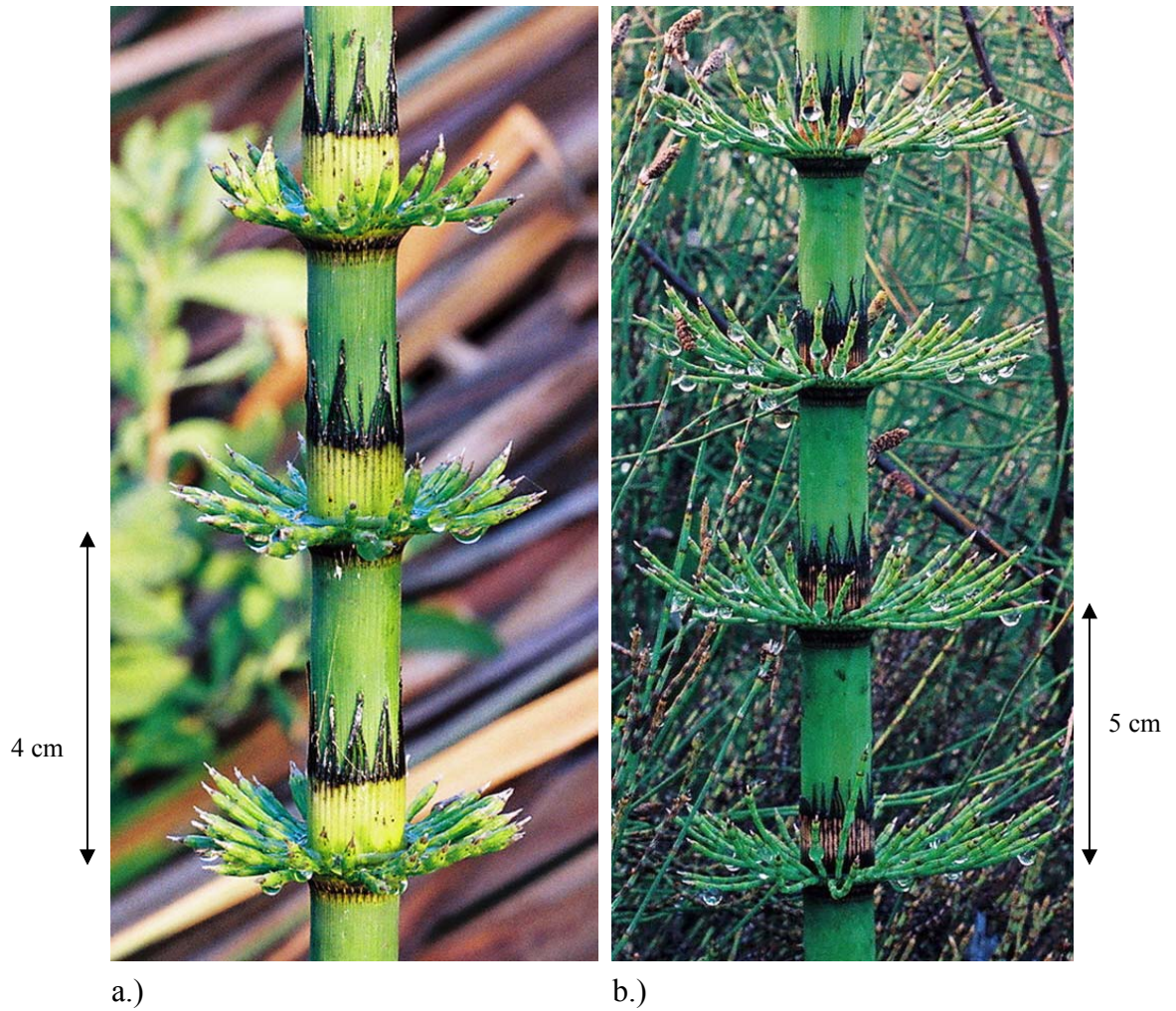


Figure 5.2 Guttation of *Equisetum giganteum* stems. (a) High salinity site near the mouth of the Rio Lluta (groundwater EC ~ 25 mS/cm) (b) Lower salinity site, Puente Tana, Camiña valley (groundwater EC ~ 3 mS/cm).



6 cm

a)



8 cm

b)

Figure 5.3 a) Deposits of guttation fluid solutes on young *E. giganteum* stem, Tarapacá valley, Tarapacá province, northern Chile. b) Developing *E giganteum* shoots. Lluta valley, Tarapacá province, northern Chile.



Figure 5.4 Rhizome of *Equisetum giganteum* growing in surface stream, Tarapacá valley near the town of Tarapacá.



Figure 5.5 Expanding shoots of *Equisetum giganteum* growing amid surface salt incrustations, Tarapacá valley, Tarapacá province, northern Chile.



Figure 5.6 *Equisetum giganteum* gametophyte (a) young sporophyte (b) and salt encrustations (c), near Molinos, Lluta valley, Tarapacá Province, northern Chile.

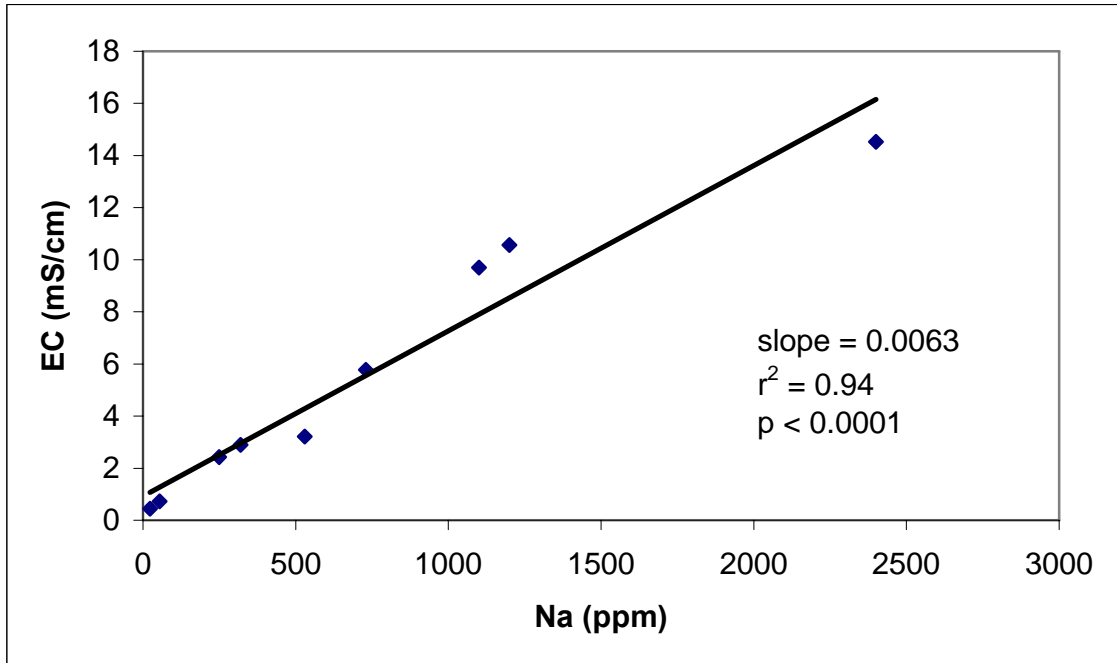


Figure 5.7 Groundwater Na concentration vs. electrical conductivity in river valleys of Tarapacá province, northern Chile, and the Elqui valley, central in the vicinity of *Equisetum giganteum* populations.

