UNIVERSIDAD AUTÓNOMA DE NUEVO LEÓN

FACULTAD DE CIENCIAS FORESTALES

SUBDIRECCIÓN DE POSTGRADO

CHARACTERIZATION OF MIXED PINE-OAK FORESTS WITH REGARD TO THE LIMITING FACTOR WATER AVAILABILITY IN THE SIERRA MADRE ORIENTAL, MEXICO

CARACTERIZACIÓN DE BOSQUES MIXTOS DE PINO-ENCINO EN LA SIERRA MADRE ORIENTAL EN MÉXICO CONSIDERANDO EL FACTOR LIMITANTE HÍDRICO

TESIS

QUE PARA OBTENER EL GRADO DE DOCTOR EN CIENCIAS CON ESPECIALIDAD EN MANEJO DE RECURSOS NATURALES

PRESENTA:

WIBKE HIMMELSBACH

LINARES, NUEVO LEÓN **DICIEMBRE, 2009**

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SRE

El trabajo de investigación con el título “Caracterización de bosques mixtos de pino-encino en la Sierra Madre Oriental en México considerando el factor limitante hídrico” fue realizado con una beca de la **Secretaría de Relaciones Exteriores** otorgada por el **Gobierno de México**.

Widmung

Gewidmet aus innigster Zuneigung meinem Sohn Lorenz Aurelio, der von klein an so oft auf seine Mutter verzichten musste.

Agradecimientos

A todas las personas e instituciones que me apoyaron físicamente, mentalmente y de manera financiera en la realización del presente trabajo.

A mi asesor principal y director de la tesis Dr. Eduardo Treviño Garza le agradezco de todo corazón su amistad, su valor de aceptarme, a una desconocida, como su tesita y su apoyo científico y logístico para realizar mis planes de investigación y para poder incorporarme en una nueva institución fuera de mi país de origen.

Al Dr. Humberto González Rodríguez le agradezco mucho su voluntad de familiarizarme con las metodologías en el campo de la fisiología vegetal aplicadas en el transcurso de la investigación y su conocimiento científico del tema.

Dr. Eduardo Estrada Castillón siempre estaba dispuesto para ayudarme con su experiencia profesional y crítica constructiva. Su opinión me ayudó muchas veces a tomar decisiones importantes, lo cual le agradezco de todo corazón.

Al Dr. Óscar Aguirre Calderón le agradezco su disponibilidad de apoyarme y colaboración en la revisión de la tesis y crítica constructiva.

A mi compañero Dino González Uribe le agradezco mucho su voluntad desinteresada de aconsejarme con su profundo conocimiento estadístico el cual me dio mucha confianza y seguridad.

A los técnicos de campo y laboratorio Elsa González, Inés Yañez, Claudia Platas, Manuel Hernández y especialmente a Juan José González Álvarez que fue indispensable para la realización del duro trabajo en el campo.

Herrn Prof. Dr. Ralph Mitlöhner danke ich von ganzem Herzen für seine selbstlose Bereitschaft und Zeit, meine Publikationen durchzulesen und während mehrerer, längere Ferngespräche ausführlichst mit mir zu besprechen. Sein Fachwissen hat entscheidend zum Gelingen der vorliegenden Arbeit beigetragen.

Meinen Eltern Hildegard und Hubert Himmelsbach danke ich ganz besonders, denn schließlich haben SIE mich gelehrt selbständig meinen Weg zu gehen, was nicht nur für den Erfolg dieser Arbeit entscheidend war, sondern ein Geschenk des Lebens ist. Es ist bestimmt nicht immer einfach gewesen, sich nicht einzumischen und einfach machen zu lassen!

Además quisiera agradecer con todo mi corazón a mi esposo Marco Aurelio González Tagle, quien estaba 24-horas disponible para apoyarme y aconsejarme profesionalmente para avanzar y terminar el trabajo exitosamente.

Finalmente, quisiera agradecer al personal académico y administrativo de la Secretaría de Relaciones Exteriores, de la Facultad de Ciencias Forestales y de la Universidad Autónoma de Nuevo León por haberme apoyado con mucho esfuerzo y paciencia para realizar y terminar mi formación profesional.

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GENERAL SUMMARY

Mexican mixed pine-oak forests are exposed to extreme environmental conditions where water availability and high accumulations of solutes in the soil are limiting factors. In order to determine the adaptation of several native tree species (*Acacia rigidula, Arbutus xalapensis, Juniperus flaccida, Pinus pseudostrobus, Quercus canbyi* and *Rhus virens*) to mentioned limiting factors, diurnal and seasonal leaf water potentials (Ψ_w), osmotic potentials (Ψ_s) and leaf pigment concentrations, such as chlorophyll (a+b) and carotenoids, were measured and analyzed under natural drought and non-drought conditions. The results are discussed in four chapters and summarized as followed:

a) Site conditions reflected by seasonal and diurnal leaf water potentials of four co-occurring tree species in the Sierra Madre Oriental, Mexico

This chapter focused on diurnal leaf water potentials (Ψ_w) of *Arbutus xalapensis, Juniperus flaccida, Pinus pseudostrobus* and *Quercus canbyi* and their relation to environmental site conditions. In general, leaf Ψ_w declined progressively with increasing drought and soil-water loss and were significantly different comparing sampling dates, sampling hours and species, while the diurnal variation was found independently of the species. Hence, diurnal fluctuations in Ψ_w were very similar comparing the four species with high values at predawn (6:00 hr) and low values after midday (12:00 hr-16:00 hr). Correlations within Ψ_w and environmental variables were highly significant for soil moisture content in the morning hours (6:00 hr and 8:00 hr) and evaporative demand components in the afternoon (12:00 hr to 16:00 hr), depending on the species. *A. xalapensis, J. flaccida* and *Q. canbyi* reflected better the measured site conditions than *P. pseudostrobus*. Finally, species employ different strategies to overcome periods of drought. *P. pseudostrobus* seems to avoid water stress by means of a deeper rooting system, while the other species have further plant physiological capacities to overcome water scarcity.

b) Adaptation of three co-occurring tree species to water stress and their role as site-indicators in mixed pine-oak forests in the Sierra Madre Oriental, Mexico

The second chapter focuses on the adaptation of native species, *Juniperus flaccida, Pinus pseudostrobus* and *Quercus canbyi*, to the limiting factors water stress and salt excess by measuring and interpreting osmotic potentials (Ψ_s) additionally to plant water (Ψ_w). The effect of two different aspects (north- and south-facing) and seasonal changes of environmental variables on water potentials, as well as the species capacity to cope with these dynamic site-conditions was aimed. In both, Ψ_w and Ψ_s significant differences were found between the two aspects, species and sampling dates, while differences between species depended on season. Seasonal and diurnal fluctuation of Ψ_w and Ψ_s were higher for *J. flaccida* and *Q. canbyi* than for *P. pseudostrobus*. Leaf Ψ_w and Ψ_s were mainly correlated with soil water content, while Ψ_s of *P. pseudostrobus* were hardly correlated with environmental variables. At last, the idea of a deeper rooting system as strategy to avoid water stress and salt excess was confirmed in the case of *P. pseudostrobus*. Furthermore, other plant physiological strategies to withstand periods of drought were applied by *J. flaccida* and *Q. canbyi,* whereas latter one had great capacity for osmotic adjustment.

c) Impact of water availability on chlorophyll and carotenoid concentrations in five tree species in Mexican pine-oak forests

The allocation of limited photosynthate between growth and defence is a crucial component of tree survival during periods of resource stress, such as drought and high levels of light. Our objectives were to describe the seasonal dynamics of chlorophyll (a+b) and carotenoids content in comparison to seasonal fluctuations in predawn leaf water- (Ψ_{wpd}) and osmotic potentials (Ψ_{spd}) of six native species, which undergo regular seasonal drought. All plant physiological variables, with exception of carotenoids content, were significantly different between two sites of different light environment and soil water availability, whereas shaded conditions had a positive effect on water potentials and leaf pigment concentrations. Furthermore, there were seasonal and plant specific differences in leaf pigment content, while *Q. canbyi* had the highest concentrations at both sites, which is surprising since *Leguminosae* like *A. rigidula* normally, tend to reach higher chlorophyll concentrations than non-*Leguminosae*. Conifer species were at a disadvantage during periods of drought in comparison to broad-leaved species, as they showed lower leaf pigment concentrations and higher seasonal variation correlated with environmental site conditions. In general, leaf chlorophyll (a+b) and carotenoids content has been related to water deficit and levels of irradiance, although the dimension of the response was different among species.

d) The relationship of water potential to air and soil water status

In this chapter, diurnal water potentials were expressed in relation to vapour pressure deficit under different conditions of soil water availability, and the importance of soil water availability or rather the capacity of soils to store water on plant water stress was emphasized. The ensuing analysis relating seasonal predawn water potentials (Ψ_{wpd}) with soil moisture content produced a critical value. Thus, water potentials declined with rapid, when soil water availability subsided 15%. This was in accordance to results of comparable studies.

All species studied are considered as suitable candidates for reforestation programs in Mexican mixed pine-oak forests. Nevertheless, their suitability depends on the environmental conditions, especially with respect to soil characteristics in the area of improvement.

RESUMEN

Los bosques mixtos de pino-encino en México están expuestos a condiciones ambientales extremas como la disponibilidad de agua y la salinización del suelo, ambas condiciones juegan un papel importante como factores limitantes en el crecimiento de las plantas. Para determinar la adaptación de varias especies arbóreas y arbustivas nativas (*Acacia rigidula, Arbutus xalapensis, Juniperus flaccida, Pinus pseudostrobus, Quercus canbyi* y *Rhus virens*) a dichos factores, se midieron y analizaron bajo condiciones naturales y dinámicas del hábitat las fluctuaciones estacionales y diarias de los potenciales hídricos (Ψ_w), osmóticos (Ψ_s) y de la concentración de pigmentos en las hojas (clorofila (a+b) y carotenoides). Los resultados fueron discutidos en cuatro capítulos y están resumidos como sigue:

a) Variación diaria y estacional de potenciales hídricos de cuatro especies de árboles nativos como indicadores de factores ambientales en la Sierra Madre Oriental, México

En este capítulo las variaciones diarias de los potenciales hídricos (Ψ_w) de *Arbutus xalapensis, Juniperus flaccida, Pinus pseudostrobus* y *Quercus canbyi* fueron enfocadas y relacionadas con las condiciones ambientales del sitio. En general, los Ψ_w disminuyeron con el incremento en la sequía y la escasa disponibilidad de agua en el suelo. Se encontraron diferencias significativas comparando los Ψ_w de las cuatro especies, estaciones y horas del día, en la cual, las variaciones diarias fueron independientes de las especies. De esta manera, las fluctuaciones diarias fueron parecidas en todas las especies con valores relativamente altos en la mañana antes del amanecer (6:00 h) y bajos después del mediodía (12:00 hasta 16:00 h). Las correlaciones entre los Ψ_w y los factores ambientales dependió de la especie y fue en general altamente significativa con el contenido de agua en el suelo en la mañana (6:00 h y 8:00 h) y con la demanda evaporativa de la atmósfera en la tarde. *A. xalapensis, J. flaccida* y *Q. canbyi* reflejaron mejor las condiciones ambientales medidas

directamente en el sitio en comparación con *P. pseudostrobus*. De tal manera que, se encontraron diferencias entre las especies con respecto a la adaptación a períodos de sequía. Parece que *P. pseudostrobus* evita los efectos negativos de una escasez de agua temporal por medio de un sistema radicular profundo, mientras que las otras especies arbóreas poseen diversas capacidades fisiológicas para superar períodos de sequía.

b) Adaptación de tres especies arbóreas nativas a la sequía y su papel como indicadores de sitio en los bosques mixtos de pino-encino en la Sierra Madre Oriental, México

El segundo capítulo trata principalmente de la adaptación de tres especies de árboles nativos, *Juniperus flaccida, Pinus pseudostrobus* y *Quercus canbyi* al estrés por escasez de agua y acumulación de solutos en el suelo, para lo cual se midieron aparte de los potenciales hídricos (Ψ_w) los potenciales osmóticos (Ψ_s). El interés fue enfocado en el impacto de factores ambientales en los potenciales hídricos, como la exposición de sitio (norte y sur), y la capacidad o estrategia de cada especie para adaptarse a cambios dinámicos del medio ambiente. Para los dos potenciales, Ψ_w y Ψ_s, se encontraron diferencias significativas comparando los resultados de las dos exposiciones, entre las especies y las estaciones del año, para lo cual, las diferencias entre las especies dependieron de la época del año. Los Ψ_w y Ψ_s de *J. flaccida* y *Q. canbyi* fueron más variables en el transcurso del día y año que los de *P. pseudostrobus*. En general y especialmente en el caso de las especies primeramente mencionadas, los dos potenciales hídricos estuvieron principalmente en correlación con el actual contenido de agua en el suelo, mientras que el Ψ_s de *P. pseudostrobus* tuvo difícilmente correlaciones con las condiciones ambientales del sitio. Esto pone finalmente énfasis en la suposición que *P. pseudostrobus* evita los efectos negativos de la falta temporal de agua y exceso de sales en el suelo por medio de un sistema radicular profundo. En cambio, *J. flaccida* y *Q. canbyi* poseen otras capacidades de superar períodos de sequía, especialmente la última mostró capacidad de adaptarse osmóticamente a un medio ambiente dinámico.

c) El efecto de la disponibilidad de agua en la concentración de clorofila y carotenoides foliar de cinco especies arbóreas en bosques mixtos de pino-encino de la Sierra Madre Oriental en México

La inversión de las limitadas tasas fotosintéticas en el crecimiento o mecanismos de defensa propios de una especie, es de vital importancia para la existencia y sobre vivencia de las plantas en regiones, donde están expuestas periódicamente a la falta de agua y en particular a una alta intensidad de luz. En este capítulo se analizaron las diferencias estaciónales en el contenido de clorofila (a+b) y carotenoides en hojas de seis especies nativas, las cuales se relacionaron con la variación estacional de los potenciales hídricos (Ψ_{wpd}) y osmóticos (Ψ_{spd}) medidos antes del amanecer. Todas las variables fisiológicas foliares, con la excepción del contenido de carotenoides, mostraron diferencias significativas entre dos sitios caracterizados por diferentes ambientes de luz y distinta disponibilidad de agua en el suelo, mientras que bajo la sombra tuvo un efecto positivo en el estatus hídrico de los árboles y la pigmentación de sus hojas. Además, se encontraron diferencias significativas en el contenido de pigmento foliar comparando las especies y diferentes estaciones del año. Sorprendentemente la concentración de pigmento foliar fue más alto en *Q. canbyi*, aunque hojas de leguminosas, como *A. rigidula*, contienen normalmente concentraciones más altas de clorofila y carotenoides que las no-leguminosas. Las confieras estuvieron en desventaja comparadas con las especies caducifolias mostrando concentraciones de pigmentación foliar considerablemente menores, las cuales tuvieron correlaciones fuertes con las variaciones estaciónales de los factores ambientales en los sitios. En general, el contenido de clorofila y carotenoide foliar fue relacionado con cambios de disponibilidad de agua y luz en el sitio. No obstante, la reacción de los árboles frente a estos factores ambientales fue diferente dependiendo de la especie.

d) Potenciales hídricos en relación con el estado hídrico del suelo y del aire

En la última parte de los resultados se relacionaron los cambios diarios del potencial hídrico con el déficit de presión de vapor del aire bajo diferentes

condiciones de disponibilidad de agua en el suelo. De esto, se destacó que el contenido de agua en el suelo, o mejor dicho la capacidad del suelo de almacenar agua juegan un papel crucial en la existencia y sobre vivencia de plantas en áreas frecuentadas por períodos de sequía. El siguiente análisis, en el cual se expresó el potencial hídrico del amanecer (Ψ_{wpd}) como función del contenido de agua en el suelo, dio como resultado un valor crítico. Por lo tanto, los Ψ_{wpd} bajaron rápidamente cuando el contenido de agua del suelo quedó por debajo del 15%. Esto está en concordancia con resultados encontrados en investigaciones semejantes.

Todas las especies arbóreas consideradas en la presente investigación son candidatos aptos para la reforestación de bosques mixtos de pino-encino en la Sierra Madre Oriental. No obstante, su recomendación depende de las condiciones ambientales, especialmente de las características del suelo, en el área de reforestación o restauración ecológica.