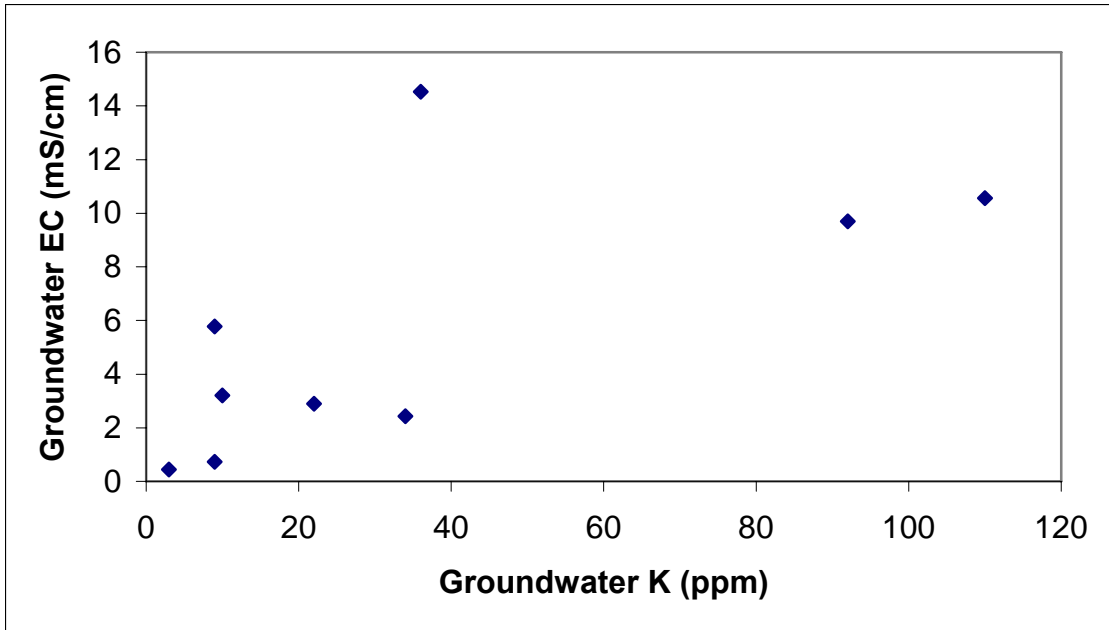


Figure 5.8 Groundwater K concentration vs. electrical conductivity in river valleys of Tarapacá province, northern Chile, and the Elqui valley, central in the vicinity of *Equisetum giganteum* populations ($p = 0.0564$).

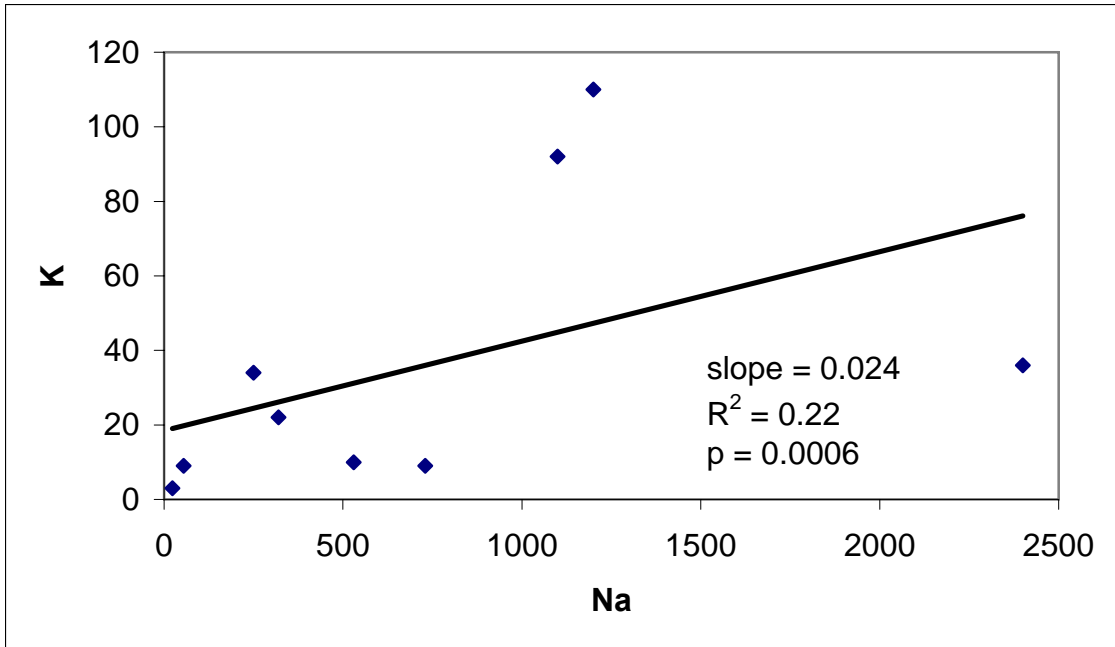


Figure 5.9 Relationship between groundwater K concentration vs. Na concentration in river valleys of Tarapacá province, northern Chile, and the Elqui valley, central in the vicinity of *Equisetum giganteum* populations.

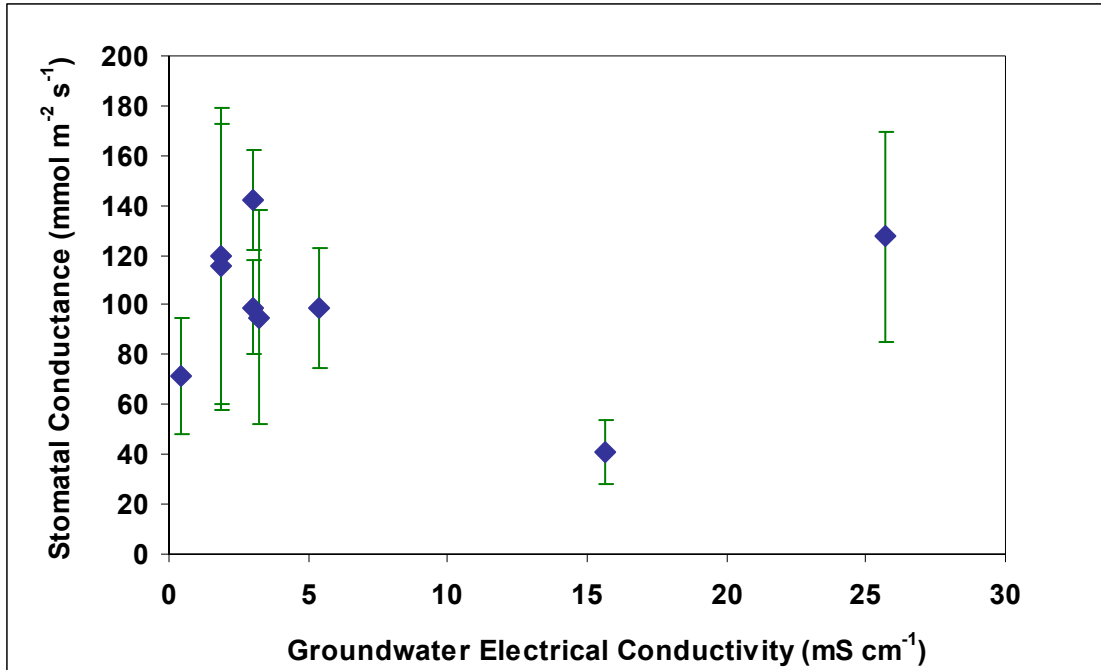


Figure 5.10 Relationship between groundwater electrical conductivity and *Equisetum giganteum* main stem stomatal conductance across sites in northern and central Chile. No significant correlation was detectable ($p = 0.651$)

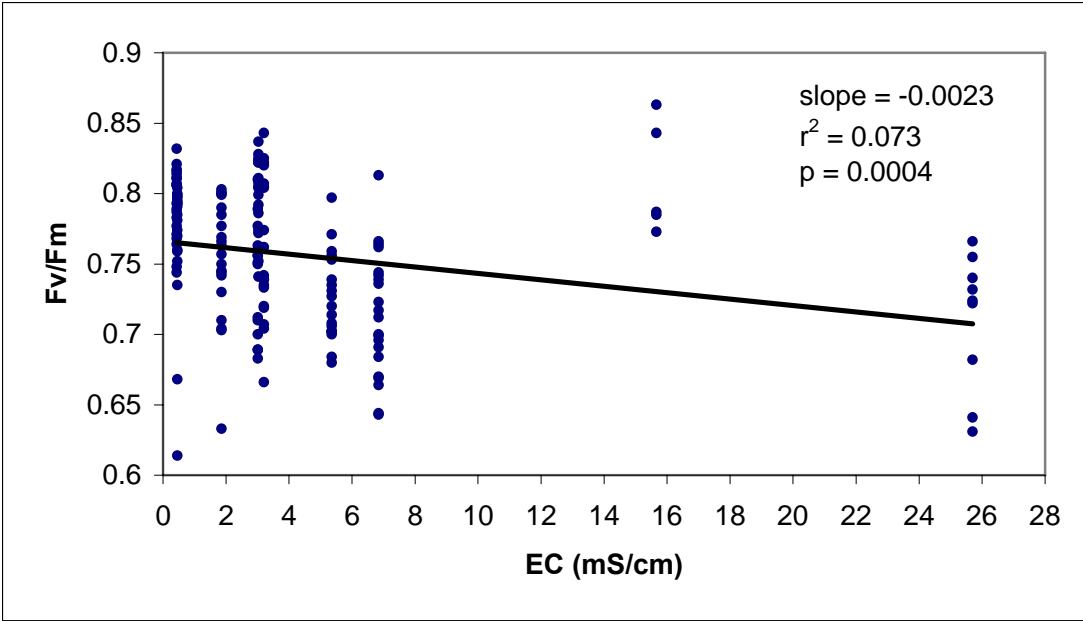


Figure 5.11 Relationship between groundwater electrical conductivity and *Equisetum giganteum* branch chlorophyll fluorescence across sites in northern Chile and northwest Argentina.

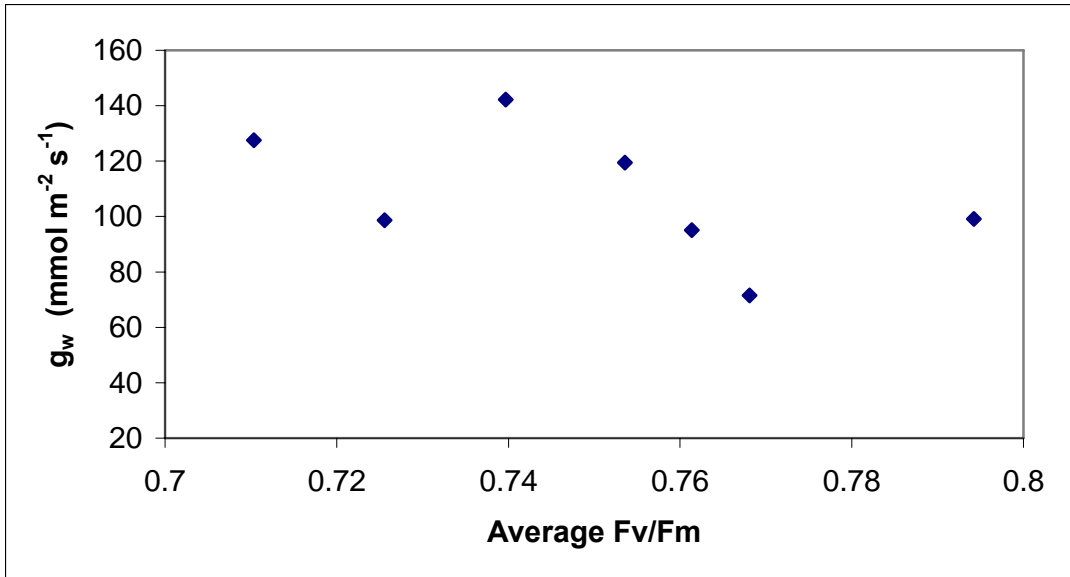


Figure 5.12 Relationship between average chlorophyll fluorescence and average stomatal conductance of *Equisetum giganteum* stems across sites in northern Chile and northwest Argentina. No significant correlation was detectable ($p = 0.233$).

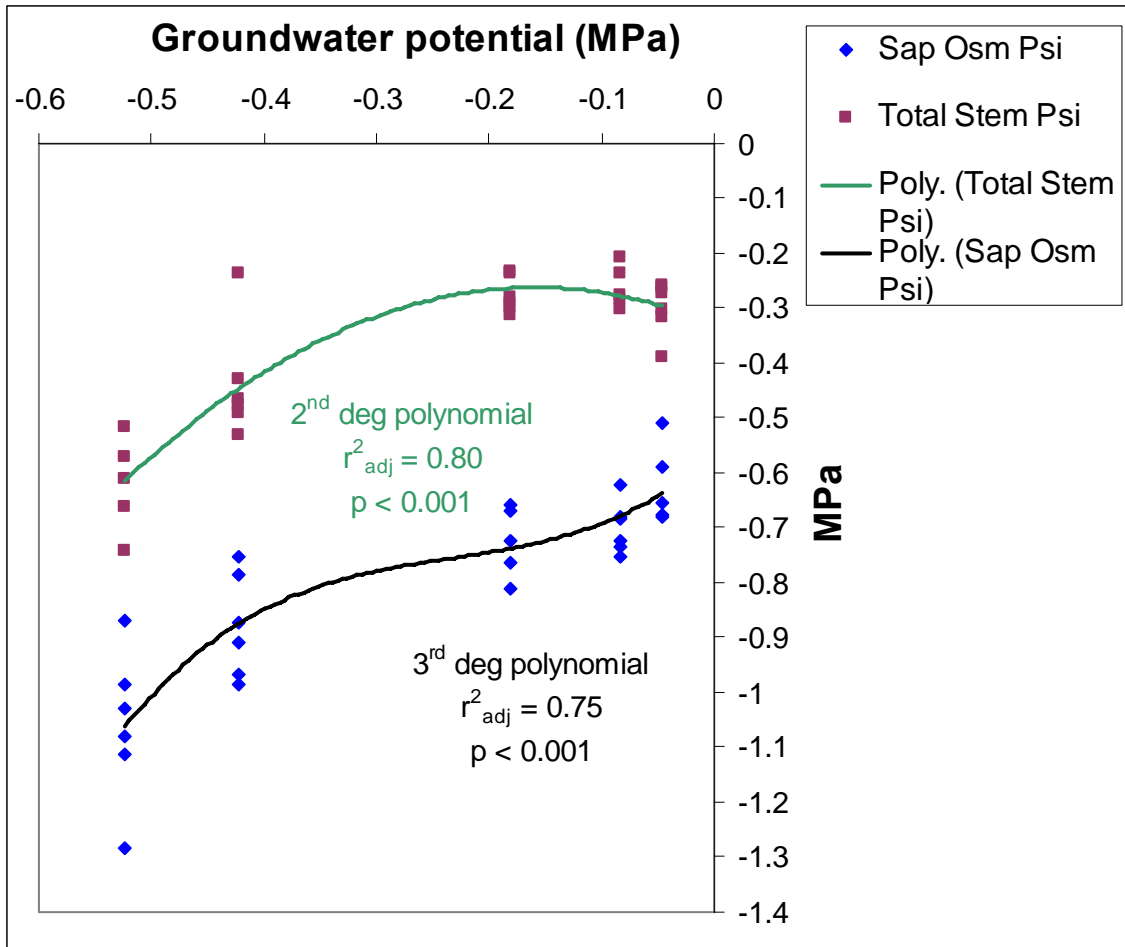


Figure 5.13. Total stem water potential and shoot sap osmotic potential for young mature stems of *Equisetum giganteum* in Chile. These are pre-dawn values.

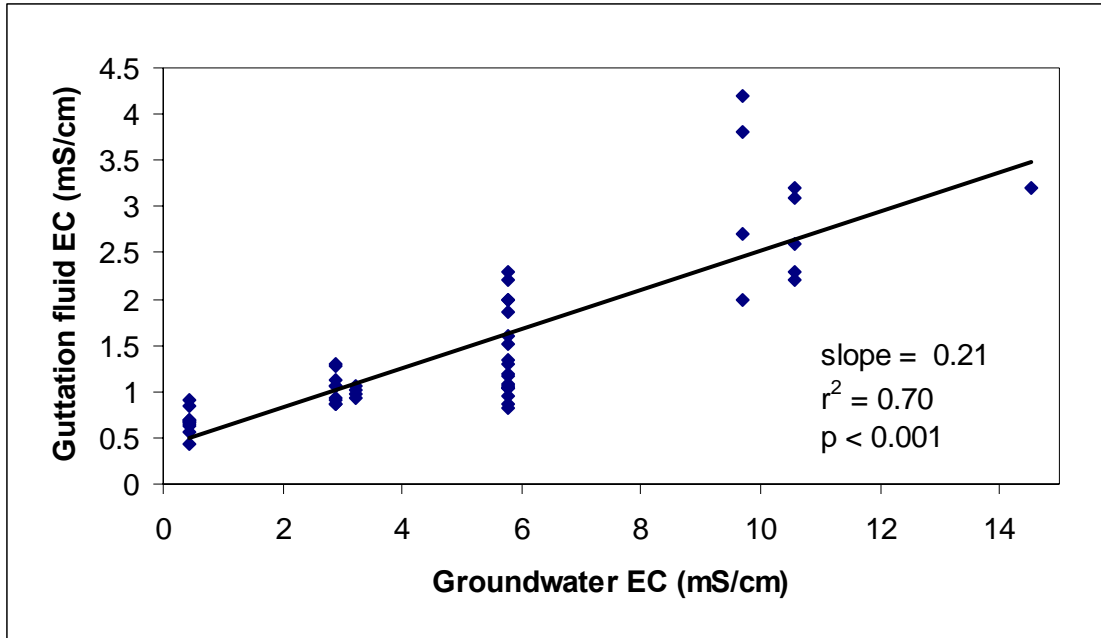


Figure 5.14. Relationship between groundwater electrical conductivity and *Equisetum giganteum* guttation fluid electrical conductivity across sites in northern and central Chile.