ZUSAMMENFASSUNG

Kiefern-Eichenmischwälder sind in Mexiko extremen Umweltbedingungen ausgesetzt, wobei Wassermangel und Bodenversalzung eine führende Rolle als limitierende Faktoren für das Pflanzenwachstum spielen. Um die Anpassung an genannte Faktoren mehrerer einheimischer Baumarten (*Acacia rigidula, Arbutus xalapensis, Juniperus flaccida, Pinus pseudostrobus, Quercus canbyi* und *Rhus virens*) zu bestimmen, wurden sowohl tägliche als auch jahreszeitliche Schwankungen der Wasserpotentiale (Ψ_w), osmotischen Potentiale (Ψ_s) und von Farbpigmenten in den Blätter (Chlorophyll- und Karotenoidgehalt) unter varierenden Umweltbedingungen im Feld untersucht und analysiert. Die Ergebnisse wurden in vier Kapiteln diskutiert und werden wie folgt zusammengefasst:

a) Wasserpotentiale im Tages- und Jahresverlauf als Indikatoren von Umweltfaktoren, gemessen an vier vergesellschafteten Baumarten in der Sierra Madre Oriental, Mexiko

In diesem Kapitel wurde Schwerpunkt auf die täglichen Schwankungen des Wasserpotentials (Ψ_w) von *Arbutus xalapensis, Juniperus flaccida, Pinus pseudostrobus* und *Quercus canbyi* gelegt und deren Relation zu den standörtlichen Umweltbedingungen. Im Allgemeinen sanken die Ψ_w mit zunehmender Trockenheit und Bodenwasserverlust und zeigten signifikante Unterschiede beim Vergleichen von Arten, Tages- und Jahreszeiten, wobei die täglichen Schwankungen artenunabhängig waren. Demnach waren die täglichen Wasserpotentialschwankungen bei allen Arten recht ähnlich, mit relative hohen Werten vor Sonnenaufgang (6:00 Uhr) und tiefen Werten am Nachmittag (12:00 bis 16:00 Uhr). Die Korrelationen zwischen Ψ_w und Umweltfaktoren war abhängig von der Art höchst significant mit dem Bodenwassergehalt in den Morgenstunden (6:00 und 8:00 Uhr) und der evaporativen Nachfrage der Atmosphäre am Nachmittag. *A. xalapensis, J. flaccida* und *Q. canbyi* gaben die gemessenen Standortsbedingungen im Vergleich zu *P. pseudostrobus* am

besten wieder. Somit wurden artspezifische Unterschiede bezüglich der Anpassung an wiederkehrende Trockenperioden festgestellt. *P. pseudostrobus* scheint vorübergehenden Wassermangel durch ein tieferes Wurzelsystem zu überbrücken, wohingegen die anderen Baumarten andere pflanzenphysiologische Eigenschaften besitzen, um Trockenperioden zu überbrücken.

b) Anpassung dreier vergesellschafteter Baumarten an Trockeheit und ihre Rolle als Standortindikatoren in Kiefern-Eichen-Mischwäldern der Sierra Madre Oriental in Mexiko

Das zweite Kapitel befasst sich überwiegend mit der Anpassung dreier, einheimischer Baumarten, *Juniperus flaccida, Pinus pseudostrobus* und *Quercus canbyi*, an die Stressoren Wassermangel und Bodenversalzung, wobei zusätzlich zu den Wasserpotentialen (Ψ_w) osmotische Potentiale (Ψ_s) gemessen wurden. Hierbei galt das Hauptinteresse den Auswirkung zweier Standorte (Nord- und Südorientierung) und der jahreszeitlichen Schwankungen von Umwelteinflüssen auf die Wasserpotentiale, als auch der artspeziefischen Fähigkeiten mit diese dynamischen Standortsbedingungen zurechtzukommen. Sowohl für die Ψ_w als auch die Ψ_s wurden significante Unterschiede zwischen den beiden Standorten, den Arten und verschiedenen Aufnahmetagen gefunden, wobei die Unterschiede zwischen den Arten jahreszeitlich abhängig waren. Tägliche und jahreszeitliche Schwankungen von Ψ_w and Ψ_s waren im Vergleich zu *P. pseudostrobus* wesentlich stärker bei *J. flaccida* und *Q. canbyi*. Im Allgemeinen und besonders für die letztgenannte Arten waren Ψ_w und Ψ_s hauptsächlich mit dem aktuellen Bodenwassergehalt korreliert, wobei das Ψ_s von *P. pseudostrobus* kaum mit standörtlichen Umweltbedinungen korreliert war. Dies bestärkt letztendlich die Vermutung, dass *P. pseudostrobus* die negativen Auswirkungen von Wassermangel und Bodenversalzung durch ein tieferes Wurzelsystem umgeht. *J. flaccida* und *Q. canbyi* dagegen besitzen andere pflanzenphysiologische Eigenschaften, um Trockenperioden unbeschadet zu überbrücken; insbesondere *Q. canbyi* zeigte Fähigkeit zur osmotischen Anpassung.

c) Einfluss von Wasserverfügbarkeit auf Chlorophyll- und Karotenoidkonzentrationen in Blättern von fünf Baumarten in mexikanischen Kiefern-Eichen-Mischwäldern der Sierra Madre Oriental

Die Investierung limitierter photosynthetischer Produkte in Pflanzenwachstum beziehungsweise pflanzenspezifischer Schutzmechanismen ist von entscheidender Bedeutung für das Überleben von Bäumen in Regionen, wo periodisch auftretender Resourcenmangel, insbesondere Trockenheit und hohe Lichtintensität, vorherrscht. In diesem Abschnitt wurde Schwerpunkt auf die Untersuchung von jahreszeitlichen Unterschieden im Chlorophyll (a+b) und Karotenoidgehalt in Blättern von sechs einheimischen Baumarten gelegt und diese mit den jahreszeitlichen Schwankungen von Wasser- (Ψ_{wpd}) und osmotischen Potentialen (Ψ_{spd}), gemessen vor Sonnenaufgang, verglichen. Alle pflanzenphysiologischen Variablen, mit Ausnahme des Karotenoidgehalts, zeigten signifikante Unterschiede beim Vergleich der beiden Standorte, die durch unterschiedliche Licht- und Bodenwasserverhältnisse gekennzeichnet waren, wobei sich Schatten positiv auf die Wasserpotentiale als auch die Blattpigmentierung auswirkte. Zudem wurden jahreszeitliche und artspezifische Unterschide im Pigmentgehalt der Blätter festgestellt. Überraschenderweise war der Blattpigmentgehalt von *Q. canbyi* auf beiden Standorten vergleichsweise am höchsten, obwohl normalerweise Blätter von Leguminosen, wie *A. rigidula*, höhere Chlorophyllkonzentrationen aufweisen als Nicht-Leguminosen. Die Koniferenarten waren im Vergleich zu den Laubbaumarten benachteiligt, da erstere wesentlich geringere Blattpigmentkonzentrationen aufwiesen und diese stark mit den jahreszeitlichen Schwankungen der standörtlichen Umweltbedingungen korreliert waren. Im Allgemeinen ließ sich ein Zusammenhang zwischen Chlorophyll- und Karotenoidgehalt und Wassermangel sowie unterschiedliche Lichtverhältnisse erkennen, allerdings war die Reaktion auf diese Umweltfaktoren von Art zu Art unterschiedlich ausgeprägt.

d) Wasserpotentiale im Zusammenhang mit Luft- und Bodenwassergehalt

Im letzten Ergebnisteil wurden die täglichen Schwankungen von Wasserpotentialen mit denen des Luftdruckdefizits bei unterschiedlichen Bodenwasserverhältnissen in Bezug gebracht. Dabei wurde betont, dass der Bodenwassergehalt bzw. die Bodenwasserspeicherkapazität eine wichtige Rolle für das Bestehen und Überleben von Pflanzen in Trockengebieten innehat. Die anschließende Analyse, bei der jahreszeitliche Wasserpotentiale, gemessen in früher Morgenstunde, als Funktion des Bodenwassergehalts ausgedrückt wurden, ergab einen kritischen Wert. So ließ sich ein starkes Abfallen der Wasserpotentiale feststellen, sobald der Bodenwassergehalt 15% unterschritt. Dies war in Übereinstimmung mit Ergebnissen vergleichbarer Studien.

Alle untersuchten Baumarten kommen als geeignete Kandidaten für die Wiederaufforstung von Kiefern-Eichen-Mischwäldern der Sierra Madre Oriental in Betracht. Allerdings hängt ihre Empfehlung bzw. Eignung von den standörtlichen Umweltbedingungen ab, insbesondere von den Bodeneigenschaften des aufzuforstenden beziehungsweise zu restaurierenden Gebietes.

CHAPTER 1

GENERAL INTRODUCTION

1. PROBLEM DESCRIPTION

Mixed pine-oak forests are widely distributed in the higher altitudes of the Eastern Sierra Madre including ecologically and economically important tree species such as *Pinus pseudostrobus* (Rzedowski 1986). These forests are exposed to extreme climatic conditions of high temperatures and low precipitation distributed irregularly through the year. Under field conditions, water stress or rather soil water availability is in general one of the major limiting factors in plant growth and affects most physiological processes in these ecosystems (Kozlowski et al. 1991, Kramer 1983, Kramer and Boyer 1995, Mitlöhner 1998, Serrano et al. 1999, González et al. 2003, Reynolds et al. 2004, Otieno et al. 2005). Moreover, water scarcity leads to salt excess due to net accumulation of solutes from weathering of parent rock material and/or anterior oceanic salt deposition in the vegetation’s rhizospheres, another serious and significant environmental problem plants have to face in arid and semiarid environments (Szabolcs 1994, Mitloehner and Koepp 2007). So far, water scarcity as well as climate and soil induced salinity can be considered natural processes and the native vegetation is normally adapted to these environmental conditions (Mitloehner and Koepp 2007).

Even so, climatic change makes conditions for plant grow even more acute. Actually, the global mean temperature (0.14-0.58°C per decade) and evapo-transpiration are increasing and changes in regional precipitation patterns are expected (Watson et al. 2000, Meehl et al. 2005). The response of forests to these changes associated with the greenhouse effect is uncertain and has the potential to greatly alter the distribution and productivity of tree species (Pastor and Post 1988, Kramer 1983, Tschaplinski et al. 1998, Watson et al. 2000, Hamilton et al. 2002, Rötzer et al. 2005, Ricker et al. 2007). Inappropriate land-use contributes further to the acceleration of forest degradation and deforestation in great parts of the mountain chain Sierra Madre Oriental due to anthropogenic pressures like repeated fire events, overgrazing, maladjusted irrigation, clearing of deep-rooted native vegetation and indiscriminate urbanization (Domínguez and Návar 2000, Cantú and González 2002, Salinas and Treviño 2002, González et al. 2005). Especially inadequate irrigation and the clearing of deep-rooted native vegetation cause secondary salinization in dryland areas worsening and/or impeding natural regeneration and reforestation (Szabolcs 1994, Munns 2002, Mitloehner and Koepp 2007). In Chiapas for instance, the thinning of the canopy in tropical highland forests by human activities increased solar radiation and exposed seedlings and juveniles to more extreme temperatures at the forest floor level, thus decreasing soil moisture availability (Ramírez-Marcial et al. 1996, Ramírez-Marcial et al. 2001). The response of forest ecosystems to such climatic and anthropogenic stresses will depend in great part on the drought (Tschaplinski et al. 1998) and salt excess tolerance capabilities of the individual species (Mitloehner and Koepp 2007).

Hence, understanding water uptake patterns, the physiological response of species to water stress, salt excess and their tolerance scope under severe environmental stress is required to explain differences among species in survival and distribution. It also allows determining the relative suitability of species for ecological restoration of degraded lands (Jurado et al. 1998, Ramírez-Marcial et al. 2001, González et al. 2003, Gebrekirstos et al. 2006). Elsewhere, previous works on plant water relations and forest restoration (Gebre et al. 1998, Jurado et al. 1998, Vertovec et al. 2001, González et al. 2003, Gebrekirstos et al. 2006, Mitloehner and Koepp 2007) emphasized the need to investigate water relations of native species and their physiological responses to changes in resource availability.

Plant internal water potential is a consequence of the soil-plant-atmospheric continuum and consists of the tension caused by the water pressure deficit of the atmosphere, regulative processes of leaves, general flow regulation resistances and the soil water potential (Philip 1966, Duhme and Richter 1977, Kozlowski et al. 1991, Kramer and Boyer 1995, Donovan et al. 2003). It is a dependable measure of plant and soil water status, and quickly measured directly in the field, a great advantage especially for plant comparison (Slatyer 1967, Sala et al. 1981, Pallardy et al. 1991, Kramer and Boyer 1995, Mitlöhner 1998, Vertovec et al. 2001, Donovan et al. 2003).

The osmotic potential (Ψ_s), in contrast, indicates the prime adaptation of plants towards spatially and periodically altered soil solute concentration (Kramer and Boyer 1995, Mitloehner and Koepp 2007). Dehydration tolerant species (according to the terminology of Kramer (1980)) reduce Ψ_s and/or accumulate solutes (osmotic adjustment; Morgan, 1980) by incorporating ionic osmotica (i.e. NaCl among others salts from the soil) as well as by processing non-ionic osmotica (i.e. other plant-born organic compounds such as sucrose) into and within their cells (Mitloehner and Koepp 2007). In this way, a plants' capacity to take up water from dry soils is enhanced and recovery after drought is facilitated (Gebre et al. 1998, Tschaplinski et al. 1998, Nguyen-Queyrens et al. 2002). Osmotic and pressure-driven water flow into plant roots occur simultaneously (Kramer and Boyer 1995). Furthermore, an osmotic (or concentration) gradient in the soil-plant continuum (Pressarakli 1991) and within the plant between its cells are a basic condition for plant life (Mitloehner and Koepp 2007).

Osmotic adjustment can be estimated by the difference between Ψ_s of well-hydrated and dehydrated plants at similar relative water content (Gebre et al. 1998). It is difficult to determine osmotic adjustment under ambient field conditions due to the need to sample well-hydrated and dehydrated plants during the same period. Therefore, studies of osmotic adjustment have been based rather on comparison of seasonal variation in Ψ_s due to difference in precipitation within a season or between seasons (Bahari et al. 1985, Abrams 1990, Gebre et al. 1998).

In general, a species' capacity of adaptation to environmental site conditions is regarded as the basic requirement for a plant's "suitability as an indicator" (Mitlöhner 1997). During the last two decades, the Mexican Government has made an effort to restore degraded forest areas, mainly using pines and introduced species instead of native ones. In 2007 for instance, the CONAFOR (Comisión Nacional Forestal) reforested close to 325 000 hectares, planting about 263 Million trees all across the country (COLPOS 2008). Nevertheless, actions are often taken without informed advice or monitoring and the efficiency of national investments is not necessarily guaranteed. However, the conservation-oriented interest in native broad-leaved species is increasing lately, and information on their biology and silvics is urgently needed (Quintana-Ascencio et al. 2004).

Leaf chlorophyll, Chl *a* and Chl *b*, content provides important information about the physiological status of plants. They are together with carotenoids virtually essential pigments converting light energy into stored chemical energy (Young and Britton 1993, Sims and Gamon 2002, Gitelson et al. 2003). Moreover, chlorophyll is a direct indicator for the photosynthetic potential, the primary production of a plant and environmental quality, since the amount of solar radiation absorbed by a leaf is a function of the photosynthetic pigment content (Curran et al. 1990, Filella et al. 1995, Carter and Spiering 2002).

Plant growth depends generally on nutrients supplied by photosynthesis and transported by water between important plant organs (Kozlowski et al. 1991). Due to the high incorporation of leaf nitrogen in chlorophyll, also the nutrient status can be estimated indirectly by the chlorophyll content (Filella et al. 1995, Moran et al. 2000).

Water stress causes stomata closure, reduces cell enlargement and influences other plant physiological processes, which inhibits photosynthesis and affects finally plant growth (Kozlowski et al. 1991). Nonetheless, the effect of water stress on photosynthesis depends on the species. Drought tolerant species for instance carry on photosynthesis to a lower water potential than those native to humid areas (Kozlowski et al. 1991, Uvalle-Sauceda et al. 2008). Many drought tolerant plant species are able to keep functioning biochemical and physiological processes such as turgor maintenance, stomatal opening, leaf expansion, photosynthesis, root growth and water absorption at even lower plant water potentials than would otherwise be possible, due to their capacity of osmotic adjustment (Kozlowski et al. 1991).