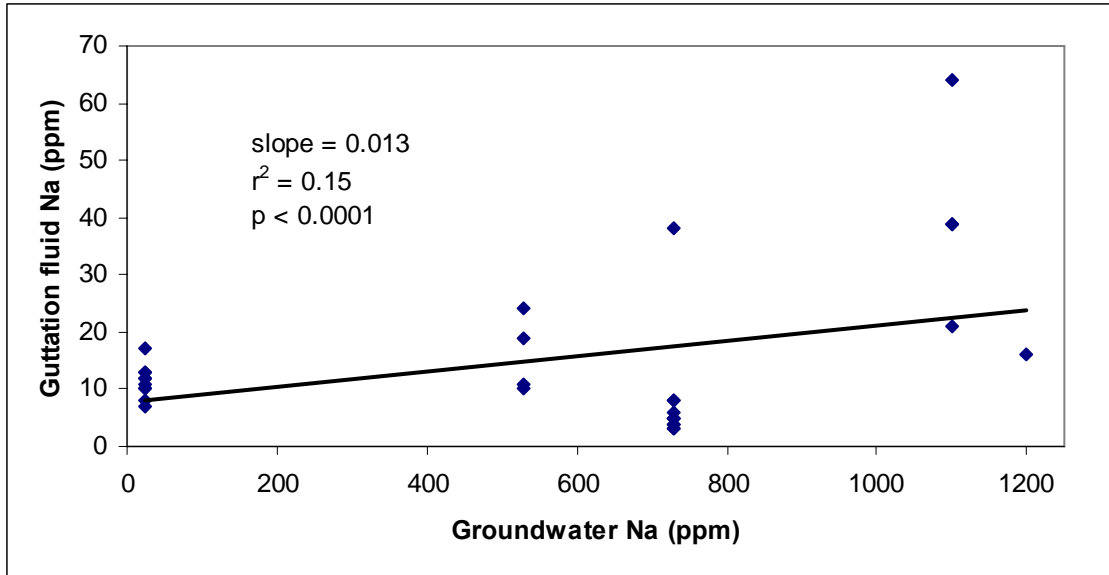


Figure 5.15 Relationship between groundwater Na concentration and *Equisetum giganteum* guttation fluid Na concentration across sites in northern and central Chile. After natural log transformation to reduce heteroskedasticity, correlation is not longer significant ($p = 0.3341$)

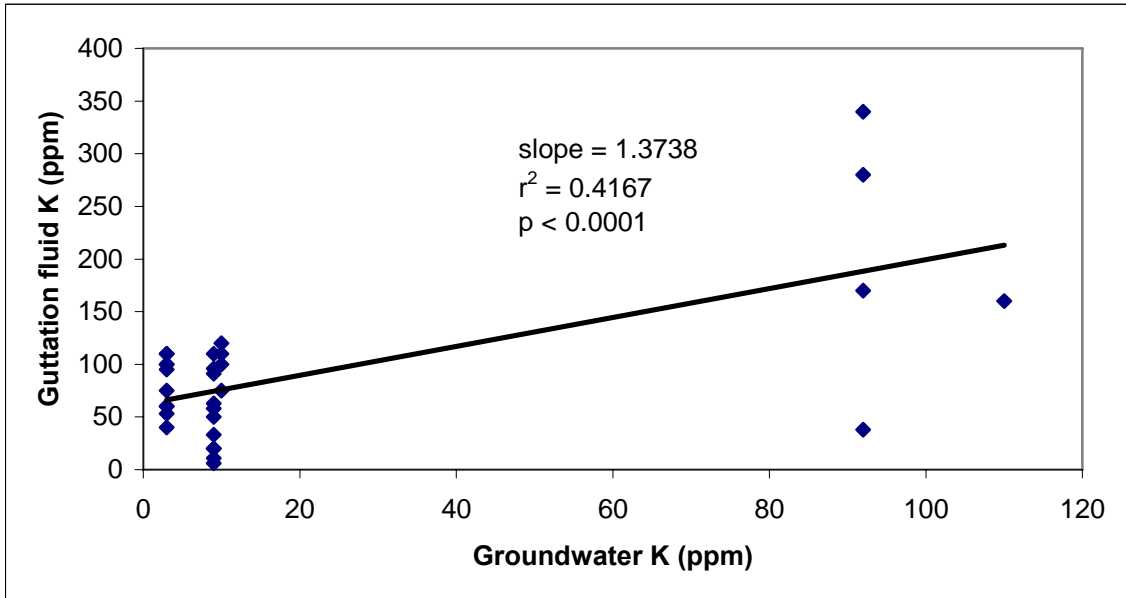


Figure 5.16 Relationship between groundwater K concentration and *Equisetum giganteum* guttation fluid K concentration across sites in northern and central Chile. When log transformed to reduce variance heteroskedasticity, $r^2 = 0.18$ and $p = 0.020$.

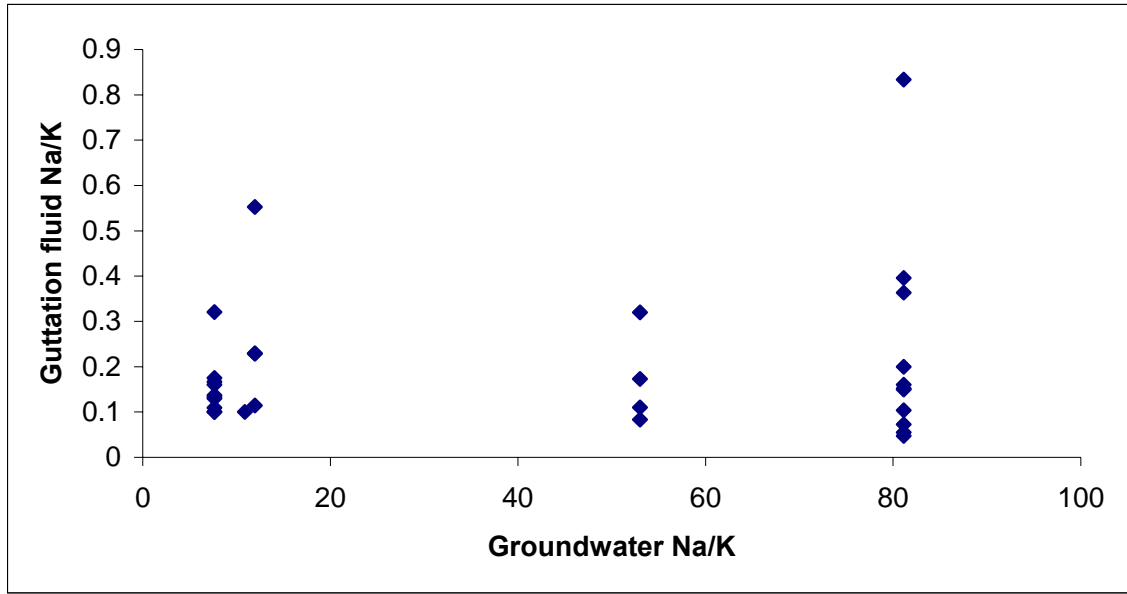


Figure 5.17 Relationship between groundwater K concentration and *Equisetum giganteum* guttation fluid K concentration across sites in northern and central Chile. No significant correlation is detectable either in the original units ($p = 0.49$) or when a square root transformation is applied to the Y axis to reduce variance heteroskedasticity ($p = 0.70$).

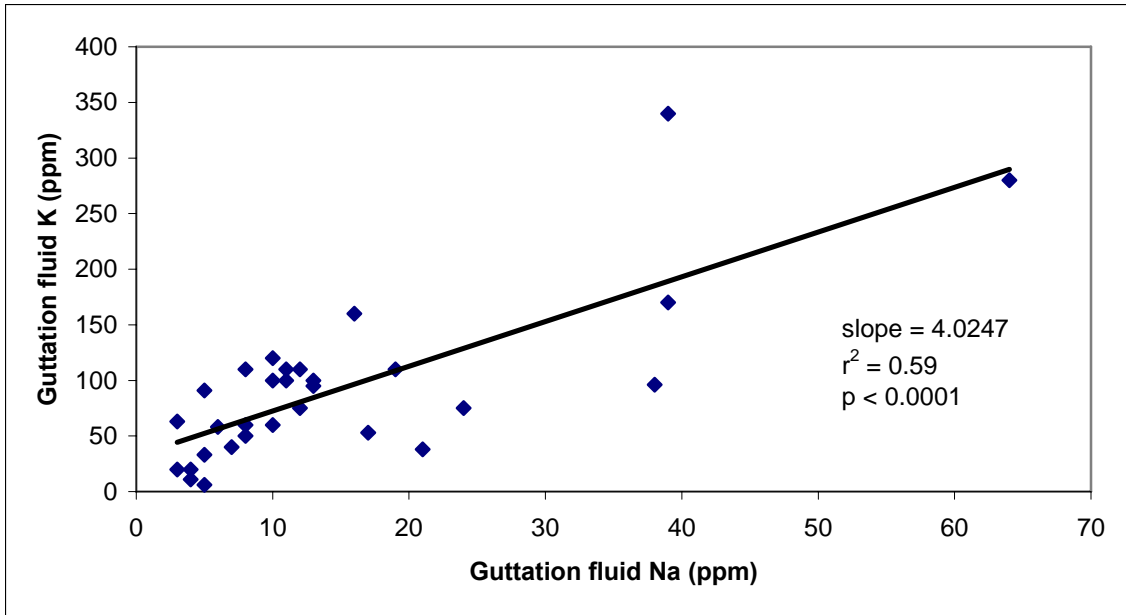


Figure 5.18 Relationship between Na and K concentrations in *Equisetum giganteum* guttation fluid across sites in northern and central Chile.

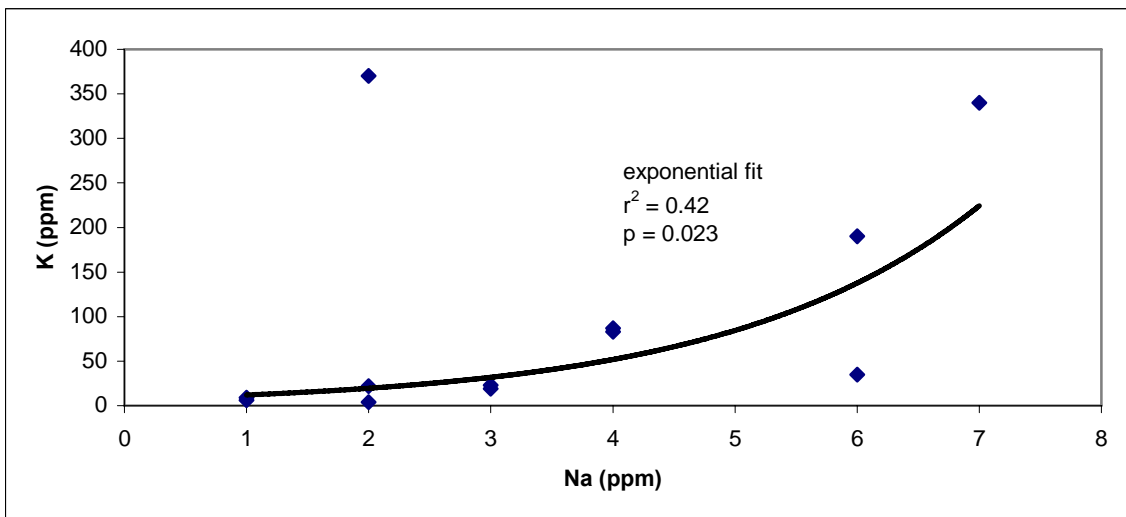


Figure 5.19 *Equisetum giganteum* stem surface deposit composition, Tarapacá Valley, Tarapacá Province, northern Chile.

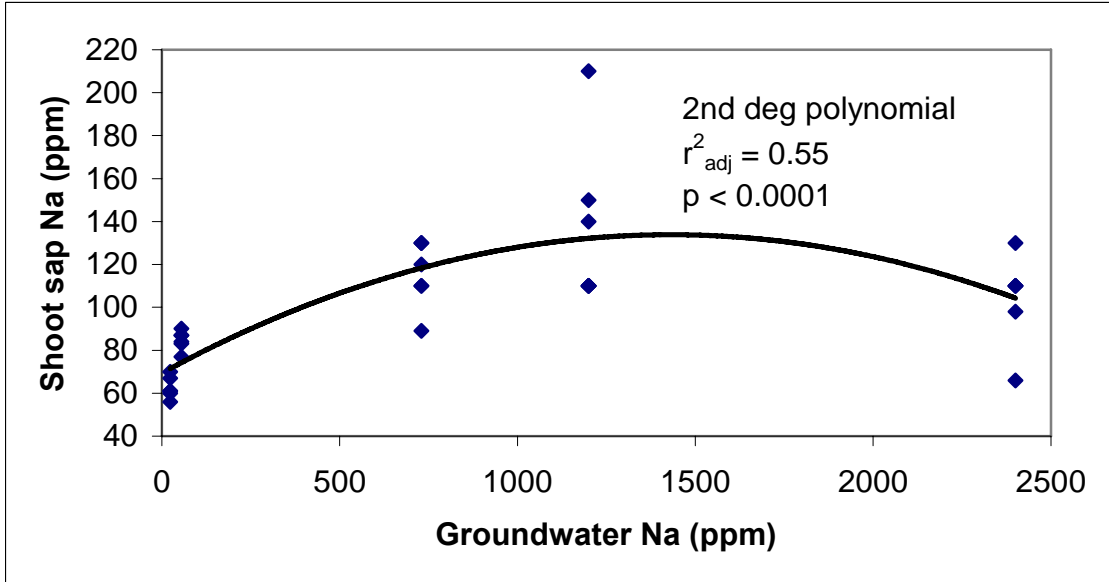


Figure 5.20 Relationship between groundwater Na concentration and *Equisetum giganteum* cell sap concentration across sites in northern and central Chile.

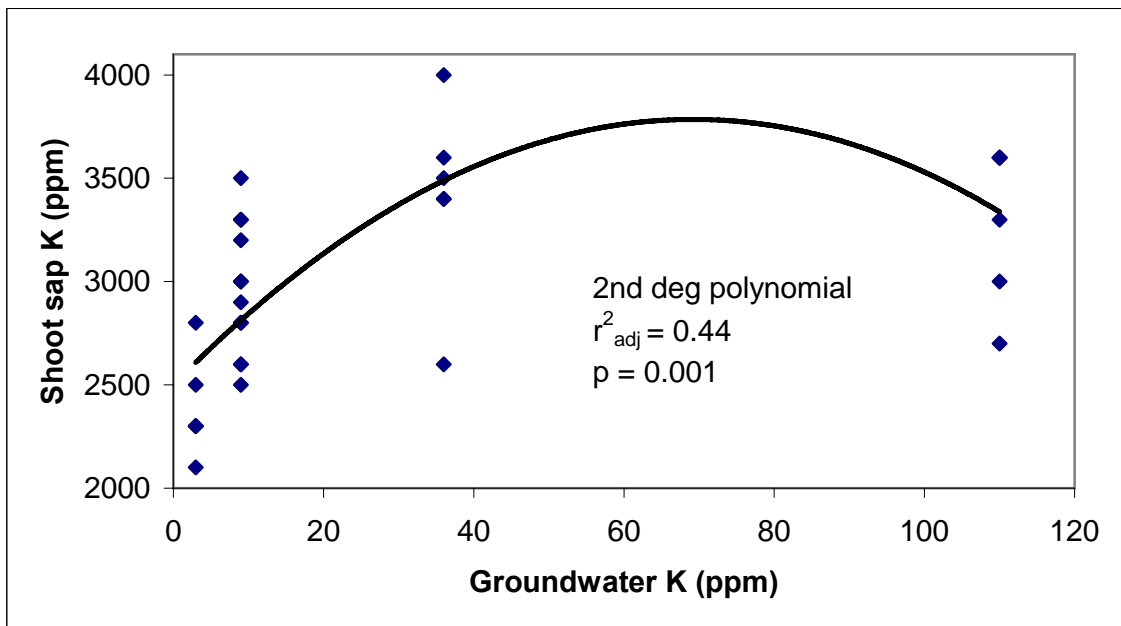


Figure 5.21 Relationship between groundwater K concentration and *Equisetum giganteum* cell sap concentration across sites in northern and central Chile.