There is a close relation between the ratio of leaf chlorophyll or rather its variation and plant physiological stress (Knipling 1970, Hendry et al. 1987, Merzlyak and Gitelson 1995, Peñuelas and Filella 1998, Merzlyak et al. 1999, Carter and Knapp 2001, Torres et al. 2002). Thus, various environmental stressors including dehydration and soil salinity (present study), can result in decrease of leaf chlorophyll contents (Hendry et al. 1987, Carter and Knapp 2001).

2. GENERAL OBJECTIVES

The main objective of the present study was to understand the functional features of mixed pine-oak forest species in relation to principal limiting factors, water scarcity and salt excess, and to determine or rather distinguish the physiological capacities of several common native tree species. The ensuing central questions were focused in order to identify drought tolerant tree species for the restoration of mixed forests in the Sierra Madre Oriental:

- Are species adapted to periods of drought?
- Can they cope with salinity?
- Has water stress an effect on photosynthesis?
- Is there a difference comparing different species and micro-sites?
- How are the species' capacities and competitiveness characterized?

Answering these questions and considering the adaptation of species to water stress and other environmental factors, criteria can be established for species selection and species site-matching, which contributes to the improvement and efficiency of reforestation activities in mountainous areas with some degree of degradation in north-eastern Mexico.

3. GENERAL HYPOTHESIS

The successful existence of a tree species at a specific site as well as the composition of species in a mixed forest ecosystem depend on the adaptability of the species to limiting factors or stressors at a site.

- Trees reflect environmental site conditions and are indicators for the latter.
- Water availability is crucial for species composition.
- Species have different capacities and strategies to cope with environmental stressors such as water scarcity or salt excess.

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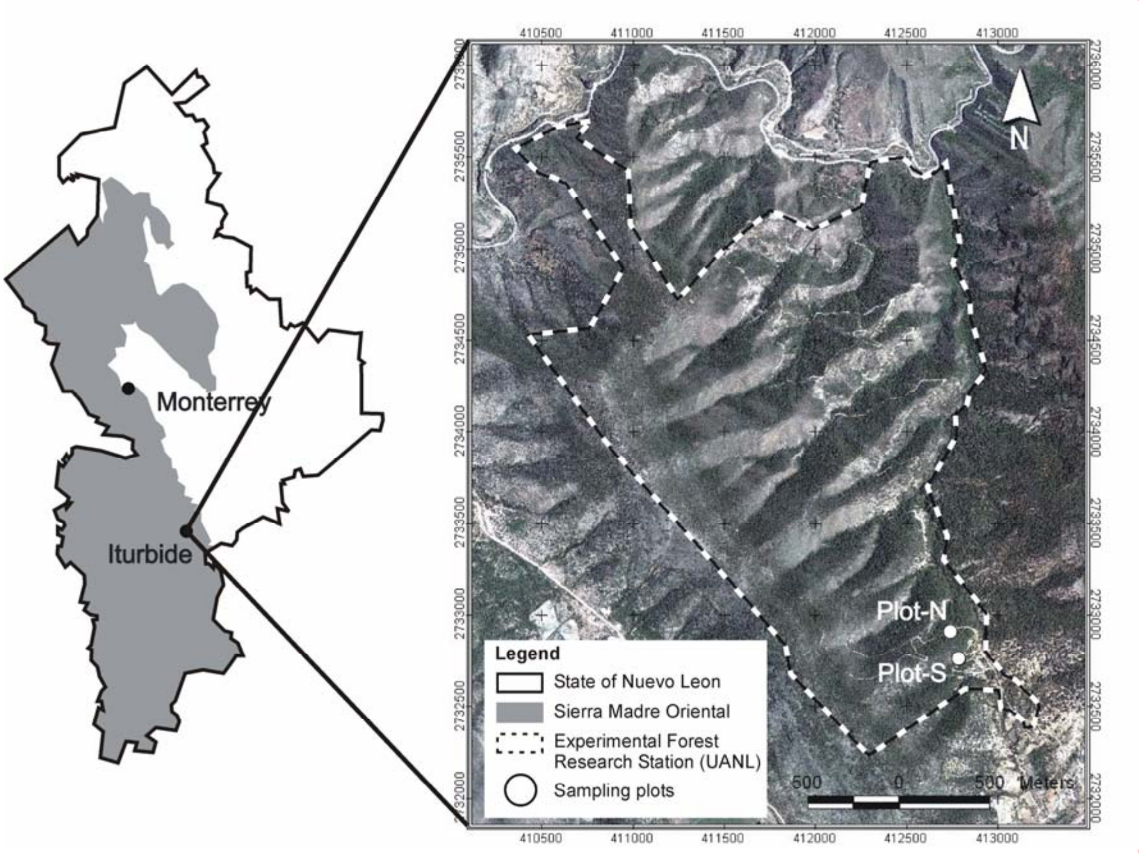
CHAPTER 2

MATERIAL AND METHODS

2.1 RESEARCH SITE

This research was carried out at the Experimental Forest Research Station of the Faculty of Forest Science, Autonomous University of Nuevo Leon (UANL) in the Sierra Madre Oriental (24º42’N; 99º51’W), located 15 km southeast of Iturbide in the state of Nuevo Leon, Mexico (Fig.2.1).

Fig.2.1 Location of the study sites at the Experimental Forest Research Station of the Faculty of Forest Science, Autonomous University of Nuevo Leon (UANL) in the Sierra Madre Oriental (24º42’N; 99º51’W), 15 km southeast of Iturbide, Nuevo Leon, Mexico.



The Experimental Forest Research Station extends over an area of about 1035 ha and due to its protection status received 20 years ago, it presents good characteristics for the study of undisturbed environmental processes. Two sites in different pine-oak forests were selected for the study: a) a major closed stand on a north-facing aspect (Plot-N) and b) an open fragmented stand on a south-facing aspect (Plot-S) (Photo 4.1). Both sites are located very close from each other on the same mountain ridge at an elevation of about 1500 m (Fig.2.1).

Photo 4.1 Vegetation at the closed, north-facing stand Plot-N (left hand side) and at the open fragmented stand Plot-S (right hand side).

The mean annual air temperature is 13.9ºC and the average annual precipitation is 639 mm, which is concentrated from May to October. The dominant soils are rocky and comprise upper cretaceous lutite or siltstone (Cantú and González 2002). Some physical and chemical properties of the soil at profile depths of 0-10 and 20-30 cm are shown in Table 2.1.

Table 2.1. Some physical and chemical soil properties at the two study sites, Plot-N and Plot-S (north- and south aspect, respectively).

Plot	Soil profile depths (cm)	Bulk density (Mg m^{-3})	Sand[a]	Silt[b]	Clay[c]	pH	EC (µScm^{-1})	OM (%)
			%					
N	0-10	0.69	25.9	47.6	26.6	7.6	159.2	11.4
N	20-30	ND	12.6	39.3	48.1	7.7	75.9	2.7
S	0-10	0.85	30.0	41.6	26.4	7.6	141.8	11.3
S	20-30	ND	39.6	43.8	16.6	7.7	72.1	2.5

ND= no data available

Remarkable is the higher electric conductivity (µScm^{-1}) at soil depth 0-10 cm compared to layer 20-30 cm and the slightly higher values at the northern in comparison to the southern aspect.

2.2 PLANT MATERIAL AND SAMPLING PROCEDURES

In each sampling plot, five individuals (replications) of each tree species were randomly selected from the understory for repetitive measurements of plant water potentials. All sampling trees were chosen within a 500 m^2 circle. The plant species were: *Juniperus flaccida* (Schltdt., *Cupressaceae*), *Pinus pseudostrobus* (Lindl., *Pinaceae*), and *Quercus canbyi* (Trel., *Fagaceae*) and both sites, while *Arbutus xalapensis* (Kunth, *Ericaceae*) and *Acacia rigidula* (Benth., *Mimosaceae*) were included only at Plot-N and Plot-S, respectively (Photo 4.2).

Photo 4.2 Species considered for the study on plant water relations: *J. flaccida, P. pseudostrobus, A. xalapensis, A. rigidula, Q. canbyi, R. virens.*

Leaf samples for the determination of the chlorophyll (a+b)- and carotenoid content were collected from surrounding understory trees of the same species, in order to prevent foliar damage of the sample trees for repetitive water potential measurements. Leaf pigments concentration were further determined for the species *Rhus virens* (Lindh., *Anacardiaceae*). For detailed information about the species included in the research, see Table 2.2.

Table 2.2. Characteristics of sampling trees: diameter at breast height (DBH) and tree height (H) both with estimated standard error (E.S.E)).

Species	North aspect (Plot-N)				South aspect (Plot-S)			
	DBH (cm)	E.S.E	H (m)	E.S.E	DBH (cm)	E.S.E	H (m)	E.S.E
J. flaccida	3.07	0.55	4.18	0.36	0.93	0.10	2.23	0.24
P. pseudostrobus	2.05	0.33	2.71	0.32	1.95	0.42	2.47	0.44
Q. canbyi	2.43	0.37	3.85	0.44	1.78	0.45	2.90	0.42
A. xalapensis	2.65	0.78	0.61	0.35	-	-	-	-
A. rigidula	-	-	-	-	shrub	shrub	2.19	0.69

The leaf water potentials (Ψ_w, MPa) were determined twice a month from January until August 2006, the osmotic potential (Ψ_s, MPa), leaf chlorophyll- and carotenoid content were measured once a month, on six and seven sampling dates, respectively, during the same period. For detailed information see sampling calendar in Table 2.3.

Table 2.3. Sampling calendar for plant physiological measurements in 2006. Sampling days for water potential measurements are marked with bolded letters (e.g. **19**), for osmotic potential- are shaded grey (▨) and for chlorophyll measurement are marked with bolded boxes (□). First mentioned days were measured in Plot-N and second days in Plot-S. Cursive bolded letters (e.g. ***1***) indicate whole day leaf water potential measurements in Plot-N.

Month	Day															
January	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	17	18	**19**	**20**	21	22	23	24	25	26	27	28	29	30	31	
February	***1***	**2**	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	17	18	19	20	21	22	***23***	**24**	25	26	27	28				
March	1	2	3	4	5	6	**7**	**8**	9	10	11	12	13	14	15	16
	17	18	19	20	21	22	23	24	25	26	27	28	29	**30**	**31**	
April	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	17	18	19	20	21	22	23	24	25	26	27	28	29	30		
May	1	2	***3***	**4**	5	6	7	8	9	10	11	12	13	14	15	16
	17	**18**	**19**	20	21	22	23	24	25	26	27	28	29	30	31	
June	1	2	3	4	5	***6***	**7**	8	9	10	11	12	13	14	15	16
	17	18	19	20	21	22	23	24	25	26	27	28	**29**	**30**		
July	1	2	3	4	5	6	7	8	9	10	11	**12**	**13**	14	15	16
	17	18	19	20	21	22	23	24	***25***	**26**	27	28	29	30	31	
August	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	***16***
	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	

In total, leaf water potentials were measured in 12 occasions twice a day at 6:00 h (Ψ_{wpd}) and 12:00 h (Ψ_{wmd}) at each aspect. In Plot-N, leaf water potentials were measured every two hours (from 6:00 hr to 18:00 hr) on six sampling dates. The osmotic potential was determined twice a day at 6:00 h

(Ψ_{spd}) and 12:00 h (Ψ_{smd}) on seven sampling dates at each aspect. Samples for the determination of leaf pigments were collected in seven occasions at both aspects in the afternoon of the same day (Table 2.3).

2.3 LEAF WATER POTENTIAL MEASUREMENT

Plant water potentials (Ψ_w) were measured immediately after cutting the leaves or terminal twigs of each sample tree according to the sampling date calendar. Measurements were made with a Scholander pressure chamber (Model 3005, Soil Moisture Equipment Corp., Santa Barbara, CA, USA) (Scholander et al. 1965), which is a widely used method (Sellin 1996), see Photo 4.3. Extensive reviews on the pressure chamber measurements are available elsewhere (Tyree and Hammel 1972, Ritchie and Hinckley 1975, Turner 1981, Boyer 1995, Richter 1997).

Photo 4.3 Leaf water potential measurements taken with a Scholander pressure chamber (Model 3005, Soil Moisture Equipment Corp., Santa Barbara, CA, USA).

Due to morphological differences among species in leaf shape, different foliar samples were required. Entire mature leaves were cut from *Q. canbyi* and *A. xalapensis*, shoots from *J. flaccida* and *A. rigidula* and fascicles of five mature needles from *P. pseudostrobus*. The brownish part of the fascicles was removed in order to prevent the needles from slipping out of the rubber seal by increasing the pressure. For safety reasons, pressure was applied to the chamber at a rate of 0.05 MPa s^{-1}.

2.4 OSMOTIC POTENTIAL MEASUREMENT

For determination of the osmotic potential (Ψ_s), plant samples were taken with a hole puncher from leaves of *Q. canbyi* and *A. xalapensis*. In the case of *J. flaccida* and *P. pseudostrobus,* parts of terminal twigs and pine needles were cut, respectively. Whole leaves were used from *A. rigidula*. Plant samples were collected and immediately saved in small tubes and shock frozen in liquid nitrogen to conserve them for later laboratorial analysis (Photo 4.4).

Photo 4.4 Sample collection in the field for osmotic potential measurement.

Once in the laboratory, the osmotic potential was determined using a Wescor HR 33T (Wescor Inc., Logan, UT) automatic scanning dew point microvoltmeter with three sample chambers (Wescor C-52) (Wilson et al. 1979). The microvolt output was monitored on a 24-channel recorder and readings after two hours equilibration were converted to bars of potential using separate calibration curves for each chamber. The chambers were kept in polystyrene boxes for extra temperature insulation, with a variation over the daily measurement period being approximately less than ± 3ºC (Photo 4.5).

Photo 4.5 Osmotic potential measurement using a Wescor HR 33T (Wescor Inc., Logan, UT) automatic scanning dew point microvoltmeter with three sample chambers (Wescor C-52).

2.5 TOTAL CHLOROPHYLLS AND CAROTENOIDS MEASUREMENT

Terminal shoots of fully expanded leafs from four randomly chosen understory trees were collected within a circle of 25 m radius, which included the sample trees for water potential measurement. Leaf samples were placed into closable plastic bags excluding most air and stored on ice under dark condition using a cooling box. Cooled samples were then transported to the laboratory for pigment analyses which were performed within 12 hr after collection (Uvalle-Sauceda et al. 2008). Quadruplicate leaf tissue samples (1.0 g of fresh weight) of each species were used for ensuing analyses. The carotenoids and total chlorophylls (a+b) were extracted in 80% ($v\ v^{-1}$) aqueous acetone, which is a mostly used solvent (Torres Netto et al. 2002), and vacuum filtered through a Whatman No. 1 filter paper. For the determination of pigment concentrations, a Perkin-Elmer Spectrophotometer (Model Lamda 1A) was used (Uvalle-Sauceda et al. 2008). The absorbance of carotenoids and chlorophylls a and b extracts was determined at wavelengths of 470, 663 and 646 nm, respectively. Concentrations of pigments were finally calculated according to the equations of Lichtenthaler and Wellburn (1983).

$$\text{Chlorophyll a } (\mu g/ml) = 12.21\ (A_{663}) - 2.81\ (A_{646})$$

$$\text{Chlorophyll b } (\mu g/ml) = 20.13\ (A_{646}) - 5.03\ (A_{663})$$

$$\text{Carotenoids } (\mu g/ml) = (1000\ A_{470} - 3.27\ [\text{chl a}] - 104\ [\text{chl b}])/227$$

2.6 REGISTRATION OF ENVIRONMENTAL DATA

Simultaneously to the plant physiological measurements, microclimatic data of air temperature (ºC) and relative humidity (%) was registered in each plot on an hourly basis by using a thermo-hygrometer (HOBO® Pro Series Weatherproof Data Loggers) that was located between the sampling trees directly in the sampling site. Likewise, daily precipitation (mm) was obtained from self-emptying rain gauges (Onset® Data Logging Rain Gauge) installed in each plot (Photo 4.6). Vapour pressure deficit (VPD) was calculated on the basis of air temperature and relative air humidity (RH). On each sampling date, soil cores at different depths of 0-10, 20-30, 40-50, and 60-70 cm were collected using a soil sampling tube (Soil Moisture Equipment Corp.) for determining the gravimetric soil water content beneath the tree canopy in each plot (Photo 4.6). Soil samples were always taken within the 500 m^2 plot, including the sampling trees. Five replications were used for each measurement and weight in the field. The soil water-content was finally determined and expressed in percent (%) by the difference of fresh and dry soil weight after drying the soil samples in an oven at 100ºC for 48 hours.