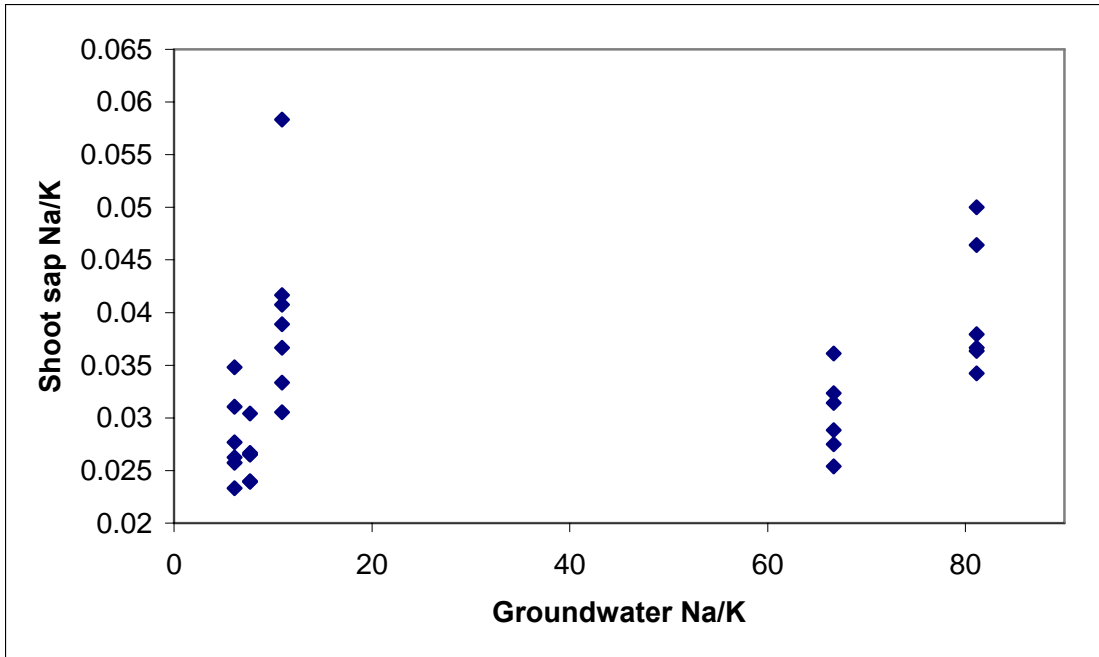


Figure 5.22 Relationship between Na/K ratios in groundwater and *Equisetum giganteum* cell sap of young shoots across sites in northern and central Chile. No significant linear correlation is detectable ($p = 0.13$).

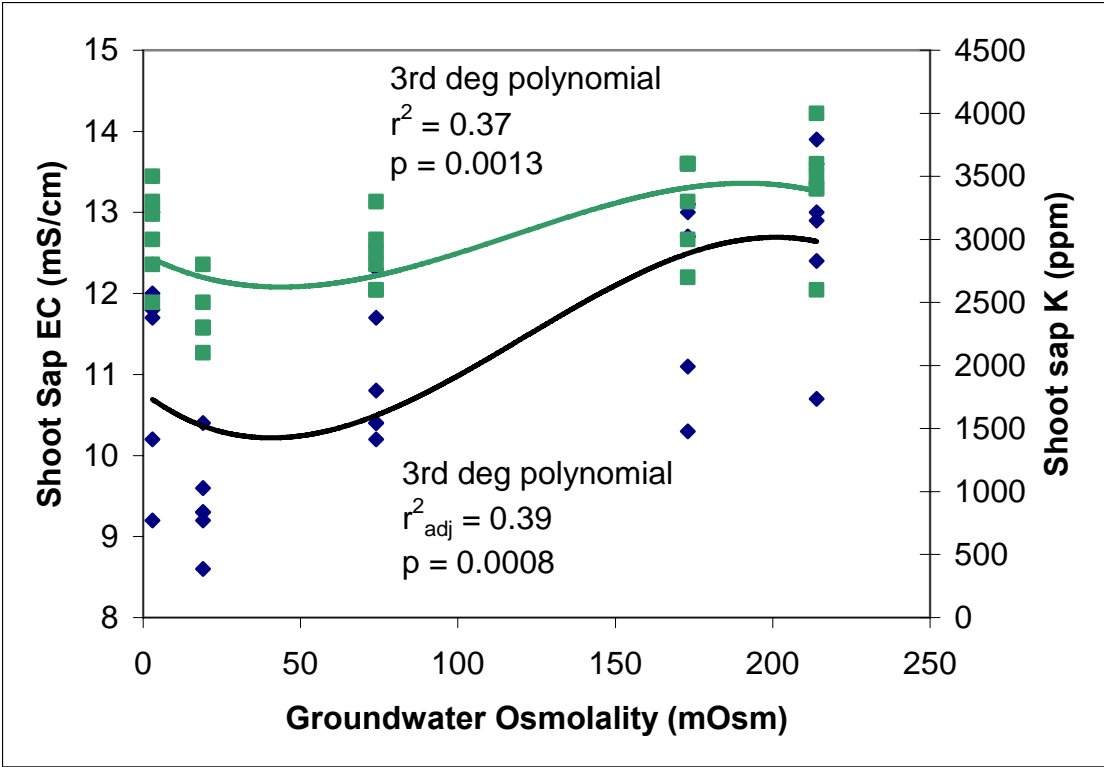


Figure 5.23 Relationships among groundwater osmolality and shoot sap EC and K concentrations for *E. giganteum* across sites in northern and central Chile.

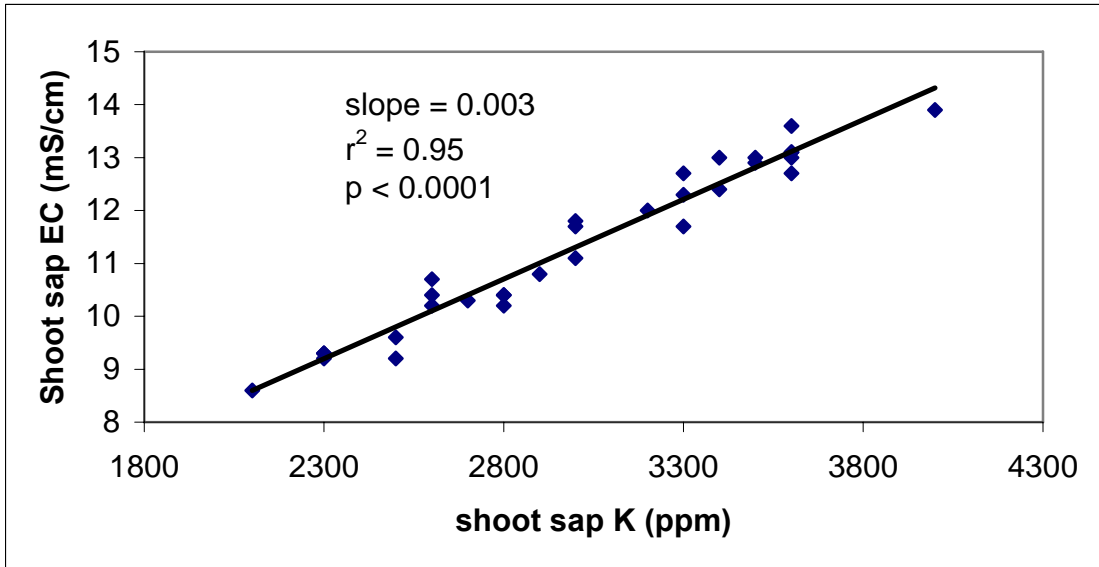


Figure 5.24 Relationship between shoot sap K concentration and shoot sap EC across sites in northern and central Chile.

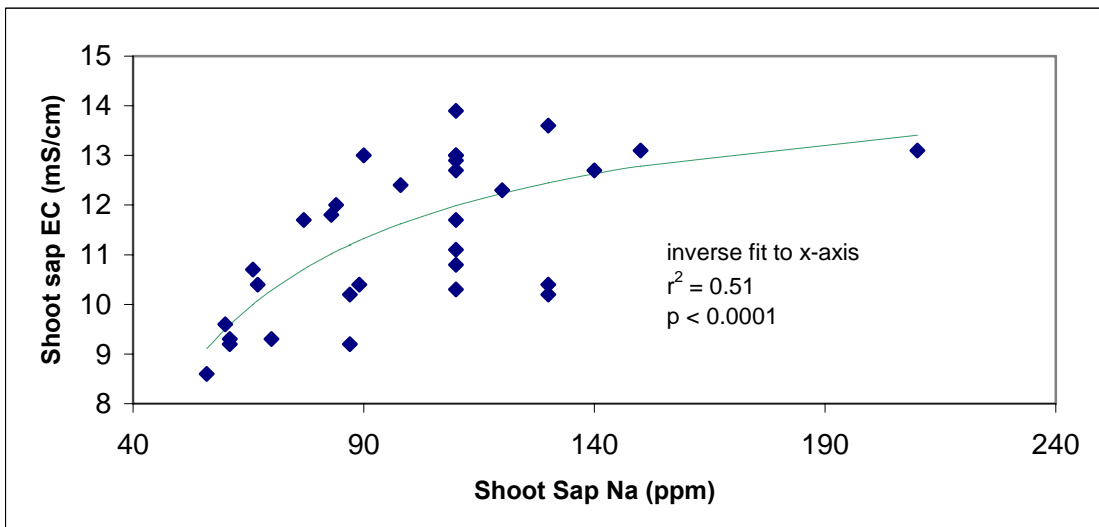


Figure 5.25 Relationship between *E. giganteum* shoot sap Na concentration and shoot sap EC across sites in northern and central Chile.

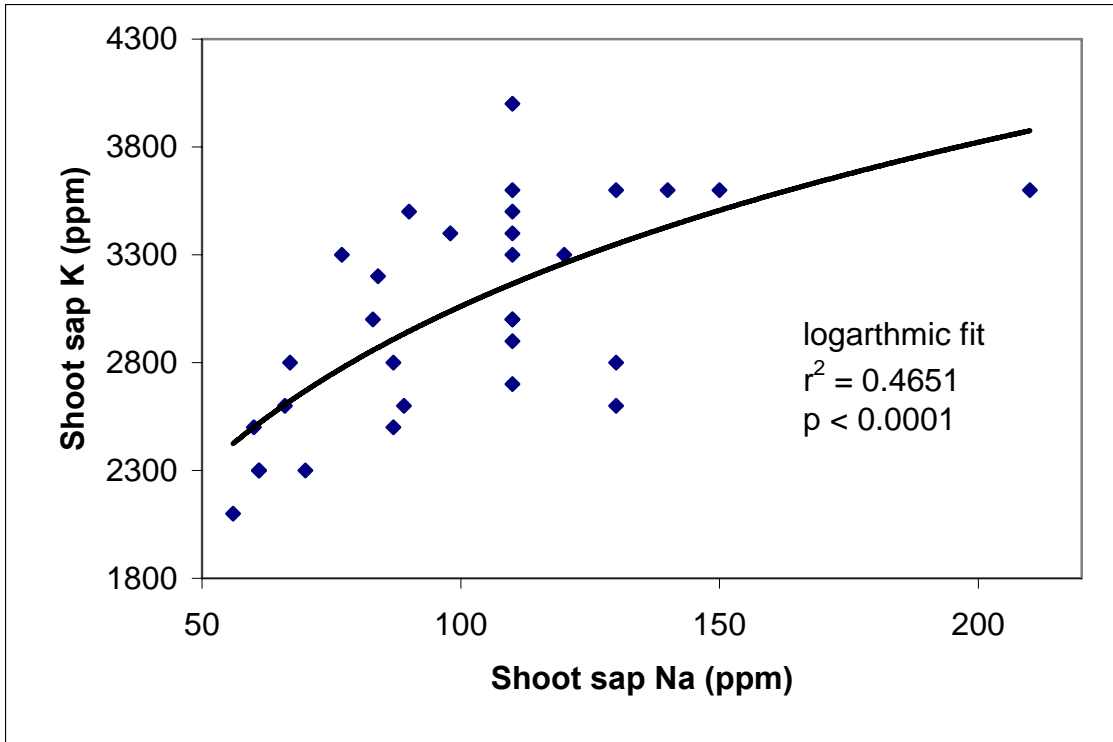


Figure 5.26 Relationship between Na and K concentrations in *Equisetum giganteum* cell sap of young shoots across sites in northern and central Chile.

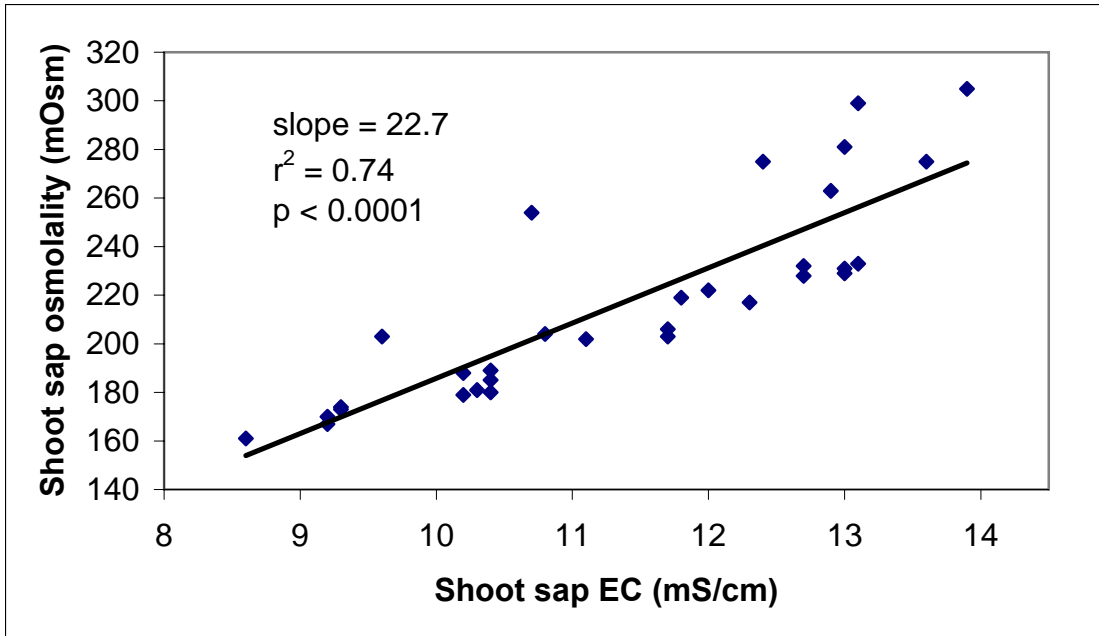


Figure 5.27 Relationship between *E. giganteum* shoot sap EC and shoot sap osmolality across sites in northern and central Chile.