Photo 4.6 Thermo-hygrometer, self-emptying rain gauge and soil sample collection in the field.

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CHAPTER 3

SITE CONDITIONS REFLECTED BY SEASONAL AND DIURNAL LEAF WATER POTENTIALS OF FOUR CO-OCCURRING TREE SPECIES IN THE SIERRA MADRE ORIENTAL, MEXICO[1]

3.1 ABSTRACT

Mixed pine-oak forests are exposed to extreme environmental conditions where water availability is a limiting factor. In order to determine the adaptation of four tree species (*Arbutus xalapensis, Juniperus flaccida, Pinus pseudostrobus, Quercus canbyi*) to water stress, diurnal leaf water potentials (Ψ_w) were measured under natural drought and non-drought conditions in the Sierra Madre Oriental. Furthermore, the relation between leaf Ψ_w and environmental variables was analyzed. The ANOVA revealed significant differences in Ψ_w between sampling dates (block variable), sampling hours and species (treatment variables) with no significant (p-value>0.05) interaction between the treatment variables. In general, all species showed high predawn and low midday values that declined progressively with increasing drought and soil-water loss. During the dry period, *J. flaccida* had the lowest Ψ_w followed by *Q. canbyi* and *A. xalapensis*, but all species recovered with rapid higher potentials after the onset of the rainy season in the end of May. *P. pseudostrobus* in comparison showed less seasonal fluctuations. Differences in leaf Ψ_w were significant (p-value<0.01) between all species, except for the two conifers (p-value>0.05). Diurnal variations in Ψ_w were very similar comparing the four species with high values at predawn (6:00 hr) and low values after midday (12:00 hr-16:00 hr).

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[1] Sent for publication to the German international journal “Forstarchiv” (TUhttp://www.forstarchiv.de/UT).

Correlations within Ψ_w and environmental variables were highly significant for soil moisture content in the morning hours (6:00 hr and 8:00 hr) and evaporative demand components in the afternoon (12:00 hr to 16:00 hr), depending on the species. Hence, *A. xalapensis, J. flaccida* and *Q. canbyi* reflected better the measured site conditions than *P. pseudostrobus*. Consequently, species employ different strategies to overcome periods of drought. *P. pseudostrobus* seems to avoid water stress by means of a deeper rooting system, while the other species have further plant physiological capacities to overcome water scarcity. In conclusion, all species are considered as suitable candidates for reforestation programs in the Sierra Madre Oriental. Nevertheless, their suitability depends on the environmental conditions, especially with respect to soil characteristics in the area of improvement.

Keywords: adaptation, *Arbutus xalapensis*, *Juniperus flaccida*, *Pinus pseudostrobus*, *Quercus canbyi*, restoration, water stress

3.2 INTRODUCTION

Mixed pine-oak forests are widely distributed ecosystems of ecological and economical importance in the mountain chain Sierra Madre Oriental, north-eastern Mexico (Rzedowski 1986). Mentioned forests are exposed to extreme climatic conditions such as high temperatures and low precipitation distributed irregularly over the year. Water availability is a limiting factor in this mountainous area controlling plant growth, survival and distribution (Kotzlowski et al. 1991, González et al. 2003). Furthermore, climatic change and anthropogenic pressures due to inappropriate land use and repeated forest fire events contribute to the acceleration of forest degradation and deforestation (Domínguez and Návar 2000, Cantú and González 2002, Salinas and Treviño 2002, González et al. 2005). The response of forest ecosystems to such environmental and anthropogenic stresses will depend on the drought tolerance capabilities of the individual species (Tschaplinski et al. 1998). There are hardly publications about plant water relations of pine-oak forest species in the north-eastern region of Mexico. Therefore, the Sierra Madre Oriental provides a rich opportunity to investigate the eco-physiological aspects of native tree species and their responses to changes in soil moisture content and atmospheric conditions in order to enable and improve sustainable tree productivity. Leaf water potentials (Ψ_w) are quickly measured in the field and a good indicator of plant water status commonly used to determine water stress (Kotzlowski et al. 1991, Mitlöhner 1997, Vertovec et al. 2001).

The objectives were to assess and quantify how diurnal and seasonal leaf water potentials are related to soil moisture availability and evaporative demand components of four native tree species that grow in the Sierra Madre Oriental.

3.3 MATERIAL AND METHODS

3.3.1 Research site

As study site a north-facing mixed pine-oak stand at about 1500 m elevation was chosen within the Experimental Forest Research Station (EF) of the Faculty of Forest Science, Autonomous University of Nuevo Leon (UANL). The EF is located 15 km southeast of Iturbide in the Sierra Madre Oriental (24º42'N; 99º51'W), Nuevo Leon, Mexico and extends over an area of about 1035 ha. The mean annual air temperature is 13.9ºC and the average annual precipitation is 639 mm, which is concentrated from May to October. The dominant soils are rocky and comprise upper cretaceous lutite or siltstone (Cantú and González, 2002).

3.3.2 Sampling procedure

Five to six individuals of each species of interest, *Arbutus xalapensis* (Kunth, Ericaceae), *Juniperus flaccida* (Schlecht., Cupressaceae), *Pinus pseudostrobus* (Lindley, Pinaceae) and *Quercus canbyi* (Trel., Fagaceace), were selected from the natural regeneration for repeated water potential measurements during the period from February until August 2006. Sampling trees varied in diameter at breast height between 2.05±0.33 cm and 3.07±0.55 cm, and in height between 2.70±0.32 m and 3.85±0.44 m, depending on the species. At each sampling date (Feb-2, Feb-23, May-3, Jun-6, Jul-25, Aug-16), measurements were taken out on the same individuals at two hour intervals from 6:00 hr until 18:00 hr immediately after cutting leaves (*A. xalapensis* and *Q. canbyi*), small twigs (*J. flaccida*) and/or fascicles of five mature needles (*P. pseudostrobus*) using a Scholander pressure chamber (Model 3005, Soil Moisture Equipment Corp., Santa Barbara, CA, USA) (Turner 1981).

3.3.3 Environmental variables

During the whole study period, a thermo-hygrometer (HOBO® Pro Series Weatherproof Data Loggers) and a self-emptying rain gauge (Onset® Data Logging Rain Gauge) were located between the sampling trees registering microclimatic data of air temperature (T, ºC), relative humidity (RH, %) and total precipitation (P, mm) on a hourly basis. Vapour pressure deficit (VPD, kPa) was calculated afterwards on the basis of T and RH. On each sampling date, soil cores at different depths of 0-10 cm (D10), 20-30 cm (D30), 40-50 cm (D50), and 60-70 cm (D70) were collected using a soil sampling tube (Soil Moisture Equipment Corp.) for determining the gravimetric soil water content beneath the tree canopy. Five replications of soil samples were taken within the 500 m^2 circle including the sampling trees.

3.3.4 Data analysis

Differences between the six sampling dates (February to August 2006), the four species and the sampling hours (6:00, 8:00, 10:00, 12:00, 14:00, 18:00 hr) were examined with a factorial analyses of variance (ANOVA) using the linear model procedure (aov) on R with the block variable “sampling date”, two treatment variables (“species” and “sampling hour”) and the interaction between the treatment variables up to two-way terms (Crawley 2007). Mean data were log transformed before ANOVA, since assumption of normality and homogeneity of variance was not true. When significant variations were revealed, results were validated using the Tukey's honestly significant difference (HSD) test ($p<0.05$), comparing the means of sampling dates, species and hours (Zar 1999, Crawley 2007).

The differences between predawn (6:00 hr) and midday (12:00 hr) leaf Ψ_w were presented as diurnal range, which indicates the species capacity to recover from daily minimum values at each sampling date. Predawn and midday values were used as the x- and y-axes, respectively. The 45° bisecting line was plotted on the same graph, representing the boundary, where the values of predawn and midday Ψ_w are equal (Gebrekirstos 2006). Values of diurnal range would approach the bisecting line, when species fail to recover from water stress in the course of the day, which could have negative effects on leaf development and tree growth (Mitlöhner 1998, 2000).

Seasonal soil moisture content (observed data) was examined with a two-factorial analysis of variance (Two-factorial ANOVA) including the interaction term (sampling date x soil depth). Also the climatic variables (T, RH and VPD) were analyzed with a two-factorial ANOVA, but without interaction term (Crawley 2007). In the case of significant differences, results were validated afterwards using the Tukey's HSD test.

Correlation coefficients between water potentials (Ψ_w) of each species and environmental variables (EV: mean D10, D30, D50, D70 per sampling date, hourly air temperature, hourly RH, hourly VPD, and monthly sum of precipitation) were analyzed with the Spearman's rank order correlation. For these analyses, observed data of each species were considered on an hourly base including data from the six sampling dates (Zar 1999, Crawley 2007).

For the visualization of the relationship between leaf water potentials and EV per sampling hour, observed data per species were analysed on an hourly bases with a detrended canonical analysis (DCA). Environmental variables were adjusted as vectors when p-value≤0.01. Ensuing to the DCA, the main influencing EV per sampling hour was considered for regression analysis for each species separately.

Before ANOVA and Correlation analysis, data were checked for normality and homogeneity of variance using the Shapiro-Wilk and Fligner-Killeen test, respectively (Zar 1999, Crawley 2007). All statistical methods were applied according to The R project for statistical computing (free statistic software, R version 2.8.1, The R Foundation for Statistical Computing, 2008).

3.4 RESULTS

3.4.1 Environmental conditions during the experimental period

Seasonal mean values (Fig.3.1d) and daily trends of air temperatures (T), relative air humidity (RH) and vapour pressure deficit (VPD) are illustrated for each sampling date in Fig.3.1a,b,c. During the experimental period, T ranged from 12.9°C (Feb-2, 6:00 hr) to 29.9°C (Jun-6, 16:00 hr), RH from 17 % (Feb-2, 12:00 hr) to 95 % (Jun-6, 6:00 hr), and VPD from 0.1 kPa (Jun-6, 6:00 hr) to 3.1 kPa (May-3, 12:00 hr). In general, mentioned climatic variables varied significantly (*p*-value<0.01) comparing different sampling dates and hours. Just RH had no significant differences (*p*-value>0.05) between sampling hours.

May-3 and Jun-6 were identified as the driest and hottest sampling dates (Fig.3.1d). Concerning diurnal variations, atmospheric conditions differed mainly comparing morning (6:00 hr, 8:00 hr) and afternoon hours (12:00 hr, 14:00 hr, 18:00 hr), depending on the EV.

Fig.3.1e shows the monthly sum of precipitation (P) and soil moisture content at four soil depths (D10, D30, D50, D70) per sampling date. The total rainfall registered at the study site was 206 mm from February until August 2006. The average soil moisture per soil depth decreased along the growing season, but increased again after the onset of the rainy season in the end of May (Fig.3.1e).

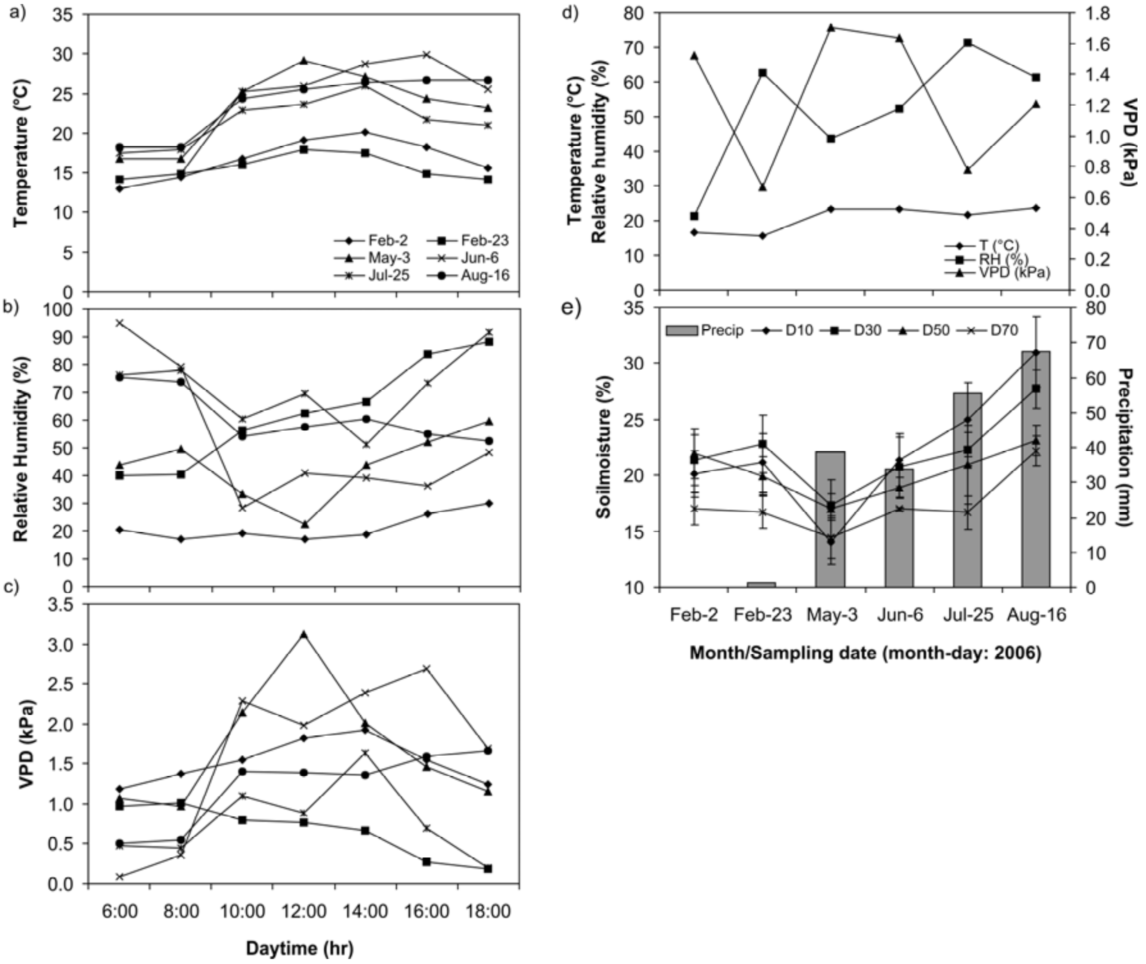

Fig.3.1. Environmental variables from February until August 2006: a) diurnal and seasonal variation in mean air temperature (°C), b) in relative humidity (%) and c) in vapour pressure deficit (kPa; VPD), d) mean values for climatic conditions at each sampling date (*n*=7) and e) monthly sum of precipitation (mm) and seasonal variation in soil moisture (%) at four soil profile depths (*n*=5, D10: 0-10 cm, D30: 20-30 cm, D50: 40-50 cm, D70: 60-70 cm). Due to technical problems, precipitation in July had to be estimated by interpolation using registered rainfall of June and August. Estimated standard errors are provided for soil moisture.

Differences between sampling dates (F_{sd}: 9.04***) and soil depths (F_{depth}: 4.34**) were statistically significant (*p*-value<0.01), although the interaction of both was not significant (*p*-value>0.80). Soil water content of the upper soil layer showed great variation and sensitivity to precipitation over the season (Fig.3.1e). The deepest layer (D70) was significantly drier than the upper layers, D10 (*p*-value<0.01) and D30 (*p*-value<0.05).

3.4.2 Variation in water potentials

Leaf water potential had highly significant differences between the sampling dates, sampling hours and species (F_{date}: 54.13***, F_{hour}: 12.8***, $F_{species}$: 45.4***). In general, leaf water potentials were much lower for all species on May-3 and Jun-6 (Fig.3.2), with one exception; *P. pseudostrobus* showed very similar water potentials independent of the sampling date (Fig.3.2).