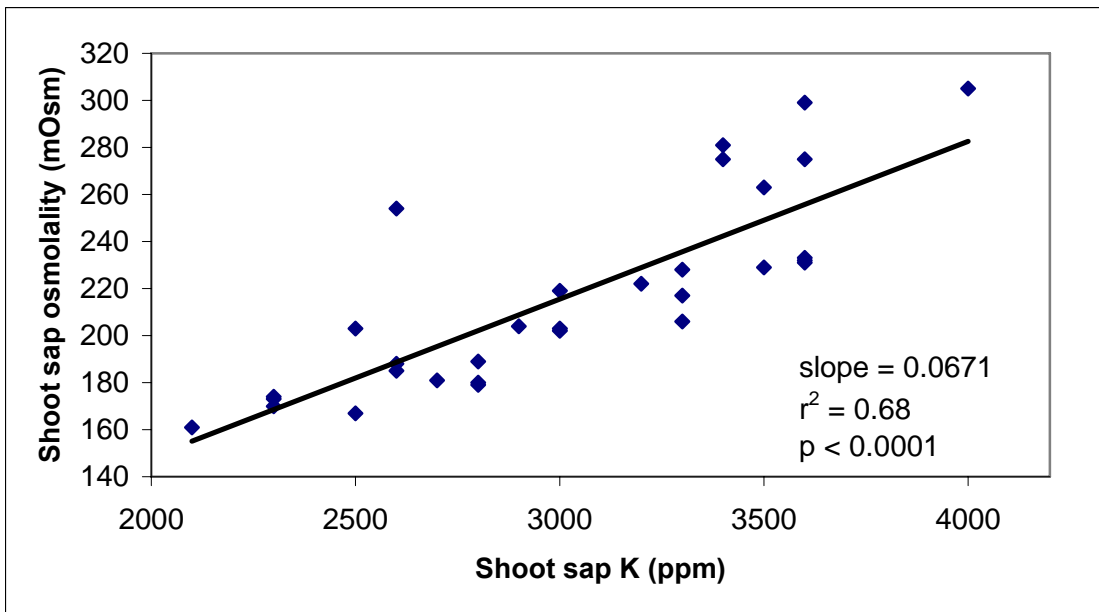


Figure 5.28 Relationship between *Equisetum giganteum* cell sap K concentration and sap osmolality for young shoots across sites in northern and central Chile.

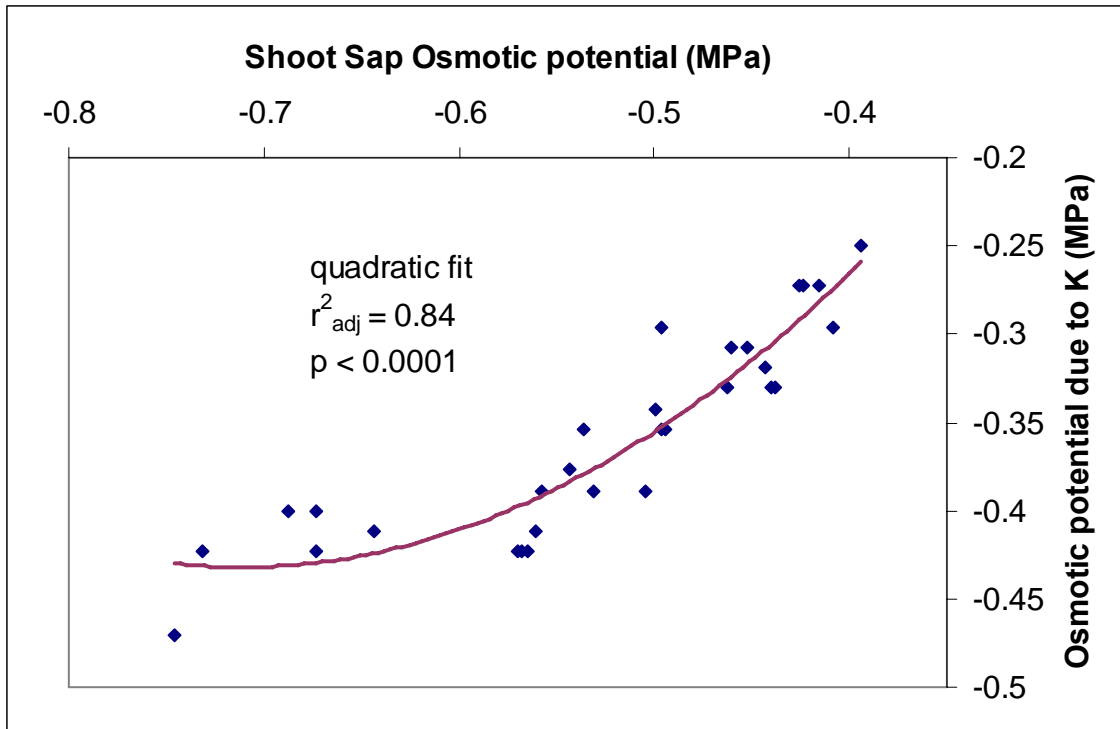


Figure 5.29 Relationship between *Equisetum giganteum* cell sap K concentration and sap osmolality for young shoots across sites in northern and central Chile.

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6. CONCLUSIONS

Equisetum giganteum occupies a wide range of habitats in southern South America, from valleys of the Atacama Desert to shaded mesic forests in Argentina. Colonies of this horsetail occupy large areas of the Atacama river valleys, including those with sufficiently high groundwater salinity to significantly inhibit floral diversity. Gametophytes and young sporophytes were observed on exposed floodplains, showing that all stages of the lifecycle are well adapted to the conditions of these valleys.

Equisetum giganteum stems exhibit characteristics of semi-self supporting plants, requiring mutual support or support of other vegetation when they grow tall. However, the flexural stiffness of its stem tissues is considerably greater than the values determined by Spatz et al. (1998a) in a greenhouse grown clone. The high stem stiffness has important implications for the study of biomechanics of the extinct ancestors of *Equisetum*, such as *Calamites* (Spatz et al. 1998b). The biomechanical properties of the stems are very similar across habitats, including greenhouse cultivation, and the stem tissue modulus of elasticity is the largest measured for any horsetail by a large margin (Spatz et al. 1998a, Speck et al. 1998, Niklas 1989). This stem construction exhibits limited phenotypic plasticity, which corresponds to the conservative nature of stem architecture and anatomy in the Sphenopsids (Niklas 1997), and indicates a “one size fits” all biomechanical strategy. Measurement of the specific elastic moduli of the component stem tissues of *Equisetum giganteum* would likely provide a more accurate estimate of the strength of the corresponding tissues in *Calamites* than the estimates obtained by Spatz et al. (1998a).

Stomatal behavior of *E. giganteum* is consistent with that of typical C3 vascular plants, although absolute values of maximum late morning stomatal conductance are very low in this species in comparison to typical C3 plants. The internode stomata exhibited strong light response, indicating that the unique stomata of *Equisetum* are fully functional in regulating transpiration in the giant horsetails. However, the environmental sensitivity of stomatal conductance was less in developing stems, which is consistent with their rapid elongation rates, which also appear insensitive to environmental differences. The high degree of variability in diurnal stomatal conductance patterns among stems suggests that there is fine regulation of stomatal opening at the individual stem level, rather than at the clone level.

Equisetum giganteum exhibits a substantial degree of salinity tolerance across a wide range of groundwater salinities in Chile and Argentina. Exclusion of Na and preferential accumulation of K at the root level appears to be the key mechanism of salinity tolerance in *E. giganteum* in the Atacama Desert. The osmolites accumulated by this plant appear to be polar, with the contribution of K being larger at lower salinities than at higher salinities. Overall stomatal conductance and chlorophyll fluorescence appear little affected by salinity, ranging from very low levels up to half strength seawater, suggesting a high degree of stress tolerance.

Overall *Equisetum giganteum* evinces a high capacity to maintain physiological homeostasis over a substantial range of habitats, from areas highly affected by salinity in the Atacama Desert to mesic forests in northwest Argentina. The ability of *E. giganteum* to adapt to such different environments likely accounts for its remarkably

large range, from the Greater Antilles to southern South America and from sea levels to 2600 m.

The remarkable ability of *Equisetum giganteum* to thrive in a range of habitats in South America provides strong evidence of the continued success of the ecological strategy of the Sphenopsids in the modern world. The remarkable persistence of this group, along with evidence that modern species have diversified relatively recently (Tertiary period, Des Marais et al. 2003) suggests a promising future for horsetails as an important component of the Earth's flora.

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