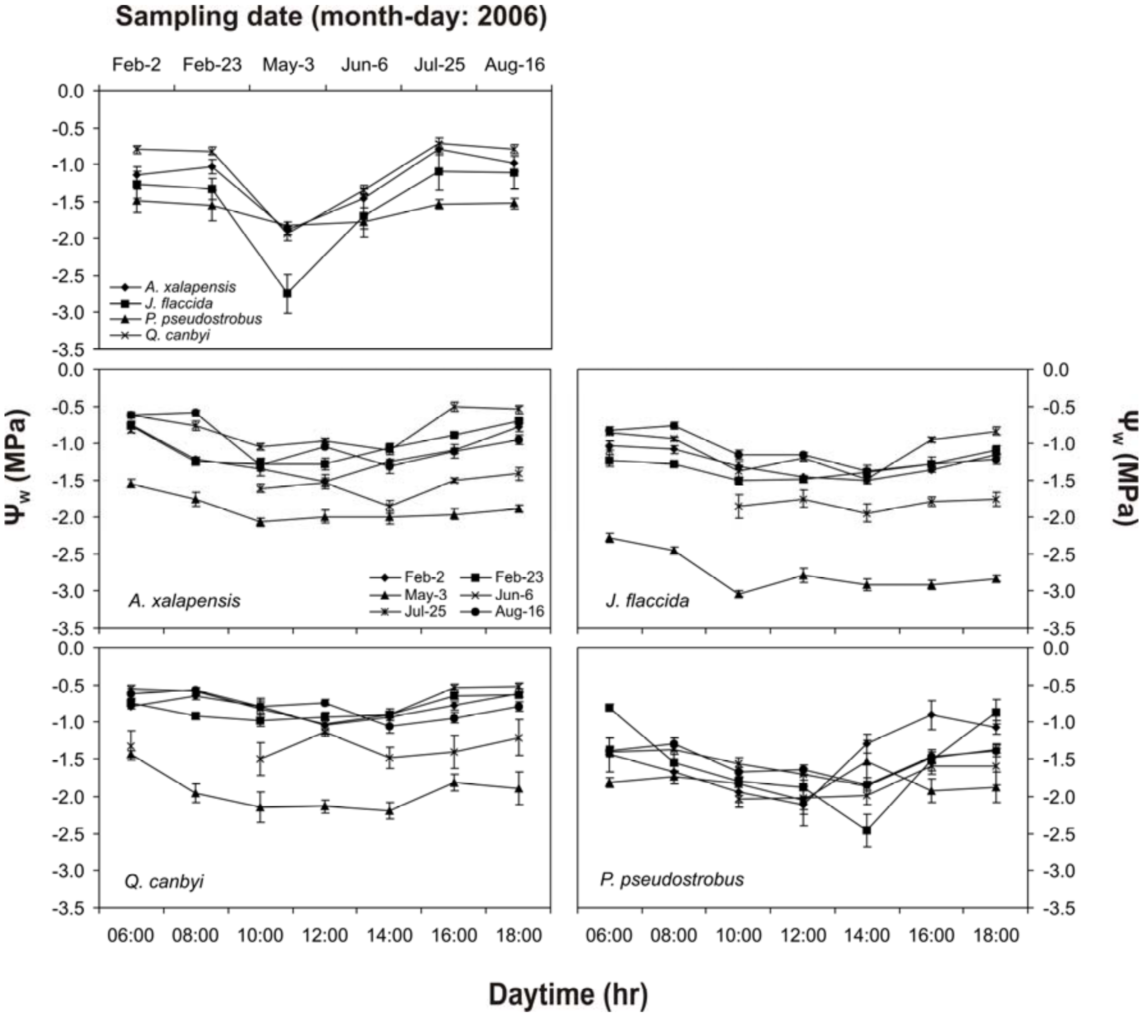

Fig.3.2. Diurnal and seasonal changes in leaf water potential (MPa; n=6) of four native tree species. In the case of seasonal changes, daily mean data per species were used (n=7). Error bars represent estimated standard error. May-3 and Jun-6 were significantly (p-value<0.001) different from the other sampling dates.

This tendency was confirmed by the ensuing Tukey test, which revealed the water potentials measured on May-3 and Jun-6 as significantly different (p-value<0.01) from any other sampling date. Mentioned sampling dates were identified as the driest and hottest ones (Fig.3.1d). Differences between the other sampling dates were not significant (p-value>0.05). Anyway, leaf Ψ_w of *J. flaccida* and *Q. canbyi* had the greatest seasonal fluctuation followed by *A. xalapensis* and *P. pseudostrobus* (Fig.3.2).

Concerning the daytime, Fig.3.2 shows clear variation in leaf water potentials in the course of the day, while lowest values were reached around midday and highest at predawn. So, Ψ_w measured at 6:00 hr and 8:00 hr were significantly higher (p-value<0.001) from those measured at 10:00 hr, 12:00 hr and 14:00 hr. Also afternoon Ψ_w (16:00 hr and 18:00 hr) differed significantly (p-value<0.05) from midday values (12:00 hr, 14:00 hr and 10:00 hr; last one only in comparison to 18:00 hr).

Furthermore, differences between the Ψ_w of the four species were statistically significant (p-value<0.001) between almost all species, mainly comparing conifers with latifolia. Nevertheless, leaf Ψ_w of *J. flaccida* and *P. pseudostrobus* differed not significantly (p-value>0.05). Conifers had in general more negative values than the latifolia (Fig.3.2).

Diurnal ranges and the species' capacity to recover from daily minimum Ψ_w values over night are presented in Fig.3.3. Values close to the bisecting line indicate a species' difficulty to cope with diurnal variation in water availability. This was the case of *Q. canbyi*, which was not able to re-saturate well during night at four sampling dates. *A. xalapensis* in contrast was identified as the species that coped best with water deficiency, followed by *P. pseudostrobus* and *J. flaccida* (Fig.3.3). The interaction between species and sampling hour was statistically not significant (p-value>0.05), which means, that the daily variation in leaf Ψ_w was not depending on the species.

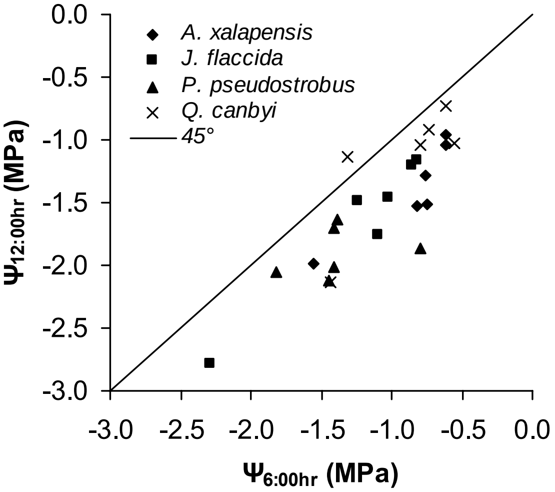

Fig.3.3. Range of daily relaxation of four tree species as a relationship between predawn (6:00 hr) and midday (12:00 hr) leaf water potential throughout the entire study period.

3.4.3 Relationship between plant water potentials and environmental variables

Correlations between leaf water potentials (Ψ_w) and environmental variables (EV) are shown in Table 3.1. In general, *A. xalapensis, J. flaccida* and *Q. canbyi* resulted as god site-indicators, reflecting the environmental site conditions much better than *P. pseudostrobus*, which leaf Ψ_w were less correlated with EVs. Focusing on the different sampling hours, Ψ_w were strongly correlated with soil moisture content, mainly the upper soil layer at predawn (6:00 hr; Table 3.1).

In the course of the day, correlations became stronger with climatic variables such as air temperature (T), relative air humidity (RH) and vapour pressure deficit (VPD). At 14:00 hr for instance, leaf Ψ_w had highly significant (*p*-value<0.001) correlations with T (*rho*=-0.59 to -0.78), VPD (*rho*=-0.65 to -0.75) and RH (*rho*=0.35 to 0.49) (Table 3.1).

Table 3.1. Spearman's correlation coefficient values (rho) for seasonal leaf water potentials (*n*=36) in relation to daily soil water-content (D10: 0-10 cm, D30: 20-30 cm, D50: 40-50 cm, D70: 60-70 cm), air temperature (T), relative humidity (RH), vapour pressure deficit (VPD), and monthly precipitation in four tree species. Only variables with significant correlations are listed.

Sampling hour	Environ. variable	*A. xalapensis* rho		*J. flaccida* rho		*P. pseudostrobus* rho		*Q. canbyi* rho	
6:00 hr	T	0.41	*	0.44	**	-0.11		0.40	*
	VPD	-0.30		-0.41	*	-0.20		-0.09	
	P	0.13		0.22		-0.38	*	0.07	
	D10	0.75	***	0.81	***	0.31		0.62	***
	D70	0.45	**	0.51	**	0.27		0.31	
8:00 hr	T	0.65	***	0.59	**	0.57	**	0.36	
	RH	0.57	**	0.49	**	0.54	**	0.35	
	VPD	-0.57	**	-0.49	**	-0.54	**	-0.35	
	D10	0.92	***	0.87	***	0.71	***	0.70	***
	D70	0.64	***	0.68	***	0.36		0.59	***
10:00 hr	T	-0.64	***	-0.50	**	-0.05		-0.58	***
	RH	0.51	**	0.19		0.34	*	0.23	
	VPD	-0.67	***	-0.44	**	-0.33		-0.49	**
	D10	0.59	***	0.63	***	0.15		0.50	**
	D30	0.02		0.13		0.35	*	0.04	
	D50	0.13		0.45	**	0.26		0.32	
	D70	0.30		0.66	***	-0.16		0.50	**
12:00 hr	T	-0.48	**	-0.44	**	0.02		-0.49	**
	RH	0.75	***	0.58	***	0.32		0.46	**
	VPD	-0.74	***	-0.65	***	-0.20		-0.63	***
	D10	0.76	***	0.82	***	0.30		0.67	***
	D50	0.04		0.35	*	0.16		0.25	
	D70	0.25		0.50	**	-0.01		0.57	***
14:00 hr	T	-0.78	***	-0.59	***	0.04		-0.69	***
	RH	0.49	**	0.46	**	-0.49	**	0.35	*
	VPD	-0.75	***	-0.70	***	0.28		-0.65	***
	D10	0.37	*	0.51	**	-0.21		0.42	*
	D50	0.08		0.18		0.36	*	0.15	
	D70	0.17		0.48	**	0.03		0.34	*
16:00 hr	T	-0.53	**	-0.39	*	-0.21		-0.57	***
	RH	0.60	***	0.55	***	-0.17		0.50	**
	VPD	-0.51	**	-0.39	*	0.07		-0.52	**
	D10	0.52	**	0.63	***	0.16		0.35	*
	D70	0.11		0.22		0.38	*	0.05	
18:00 hr	T	-0.52	**	-0.39	*	-0.52	**	-0.50	**
	RH	0.37	*	0.41	*	0.01		0.28	
	VPD	-0.49	**	-0.44	**	-0.32		-0.44	**
	P	-0.35	*	-0.21		-0.51	**	-0.36	*
	D10	0.40	*	0.52	**	-0.02		0.31	

These results are confirmed by the DCA (Fig.3.4), where D10 explained between 57% and 72% (*p*-value<0.001) of the species' leaf Ψ_w at 6:00 hr and 8:00 hr, respectively. From 10:00 hr to 14:00 hr, the VPD explained mainly the Ψ_w with 52%, 74% and 43% (*p*-value<0.001), respectively. At 16:00 hr, RH had the greatest influence (46%, p-value<0.001) on leaf Ψ_w, while T with 38% (p-value<0.001) at 18:00 hr (Fig.3.4). Sampling date (SD) was especially important in the early morning (6:00 hr and 8:00 hr) and the afternoon (12:00 hr and 16:00 hr), indicating that the variables' influences on leaf Ψ_w is dependent on the season.

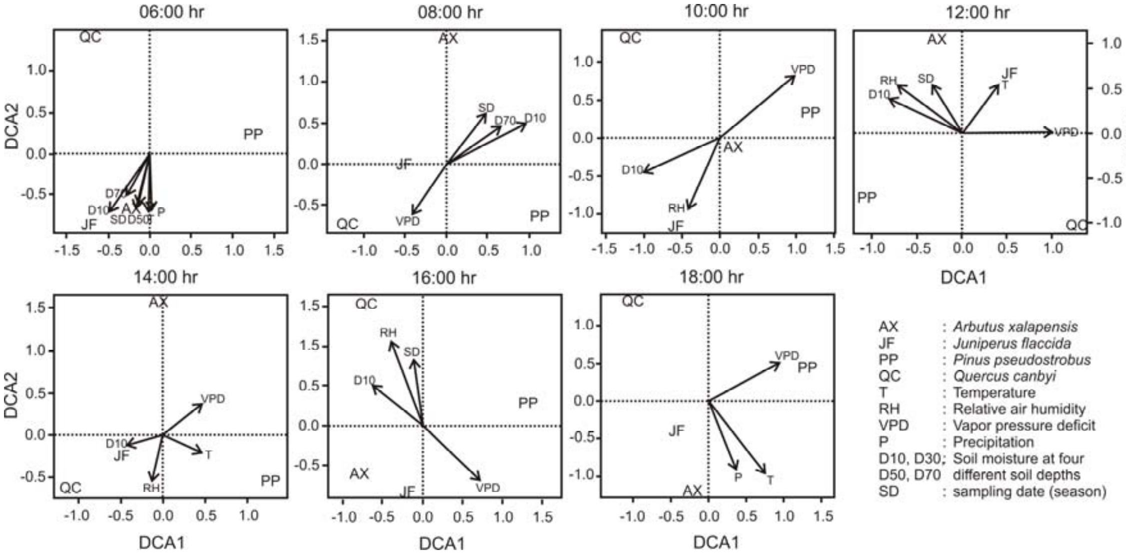

Fig.3.4. Result of the detrended canonical analysis (DCA) at each sampling hour including data from the six sampling dates. Environmental variables were adjusted as vectors when p-value≤ 0.01.

Leaf Ψ_w were related for each species separately by regression to the EV, which had, according to the DCA, the main influence on the leaf Ψ_w at a given sampling hour (Table 3.2). *A. xalapensis* resulted to reflect best the site's environmental conditions (r^2=0.22 to 0.85) at almost every sampling hour, followed by *J. flaccida* (r^2=0.05 to 0.66) and *Q. canbyi* (r^2=0.09 to 0.44). In general, leaf Ψ_w of *P. pseudostrobus* were hardly influenced by EVs, variation in r^2-values between 0.00 and 0.42 (Table 3.2).

Also, the relationship between $\Psi_{6:00\ hr}$ and $\Psi_{12:00\ hr}$ was described for each species separately and highly significant (p-value<0.001) for almost all species (Table 3.2). Only the relationship between leaf Ψ_w of *P. pseudostrobus* was not significant (p-value=0.43). This would mean that a change in $\Psi_{6:00\ hr}$ has no influence on the leaf Ψ_w at 12:00 hr. Predawn leaf Ψ_w of *J. flaccida* in comparison explain about 80% of the total seasonal variability in $\Psi_{12:00\ hr,}$ and in the case of *A. xalapensis* and *Q. canbyi* 63% and 28%, respectively (Table 3.2).

Table 3.2. Least-squares coefficients for diurnal leaf water potential (Ψ_w) in relation to environmental variables (*EV*: soil water content at 0-10 cm depth (D10), vapour pressure deficit (VPD), relative air humidity (RH) and air temperature (T)), which influenced mainly the Ψ_w (compare Fig.3.4) in four tree species at a given daytime.

Daytime and variable	Tree species	Least-squares statistics*						
		y-axis intercept			Slope of regression model			Adjusted r^2
		$\hat{\beta}_0$	E.S.E.	*p*-value	$\hat{\beta}_1$	E.S.E.	*p*-value	
6:00 hr	*A. xalapensis*	4.12	0.79	<0.0001	-1.39	0.25	<0.0001	0.47
D10	*J. flaccida*	5.01	0.83	<0.0001	-1.57	0.27	<0.0001	0.50
	P. pseudostr.	0.64	0.93	0.5000	-0.11	0.30	0.7148	-0.03
	Q. canbyi	4.78	1.02	<0.0001	-1.59	0.33	<0.0001	0.41
8:00 hr	*A. xalapensis*	6.67	0.53	<0.0001	-2.12	0.17	<0.0001	0.85
D10	*J. flaccida*	6.09	0.82	<0.0001	-1.89	0.26	<0.0001	0.66
	P. pseudostr.	2.30	0.42	<0.0001	-0.60	0.13	0.0001	0.42
	Q. canbyi	5.63	1.23	0.0001	-1.86	0.39	<0.0001	0.44
10:00 hr	*A. xalapensis*	0.15	0.05	0.0078	0.47	0.10	<0.0001	0.41
VPD	*J. flaccida*	0.28	0.08	0.0007	0.51	0.14	0.0012	0.26
	P. pseudostr.	0.52	0.05	<0.0001	0.10	0.09	0.3225	0.00
	Q. canbyi	-0.22	0.10	0.0383	0.71	0.19	0.0007	0.29
12:00 hr	*A. xalapensis*	0.13	0.04	0.0025	0.43	0.06	<0.0001	0.58
VPD	*J. flaccida*	0.25	0.05	<0.0001	0.48	0.08	<0.0001	0.53
	P. pseudostr.	0.58	0.05	<0.0001	0.09	0.08	0.2396	0.01
	Q. canbyi	-0.12	0.08	0.1141	0.52	0.12	0.0002	0.34
14:00 hr	*A. xalapensis*	0.14	0.06	0.0232	0.43	0.09	<0.0001	0.38
VPD	*J. flaccida*	0.37	0.07	<0.0001	0.37	0.11	0.0021	0.24
	P. pseudostr.	0.72	0.07	<0.0001	-0.34	0.12	0.0102	0.16
	Q. canbyi	-0.07	0.09	0.4566	0.47	0.15	0.0037	0.21
16:00 hr	*A. xalapensis*	2.35	0.76	0.0040	-0.58	0.19	0.0049	0.20
RH	*J. flaccida*	1.50	0.65	0.0281	-0.28	0.17	0.1012	0.05
	P. pseudostr.	-1.10	0.56	0.0584	0.37	0.14	0.0138	0.15
	Q. canbyi	1.60	0.82	0.0597	-0.43	0.21	0.0477	0.09
18:00 hr	*A. xalapensis*	-2.97	0.92	0.0029	0.96	0.30	0.0033	0.22
T	*J. flaccida*	-1.73	0.89	0.0604	0.67	0.29	0.0277	0.12
	P. pseudostr.	-2.62	0.74	0.0013	0.95	0.24	0.0005	0.30
	Q. canbyi	-3.07	1.04	0.0059	0.95	0.34	0.0090	0.17
$\Psi_{6:00\ hr.}$	*A. xalapensis*	0.44	0.03	<0.0001	0.63	0.08	<0.0001	0.63
$\Psi_{12:00\ hr.}$	*J. flaccida*	0.35	0.03	<0.0001	0.76	0.07	<0.0001	0.80
	P. pseudostr.	0.58	0.05	<0.0001	0.11	0.13	0.4279	-0.01
	Q. canbyi	0.18	0.07	0.0105	0.55	0.15	0.0009	0.28

Least-squares estimates have indicated that the best fitted model to relate Ψ_w as a function of different EV corresponded to a power ($\hat{\Psi}_w = \hat{\beta}_0 \times EV^{\hat{\beta}_1}$; where $\hat{\Psi}_w = -\Psi_w$) mathematical function. $\hat{\beta}_0$ and $\hat{\beta}_1$ are the *y*-axis intercept and slope of regression model, respectively. Estimated standard errors (E.S.E.'s), *p*-values and adjusted coefficient of determination (r^2) values are provided. *n=5 to 6. Furthermore, the relationship between predawn ($\Psi_{6:00\ hr}$) and midday ($\Psi_{12:00\ hr}$) leaf water potentials was analysed for each species separately.

3.5 DISCUSSION

Diurnal and seasonal water potentials (Ψ_w) studied in the course of seven months revealed significant differences between sampling dates, sampling hours and species; owing to which tree species seem to employ different strategies to offset the harmful effect of drought periods. An understanding of these strategies is of importance for the selection of suited tree species for reforestation of forest areas, where water is a limiting factor (Gebrekirstos et al. 2006).

Leaf Ψ_w showed a pattern of progressive decline during the dry season and recovered after the onset of precipitation in the end of May. Water potentials of *J. flaccida* showed the greatest seasonal variation followed by *Q. canbyi, A. xalapensis* and *P. pseudostrobus* (Fig.3.2). Observed fluctuations were very similar to the variation in soil moisture content of the upper two soil layers (0-10 cm and 20-30 cm) with respect to the three first mentioned species. Especially water potentials of *J. flaccida* had strong correlations with soil moisture content throughout the day (Table 3.1).

All species presented their lowest values around midday, the time when all environmental influences on the plant’s internal water balance are integrated and reflected, indicating the least favourable condition for a plant (Mitlöhner 1997, Gebre et al. 1998, Mitlöhner 1998, Gebrekirstos et al. 2006). Many authors consider predawn Ψ_w as an estimate of soil water potential (Hinckley et al. 1978, Bréda et al. 1995, Sellin 1998, Mitlöhner 1998, Zobel et al. 2001, Gebrekirstos et al. 2006), since plants recover over night from daily minimum values. Others in comparison doubt that plants necessarily equilibrate with soil water potential during their phase of relaxation (Richter 1997, Donovan et al. 2001, 2003).

As the Spearsman’s correlations and DCA proved, leaf Ψ_w of *J. flaccida*, *Q. canbyi* and *A. xalapensis* were highly correlated with soil moisture content in the upper soil layers at predawn (*rho*=0.62 to 0.81, *p*-value<0.001) and even at

8:00 hr (*rho*=0.70 to 0.92, *p*-value<0.001), suggesting some use of summer precipitation (Table 3.1, Fig.3.4). Hence, predawn Ψ_w are assumed to be an estimate of soil water potential in the present study with regard to the three mentioned species. Strong correlations of predawn Ψ_w with soil moisture content in the topsoil were also found in Arizonian pine-oak forests (Kolb and Stone 2000) and Mexican thornscrub shrub species (González et al. 2004).

In the course of the day, the effect of evaporative demand components (T, RH and VPD) on leaf Ψ_w increased (Table 3.1, Fig.3.4) and reached its peak between 12:00 hr and 16:00 hr, depending on the species. So, *A. xalapensis* showed great sensitivity to atmospheric changes followed by *Q. canbyi* and *J. flaccida* (Table 3.1), reflecting best the environmental conditions measured at the site (Table 3.2). Water potentials of *P. pseudostrobus* in comparison were hardly correlated with environmental variables (Table 3.1). Nevertheless, *P. pseudostrobus* seemed to come through periods of water stress, taking advantage of stored soil moisture in deeper soil horizons (>70 cm depth), which were not included in the study, an explanation for its capacity to re-saturate over night (Fig.3) and the equalized seasonal fluctuation in leaf Ψ_w (Fig.3.2). *J. flaccida, A. xalapensis,* and *Q. canbyi* in comparison, characterized by more superficial rooting systems, depended more on seasonal fluctuations in precipitation and seemed to use further plant physiological strategies to overcome drought. Particularly *J. flaccida,* which's Ψ_w was highly correlated with soil moisture content throughout the day (Table 3.1).

Significant seasonal (*p*-value<0.01) effects on the leaf Ψ_w were found principally for *A. xalapensis* and *J. flaccida* (Fig.3.4) during the extreme sampling hours (6:00 hr, 8:00 hr versus 12:00 hr, 16:00 hr). Particularly the seasonal fluctuations in Ψ_w of *J. flaccida* and their strong correlations with soil moisture in the topsoil are an indicator for a quite superficial rooting system. Daily fluctuations in Ψ_w were very similar comparing the four species (Fig.3.2), which is in accordance with comparative studies (González et al. 2004).

The species comparison resulted that leaf Ψ_w of *Q. canbyi* were significantly (*p*-value<0.01) less negative than the ones of the other species, followed by *A. xalapensis*, *J. flaccida* and *P. pseudostrobu*s (Fig.3.2). Also in Arizona oak trees had higher Ψ_w and showed greater diurnal variation than pine trees in comparison (Kolb and Stone 2000). On the other hand, *Q. canbyi* had more difficulties to re-saturate during night than the other species in the present study (Fig.3).

3.6 CONCLUSION

The results obtained for *A. xalapensis, J. flaccida* and *Q. canbyi* support the hypothesis that predawn Ψ_w reflects the actual retention of water by the soil, integrating and characterizing the soil matrix potentials, at least in the upper soil horizons. Furthermore, midday Ψ_w of the same species revealed the internal water availability in the plant under the influence of atmospheric water saturation deficits. All species are adapted to periods of water stress but possess quite different strategies to cope with it.

Seedlings of the deep rooting *P. pseudostrobus* should be produced for reforestation in sufficient big containers and transplanted in soil with deeper profiles promising best transplantation results. *J. flaccida* resulted to be a very superficial rooter, suffering water stress during dry periods, but being able to take advantage spontaneously of topsoil water after the onset of precipitation. This species is optimal for reforestation on xeric sites with low soil profiles. Water potentials of *Q. canbyi* and *A. xalapensis* presented similar seasonal and diurnal behaviour, although Ψ_w of *Q. canbyi* reached slightly more negative values during the dry and hot period. Both species showed great physiological flexibility and capacity to deal with water scarcity. *A. xalapensis* was found to be the species indicating best the site's environmental conditions. Considering furthermore, that *A. xalapensis* is listed in the IUCN "Red List of Threatened Species" (Ramirez-Marcial and González-Espinosa 1998) as a species dependent on conservation; it is strongly recommended to include *A. xalapensis* in reforestation programs of pine-oak forest, its natural habitat.

Acknowledgement

The study was funded in part by the Comisión Nacional Forestal and Consejo Nacional de Ciencia y Tecnología (CONAFOR-CONACYT, No. 2002-C01-5951) and the Universidad Autónoma de Nuevo León (UANL-PAICYT, No. CN 1133-05). We further would like to thank the Secretaría de Relaciones Exteriores (SRE) for the support by scholarship. We gratefully acknowledge the anonymous reviewers for their enriching comments. Valuable technical assistance provided by Juan José González Álvarez is gratefully recognized.

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CHAPTER 4

ADAPTATION OF THREE CO-OCCURRING TREE SPECIES TO WATER STRESS AND THEIR ROLE AS SITE-INDICATORS IN MIXED PINE-OAK FORESTS IN THE SIERRA MADRE ORIENTAL, MEXICO[1]

4.1 ABSTRACT

Water availability and salt excess are limiting factors in Mexican mixed pine-oak forest. In order to characterize the adaptation of native species to these stresses, plant water (Ψ_w) and osmotic potentials (Ψ_s) of *Juniperus flaccida, Pinus pseudostrobus* and *Quercus canbyi* were measured under natural drought and non-drought conditions under two different aspects in the Sierra Madre Oriental. Factorial ANOVA revealed significant differences in Ψ_w and Ψ_s between two aspects, species and sampling dates. In general, all species showed high predawn and low midday values that declined progressively with increasing drought and soil-water loss. Seasonal and diurnal fluctuation of Ψ_w and Ψ_s were higher for *J. flaccida* and *Q. canbyi* than for *P. pseudostrobus*. Leaf Ψ_w and Ψ_s were mainly correlated with soil water content, while Ψ_s of *P. pseudostrobus* were hardly correlated with environmental variables. Thus, species have different strategies to withstand drought. *P. pseudostrobus* avoids water stress by means of a deeper rooting system, while *J. flaccida* and *Q. canbyi* possess further plant physiological capacities. All species are considered as suitable candidates for reforestation programs in Mexican mixed pine-oak forests. Nevertheless, their suitability depends on the environmental

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[1] Sent for publication to the German international journal “Trees- Structure and Function” of the Springer-Verlag (http://www.springerlink.com/content/100387/).

conditions, especially with respect to soil characteristics in the area of improvement.

Keywords: Juniperus flaccida, Pinus pseudostrobus, *Quercus canbyi*, restoration, water potential, osmotic potential

4.2 INTRODUCTION

Mixed pine-oak forests are widely distributed in the higher altitudes of the Eastern Sierra Madre, including ecologically and economically important tree species such as *Pinus pseudostrobus* (Rzedowski 1986). These forests are exposed to extreme climatic conditions of high temperatures and low precipitation distributed irregularly through the year. Under field conditions, water stress or rather soil water availability is in general one of the major limiting factors in plant growth and affects most physiological processes in these ecosystems (Kozlowski et al. 1991, Kramer 1983, Kramer and Boyer 1995, Mitlöhner 1998, Serrano et al. 1999, González et al. 2003, Reynolds et al. 2004, Otieno et al. 2005). Moreover, water scarcity leads to salt excess in the vegetation's rhizospheres, another serious and significant environmental problem plants have to face in arid and semiarid environments (Szabolcs 1994, Mitloehner and Koepp 2007). So far, water scarcity as well as climate and soil induced salinity can be considered natural processes and the native vegetation is normally adapted to these environmental conditions (Mitloehner and Koepp 2007).

Even so, climatic change makes conditions for plant grow even more acute. The response of forests to these changes associated with the greenhouse effect is uncertain and has the potential to greatly alter the distribution and productivity of tree species (Pastor and Post 1988, Kramer 1983, Tschaplinski et al. 1998, Rötzer et al. 2005, Ricker et al. 2007). Inappropriate land-use contributes further to the acceleration of forest degradation and deforestation in great parts of the mountain chain Sierra Madre

Oriental due to anthropogenic pressures (Domínguez and Návar 2000, Cantú and González 2002, González et al. 2005). Especially inadequate irrigation and the clearing of deep-rooted native vegetation cause secondary salinization in dryland areas worsening and/or impeding natural regeneration and reforestation (Szabolcs 1994, Munns 2002, Mitloehner and Koepp 2007). In Chiapas, southern Mexico, the thinning of the canopy in tropical highland forests by human activities increased solar radiation and exposed seedlings and juveniles to more extreme temperatures at the forest floor level, thus decreasing soil moisture availability (Ramírez-Marcial et al. 1996, Ramírez-Marcial et al. 2001). The response of forest ecosystems to such climatic and anthropogenic stresses will depend in great part on the drought (Tschaplinski et al. 1998) and salt excess tolerance capabilities of the individual species (Mitloehner and Koepp 2007).

Hence, understanding water uptake patterns, the physiological response of species to water stress, salt excess and their tolerance scope under severe environmental stress is required to explain differences among species in survival and distribution. It also allows determining the relative suitability of species for ecological restoration of degraded lands (Jurado et al. 1998, Ramírez-Marcial et al. 2001, González et al. 2003, Gebrekirstos et al. 2006). Elsewhere, previous works on plant water relations and forest restoration (Gebre et al. 1998, Jurado et al. 1998, Vertovec et al. 2001, González et al. 2003, Gebrekirstos et al. 2006, Mitloehner and Koepp 2007) emphasized the need to investigate water relations of native species and their physiological responses to changes in resource availability.

Plant internal water potential is a consequence of the soil-plant-atmospheric continuum and consists of the tension caused by the water pressure deficit of the atmosphere, regulative processes of leaves, general flow regulation resistances and the soil water potential (Philip 1966, Duhme and Richter 1977, Kozlowski et al. 1991, Kramer and Boyer 1995, Donovan et al. 2003). It is a dependable measure of plant and soil water status, and quickly measured directly in the field, a great advantage especially for plant comparison (Slatyer 1967, Sala et al. 1981, Pallardy et al. 1991, Kramer and Boyer 1995, Mitlöhner 1998, Vertovec et al. 2001, Donovan et al. 2003).

The osmotic potential (Ψ_s), in contrast, indicates the prime adaptation of plants towards spatially and periodically altered soil solute concentration (Kramer and Boyer 1995, Mitloehner and Koepp 2007). Dehydration tolerant species (according to the terminology of Kramer (1980)) reduce Ψ_s and/or accumulate solutes (osmotic adjustment; Morgan 1980) by incorporating ionic osmotica (i.e. NaCl among others salts from the soil) as well as by processing non-ionic osmotica (i.e. other plant-born organic compounds such as sucrose) into and within their cells (Mitloehner and Koepp 2007). In this way, a plants' capacity to take up water from dry soils is enhanced and recovery after drought is facilitated (Gebre et al. 1998, Tschaplinski et al. 1998, Nguyen-Queyrens et al. 2002). Osmotic and pressure-driven water flow into plant roots occur simultaneously (Kramer and Boyer 1995). Furthermore, an osmotic (or concentration) gradient in the soil-plant continuum (Pressarakli 1991) and within the plant between its cells are a basic condition for plant life (Mitloehner and Koepp 2007).

Osmotic adjustment can be estimated by the difference between Ψ_s of well-hydrated and dehydrated plants at similar relative water content (Gebre et al. 1998). It is difficult to determine OA under ambient field conditions due to the need to sample well-hydrated and dehydrated plants during the same period. Therefore, studies of osmotic adjustment have been based rather on comparison of seasonal variation in Ψ_s due to difference in precipitation within a season or between seasons (Bahari et al. 1985, Abrams 1990, Gebre et al. 1998).

In general, a species' capacity of adaptation to environmental site conditions is regarded as the basic requirement for a plant's "suitability as an indicator" (Mitlöhner 1997). During the last two decades, the Mexican Government has made an effort to restore degraded forest areas, mainly using pines and introduced species instead of native ones. However, the conservation-oriented interest in native broad-leaved species is increasing lately, and information on their biology and silvics is urgently needed (Quintana-Ascencio et al. 2004).

Hence, the specific objectives of our study were to a) compare three dominant tree species of mixed pine-oak forests in the Sierra Madre Oriental with respect to their adaptation to water deficit and their salt tolerance scope; b) compare the effect of two different sites on the water status of the species and c) identify their strategies to withstand periods of drought.

4.3 MATERIAL AND METHODS

4.3.1 Research site

This research was carried out at the Experimental Forest Research Station of the Faculty of Forest Science, Autonomous University of Nuevo Leon (UANL) in the Sierra Madre Oriental (24º42’N; 99º51’W), located 15 km southeast of Iturbide in the state of Nuevo Leon, Mexico. The Experimental Forest Research Station extends over an area of about 1035 ha and due to its protection status received 20 years ago. Two sites in different pine-oak forests were selected for the study: a) a major closed stand on a north-facing aspect (Plot-N) and b) an open fragmented stand on a south-facing aspect (Plot-S). Both sites are located very close from each other on the same mountain ridge at an elevation of about 1500 m. The mean annual air temperature is 13.9ºC and the average annual precipitation is 639 mm, which is concentrated from May to October. The dominant soils are rocky and comprise upper cretaceous lutite or siltstone (Cantú and González 2002). Some physical and chemical properties of the soil at profile depths of 0-10 and 20-30 cm are shown in Table 4.1.

Remarkable is the higher electric conductivity (µScm^{-1}) at soil depth 0-10 cm compared to layer 20-30 cm and the slightly higher values at the northern in comparison to the southern aspect.

Table 4.1. Some physical and chemical soil properties at the two study sites (north- and south aspect). (EC=electric conductivity; OM=organic material).

Sampling site	Soil profile depths (cm)	Bulk density ($Mg\ m^{-3}$)	Sand[a]	Silt[b]	Clay[c]	pH	EC (μScm^{-1})	OM (%)
			%					
Plot-N	0-10	0.69	25.9	47.6	26.6	7.6	159.2	11.4
Plot-N	20-30	ND*	12.6	39.3	48.1	7.7	75.9	2.7
Plot-S	0-10	0.85	30.0	41.6	26.4	7.6	141.8	11.3
Plot-S	20-30	ND*	39.6	43.8	16.6	7.7	72.1	2.5

*ND= no data available; each value is the mean of five replications.

[a] Soil particle size: 0.063-2.0 mm.

[b] Soil particle size: 0.002-0.063 mm.

[c] Soil particle size: <0.002 mm.

4.3.2 Sampling procedures and water potential measurement

In each sampling plot, five individuals (replications) of each tree species were randomly selected within a 500 m^2 circle from the understory for repetitive measurements of plant water potentials. The plant species were: *Juniperus flaccida* (Schltdl., Cupressaceae), *Pinus pseudostrobus* (Lindl., Pinaceae), and *Quercus canbyi* (Trel. Fagaceae). Sampling trees varied in diameter at breast height between 2.05±0.33 cm and 3.07±0.55 cm, and in height between 2.70±0.32 m and 3.85±0.44 m, depending on the species. The leaf water potentials (Ψ_w, MPa) were determined twice a month from January until August 2006, and the osmotic potential (Ψ_s, MPa) was measured once a month during the same period. Plant water potentials were measured with a Scholander pressure chamber (Model 3005, Soil Moisture Equipment Corp., Santa Barbara, CA, USA) (Scholander et al. 1965) immediately after cutting the leaves or terminal twigs of each sample tree and monitored twice a day, at 6:00 hr (Ψ_{wpd}) and 12:00 hr (Ψ_{wmd}), which is a widely used method (Sellin 1996).

For determination of the osmotic potential (Ψ_s), plant samples were taken with a hole puncher from leaves of *Q. canbyi*. In the case of *J. flaccida* and *P. pseudostrobus,* parts of terminal twigs and pine needles were cut, respectively. Plant samples were collected at 6:00 hr (Ψ_{spd}) and 12:00 hr (Ψ_{smd}) and immediately saved in small tubes and shock frozen in liquid nitrogen to conserve them for later laboratorial analysis. Once in the laboratory, the osmotic potential was determined using a Wescor HR 33T (Wescor Inc., Logan, UT) automatic scanning dew point microvoltmeter with three sample chambers (Wescor C-52) (Wilson et al. 1979).

4.3.3 Environmental data

Simultaneously, microclimatic data of air temperature (ºC) and relative humidity (%) was registered in each plot on an hourly basis by using a thermo-hygrometer (HOBO® Pro Series Weatherproof Data Loggers) that was located between the sampling trees directly in the sampling site. Likewise, daily precipitation (mm) was obtained from self-emptying rain gauges (Onset® Data Logging Rain Gauge) installed in each plot. Vapour pressure deficit (VPD) was calculated on the basis of air temperature and relative air humidity (RH). On each sampling date, soil cores at different depths of 0-10, 20-30, 40-50, and 60-70 cm (five replications each) were collected using a soil sampling tube (Soil Moisture Equipment Corp.) for determining the gravimetric soil water content beneath the tree canopy in each plot. The soil water-content was finally determined and expressed in percent (%).

4.3.4 Statistical analysis

The desire was to test the null hypothesis of no effect of tree species, site and sampling date on the water potentials (Ψ_w and Ψ_s each at predawn and midday) of common species in pine-oak forests in the Sierra Madre Oriental.

Differences between the two aspects (N and S), the tree species (or rather soil depths) and the sampling dates (January to August 2006) were examined with a factorial analyses of variance (ANOVA) using the linear model procedure (aov) on R (R version 2.8.1, The R Foundation for Statistical Computing 2008) with the block variable “aspect”, two treatment variables (“sampling date” and “species”) and the interaction between the treatment variables up to two-way terms (Crawley 2007). The ANOVA was conducted for predawn and midday data separately in the case of water and osmotic potentials. Data were log transformed before ANOVA, since assumption of normality and homogeneity of variance was not true.

When significant variations were revealed, results were validated using the Tukey's honestly significant difference (HSD) test ($p<0.05$), comparing the means of the two aspects (for each variable separately) and the means of species (or rather soil depths) for each aspect separately (Zar 1999, Crawley 2007).

The significance of seasonal differences (ΔSD) in Ψ_{spd} and Ψ_{smd} was tested with the Wilcoxon's rank sum test (Crawley 2007), with each having a representative dry (Apr-5) and moist (Aug-16/17) sampling date.

Correlation coefficients between water- and osmotic potentials ($\Psi_{wpd,}$ $\Psi_{wmd,}$ Ψ_{spd} and Ψ_{smd}) and environmental variables (monthly mean soil water content, monthly mean air temperature, monthly mean RH, monthly mean VPD, and monthly sum of precipitation) were quantified by the Spearman's rank order correlation analyses, since the null hypothesis of normality was rejected at *p*-value<0.05. For these analyses, real data of each species summarizing both plots was considered on a seasonal base (Zar 1999, Crawley 2007).

Moreover, the interrelationship between water potentials (dependent variables) and environmental variables (independent variables) was analyzed for each species (summarized data of both plots) using the procedure of stepwise regression including up to three-way interaction terms (Crawley 2007). For each dependent variable (real data of $\Psi_{wpd,}$ $\Psi_{wmd,}$ Ψ_{spd} and Ψ_{smd}) a stepwise regression was conducted considering the same independent variables (mean soil water content per sampling date, monthly mean air temperature, monthly mean RH, monthly mean VPD, and monthly sum of precipitation).

Before ANOVA and the regression analysis, data was checked for normality and homogeneity of variance using the Shapiro-Wilk test and Fligner-Killeen test, respectively (Zar 1999, Crawley 2007). All statistical methods were applied according to The R project for statistical computing (free statistic software, R version 2.8.1, The R Foundation for Statistical Computing 2008).

4.4 RESULTS

4.4.1 Environmental conditions during the experimental period:

Trends of monthly mean air temperatures, relative air humidity, vapour pressure deficit, total precipitation and soil water content are illustrated in Fig.4.1. During the experimental period, mean air temperatures ranged from 12.6ºC (February) to 20.7ºC (June) in Plot-N and from 14.6°C (February) to 21.2°C in Plot-S (Fig.4.1$_b$). Fig.4.1$_c$ further illustrates the monthly means of relative humidity ranging from 53% (February) to 77% (August) in Plot-N and from 49% (February) to 76% (August) in Plot-S. Concerning the vapour pressure deficit, monthly mean values ranged from 0.7 kPa (August) to 1.3 kPa (April) in Plot-N and from 0.7 kPa (August) to 1.5 kPa (April) in Plot-S (Fig.4.1).

The total rainfall was 206 mm in the North and 272 mm in the South (Fig.4.1). Concerning the total rainfall at the northern aspect, due to technical problems the monthly rainfall in July had to be estimated by interpolation using registered precipitation of June and August. The comparison of the two sites revealed no statistical differences in climatic variables (one-way ANOVA: *p*-value>0.18).

Soil moisture content was significantly higher at the northern aspect than at the southern (Table 4.2; *F*-value: 419.6***). Comparing the results of different sampling dates and soil depths, soil moisture content was significantly different (*F*-value: 11.2*** and 10.0***, respectively). Furthermore, soil moisture content at different soil depths is influenced by the season interpreting the significant interaction between soil depth and sampling date (*F*-value: 1.6*). The ensuing Tukey HSD test specified soil moisture content significantly higher in the first soil depth (0-10 cm) than in depth 40-50 cm and 60-70 cm in Plot-S (Fig.4.1). In

Plot-N, depth 60-70 cm was significantly drier than the upper layers 0-10 cm and 20-30 cm (Fig.4.1).

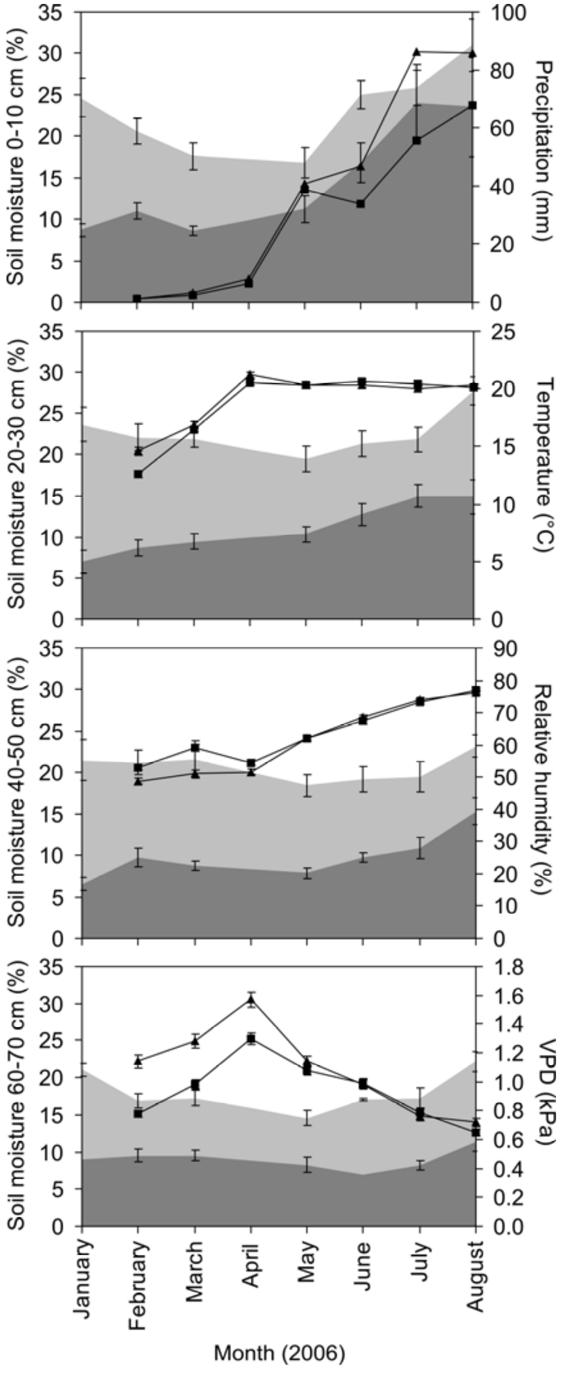

Fig.4.1 Seasonal variation in mean air temperature (°C), relative humidity (%), vapour pressure deficit (VPD; kPa), actual precipitation (mm) and mean soil moisture (%) at four soil profile depths (*n*=5) during the study period from February until August 2006; bars represent standard errors

The average soil moisture per depth was decreasing along the growing season at both sites, but increasing again after the onset of the rainy season in the end of May, especially in the upper soil horizons.

Table 4.2. *F*-statistics of analyses of variance for factors effects on leaf water- (Ψ_{wpd} and Ψ_{wmd}), leaf osmotic potentials (Ψ_{spd} and Ψ_{smd}) and on soil water content during the study period from January until August 2006. *n*=2 (aspect), *n*=3 (species; with five replicates each), *n*=12 (sampling date: SD) and *n*=4 (soil depth, with five replicates each).

Factors	Dependent variables									
	Ψ_{wpd} (MPa)		Ψ_{wmd} (MPa)		Ψ_{spd} (MPa)		Ψ_{smd} (MPa)		Soilmoisture (%)	
Aspect	128.11	<2.2e-16	195.28	<2.2e-16	12.27	0.00058	1.30	0.2548	419.6	<2.2e-16
Species*	188.20	<2.2e-16	149.92	<2.2e-16	10.92	3.3e-05	8.38	0.0003	10.0	2.6e-06
SD	45.75	<2.2e-16	32.89	<2.2e-16	14.06	3.4e-13	9.13	9.2e-09	11.2	2.3e-16
Species* x SD	7.56	<2.2e-16	6.98	<2.2e-16	0.14	0.0015	1.76	0.0569	1.6	0.0270

*In the case of soil moisture the factor "species" is replaced by "soil depth".

4.4.2 Variation in leaf water potentials

Leaf water potential had highly significant differences between the two aspects for both before dawn (Ψ_{wpd}) and midday (Ψ_{wmd}) (Table 4.2; F_{wpd}: 128.11***; F_{wmd}: 195.28***). In general, leaf water potentials were much lower in Plot-S (Fig.4.2). Furthermore, there was a highly significant effect of species (F_{wpd}: 188.20***; F_{wmd}: 149.92***) and sampling date (F_{wpd}: 45.75***; F_{wmd}: 32.89***) on both Ψ_{wpd} and Ψ_{wmd} (Fig.4.2, Table 4.2).

All species differed significantly (*p*-value<0.01) in Ψ_{wpd} in Plot-N (Fig.4.2$_{a}$) and Plot-S (Fig.4.2$_{b}$); with one exception in Plot-S, where Ψ_{wpd} of *P. pseudostrobus* and *J. flaccida* were not significantly different (*p*-value>0.05). Concerning the $\Psi_{wmd,}$ differences were significant between *Q. canbyi* and both conifers (*p*-value<0.001) (Fig.4.2$_{c}$), while there were no significant differences (*p*-value>0.05) between *J. flaccida* and *P. pseudostrobus* in both plots (Fig.4.2$_{c,d}$). *Q. canbyi* had in general the highest predawn and midday leaf water potentials at the northern aspect throughout the study period (Fig.4.2$_{a,c}$).

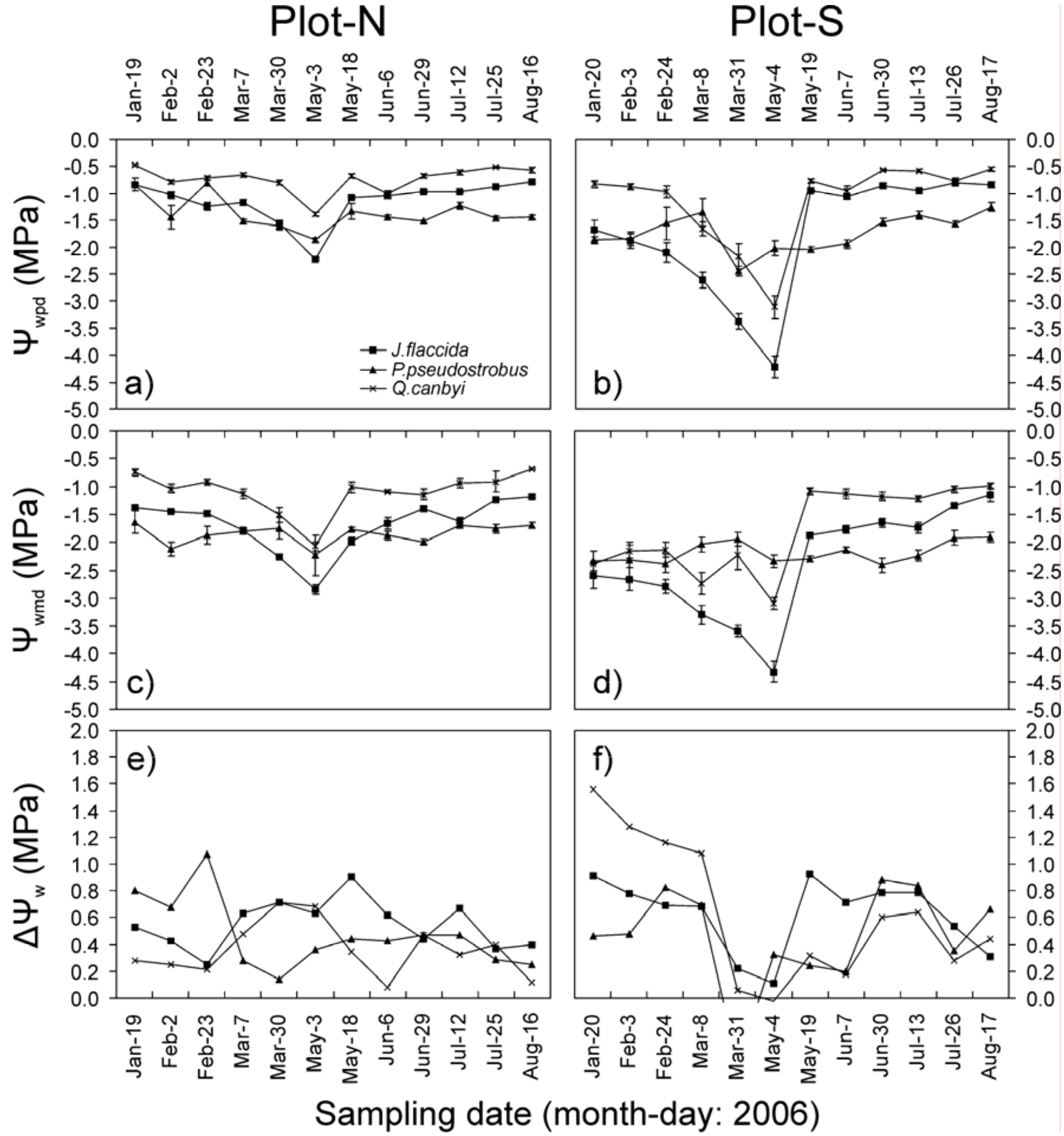

Fig.4.2 Seasonal variation in predawn (Ψ_{wpd}) and midday (Ψ_{wmd}) leaf water potentials as well as the difference between midday and predawn ($\Delta\Psi_w$) leaf water potential in three tree species at the north (Plot-N) and south aspect (Plot-S); values are means (*n*=5), bars represent standard error.

At the southern site in comparison *P. pseudostrobus* maintained highest values during the dry period from March to May, but was topped by *Q. canbyi* and *J. flaccida* after the onset of precipitation (Fig.4.2$_{b,d}$).

In general, Ψ_{wpd} varied between -0.47±0.02 MPa (*Q. canbyi*) and -2.22±0.03 MPa (*J. flaccida*) in Plot-N (Fig.4.2$_a$) compared to -0.55±0.03 MPa (*Q. canbyi*) and -4.32±0.19 MPa (*J. flaccida*) in Plot-S (Fig.4.2$_b$).

Hence, *J. flaccida* reached most negative values in both study sites. Furthermore, water stress appeared earlier in Plot-S than in Plot-N. For instance in Plot-S, Ψ_{wpd} began to decline drastically in early March (Ψ_{wpd}<-1.35±0.26 MPa) and in Plot-N towards the end of March (Ψ_{wpd}<-0.80±0.05 MPa). Midday leaf water potentials were more negative but with similar tendencies as observed in predawn potentials (Fig.4.2$_{c,d}$). Moreover, tree species suffered less water stress in Plot-N (Fig.4.2$_e$) than in Plot-S (Fig.4.2$_f$), where $\Delta\Psi_w$ were close to zero for all species on Mar-31 and May-4.

4.4.3 Variation in osmotic potentials

The osmotic potential had highly significant differences between the two aspects for Ψ_{spd}, but no statistically significant differences in Ψ_{smd} (Table 4.2; F_{spd}: 12.27***). As observed before in leaf water potentials, there was a highly significant effect of species (F_{spd}: 10.92***; F_{smd}: 8.38***) and sampling date (F_{spd}: 14.06***; F_{smd}: 9.13***) on both Ψ_{spd} and Ψ_{smd} (Fig.4.3), the interaction term "species x sampling date" was only statistically significant for predawn data (F_{spd}: 0.14**; Table 4.2).

Concerning the site comparison, osmotic potentials were much lower at the southern aspect (Fig.4.3$_{b,d}$) than at the northern (Fig.4.3$_{a,c}$). In contrast to leaf water potentials species hardly differed significantly in osmotic potentials at both aspects. There were just four exceptions: Ψ_{spd} was significantly different for *Q. canbyi* and *J. flaccida* (*p*-value=0.015) in Plot-N (Fig.4.3$_a$), at the southern aspect Ψ_{spd} was significantly different (*p*-value=0.009) for *Q. canbyi* and *P. pseudostrobus* (Fig.4.3$_b$).

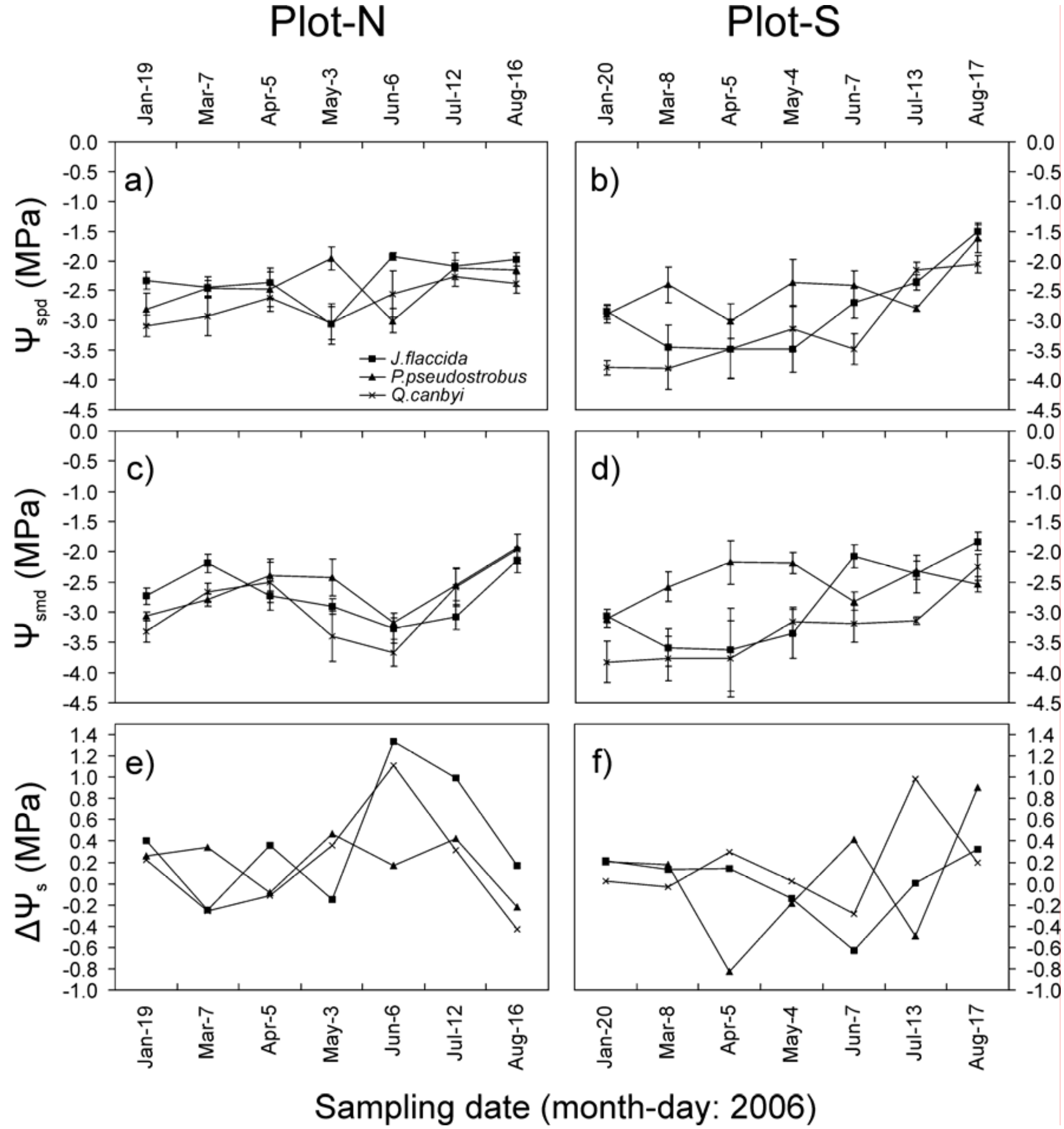

Fig.4.3 Seasonal variation in predawn (Ψ_{spd}) and midday (Ψ_{smd}) osmotic potential in three tree species at the north (Plot-N) and south aspect (Plot-S); values are means (n=5), bars represent standard error.

Ψ_{smd} in comparison differed significantly between *Q. canbyi* and both conifers, *P. pseudostrobus* (*p*-value=0.001) and *J. flaccida* (*p*-value=0.033) in Plot-N (Fig.4.3$_c$). In Plot-S (Fig.4.3$_d$), where differences between species were a little greater, *P. pseudostrobus* maintained generally higher osmotic potentials. Furthermore, osmotic potentials declined slightly in the course of the study and recover higher values at the end of the study period. In general, Ψ_{spd} varied between -1.92±0.07 MPa (*J. flaccida*) and -3.09±0.18 MPa (*Q. canbyi*) in Plot-N (Fig.4.3$_a$) compared to -1.51±0.14 MPa (*J. flaccida*) and -3.80±0.25 MPa (*Q. canbyi*) in Plot-S (Fig.4.3$_b$). Midday osmotic potentials were more negative but with similar tendencies as observed in predawn potentials (Fig.4.3$_{c,d}$).

Concerning the $\Delta\Psi_s$, it is difficult to see a clear tendency, especially for *P. pseudostrobus* (Fig.4.3$_{e,f}$) In the case of *J. flaccida* and *Q. canbyi*, $\Delta\Psi_s$ had wide ranges during the moist and small ranges during the dry period (Apr-5) in Plot-N (Fig.4.3$_e$). In Plot-S in comparison, *Q. canbyi* and *J. flaccida* had relatively wide $\Delta\Psi_s$ during the dry period (Fig.4.3$_f$). Here, the major decrease in $\Delta\Psi_s$ was observed later (Jun-7) recovering wider ranges again in August.

Seasonal differences (ΔSD) in osmotic potentials (Ψ_{spd} and Ψ_{smd}) between a dry (Apr-5) and moist (Aug-16/17) sampling date were significant for all species concerning Ψ_{spd} (*p*-value<0.01), while Ψ_{smd} were significantly different only for *J. flaccida* (ΔSD=1.19 MPa; *p*-value=0.007) and *Q. canbyi* (ΔSD=1.03 MPa; *p*-value=0.038).

4.4.4 Relationships between plant water potentials and environmental variables

Correlations between leaf water potentials (Ψ_{wpd} and Ψ_{wmd}), osmotic potentials (Ψ_{spd} and Ψ_{smd}) and environmental variables are shown in Table 4.3. In general, highly significant (*p*-value<0.05) and positive correlations were found between leaf water potentials, both Ψ_{wpd} and Ψ_{wmd}, and soil water content, whereas correlations were strongest within the first three soil depths. Concerning the climatic variables, Ψ_{wpd} and Ψ_{wmd} were positively correlated with monthly mean relative air humidity (RH) and negatively with monthly mean vapour pressure deficit (VPD). In the case of *J. flaccida* and *Q. canbyi* correlations were even highly significant (*p*-value<0.001) and water potentials of both species were furthermore positively correlated with precipitation (*p*-value<0.01) and monthly mean air temperature (*p*-value<0.05).

Table 4.3. Spearman's correlation coefficient values (*rho*) for predawn (PD) and midday (MD) leaf water- (observed data of both plots; *n*=110) and osmotic potential (observed data of both plots; *n*=70) in relation to mean soil water content at four depths (SWC), monthly mean air temperature, monthly mean relative humidity (RH), monthly mean vapour pressure deficit (VPD), and monthly precipitation in three tree species.

Environmental variable	Species					
	J. flaccida		*P. pseudostrobus*		*Q. canbyi*	
	PD	MD	PD	MD	PD	MD
	Leaf water potential (Ψ_{wpd} and Ψ_{wmd})					
SWC: 0-10 cm	0.79***	0.86***	0.53***	0.36***	0.71***	0.76***
SWC: 20-30 cm	0.48***	0.66***	0.44***	0.50***	0.53***	0.71***
SWC: 40-50 cm	0.40***	0.57***	0.38***	0.40***	0.45***	0.62***
SWC: 60-70 cm	0.21*	0.42***	0.35***	0.50***	0.25*	0.48***
Temperature	0.39***	0.27**	0.02	0.09	0.21*	0.25**
RH	0.76***	0.66***	0.24*	0.15	0.56***	0.54***
VPD	-0.72***	-0.81***	-0.51***	-0.26**	-0.66***	-0.68***
Precipitation	0.53***	0.32***	-0.04	-0.06	0.30**	0.25**
	Osmotic potential (Ψ_{spd} and Ψ_{smd})					
SWC: 0-10 cm	0.70***	0.43***	0.10	-0.01	0.66***	0.44***
SWC: 20-30 cm	0.54***	0.34*	0.09	-0.15	0.39**	0.45***
SWC: 40-50 cm	0.52***	0.33*	0.06	-0.12	0.34*	0.44***
SWC: 60-70 cm	0.50***	0.18	0.12	-0.12	0.31*	0.42**
Temperature	0.23*	-0.05	0.11	-0.27*	0.22	-0.12
RH	0.56***	0.43***	0.23	0.10	0.57***	0.37**
VPD	-0.66***	-0.56***	-0.23	-0.10	-0.61***	-0.55***
Precipitation	0.23	0.35**	0.20	0.21	0.35**	0.13

Not significant $p > 0.05$; $^*p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

Similar tendencies as in water potentials were observed in osmotic potentials. Although osmotic potentials of *P. pseudostrobus* were neither correlated with environmental variables nor with water potentials, with one exception: negative correlation was found between Ψ_{smd} and monthly mean air temperature. *J. flaccida* and *Q. canbyi* in comparison showed significantly strong correlations with soil water content and climatic variables such as monthly mean vapour pressure deficit (*p*-value<0.001), monthly mean relative air humidity (*p*-value<0.01) and precipitation (partly; *p*-value<0.01).

In general, *J. flaccida* (*rho*≤0.86) reflected best environmental conditions followed by *Q. canbyi* (*rho*≤0.76) and *P. pseudostrobus* (*rho*≤0.53) while last mentioned species showed least correlations in both leaf water- and osmotic potentials (Table 4.3).

Stepwise regression analysis resulted in very complex models including several predictors and interaction terms (results are not shown here). To give an impression about the complexity of the relationship between water potentials and explanatory variables see Fig.4.4. Models could be adjusted in the case of *P. pseudostrobus* to 52% (*p*-value=8.41e-11) and 25% (*p*-value=5.15e-06), in the case of *Q. canbyi* 91% (*p*-value=2.2e-16) and 83% (*p*-value=2.2e-16) and *J. flaccida* to 95% (*p*-value=2.2e-16) and 93% (*p*-value=2.2e-16) for Ψ_{wpd} and Ψ_{wmd}, respectively.

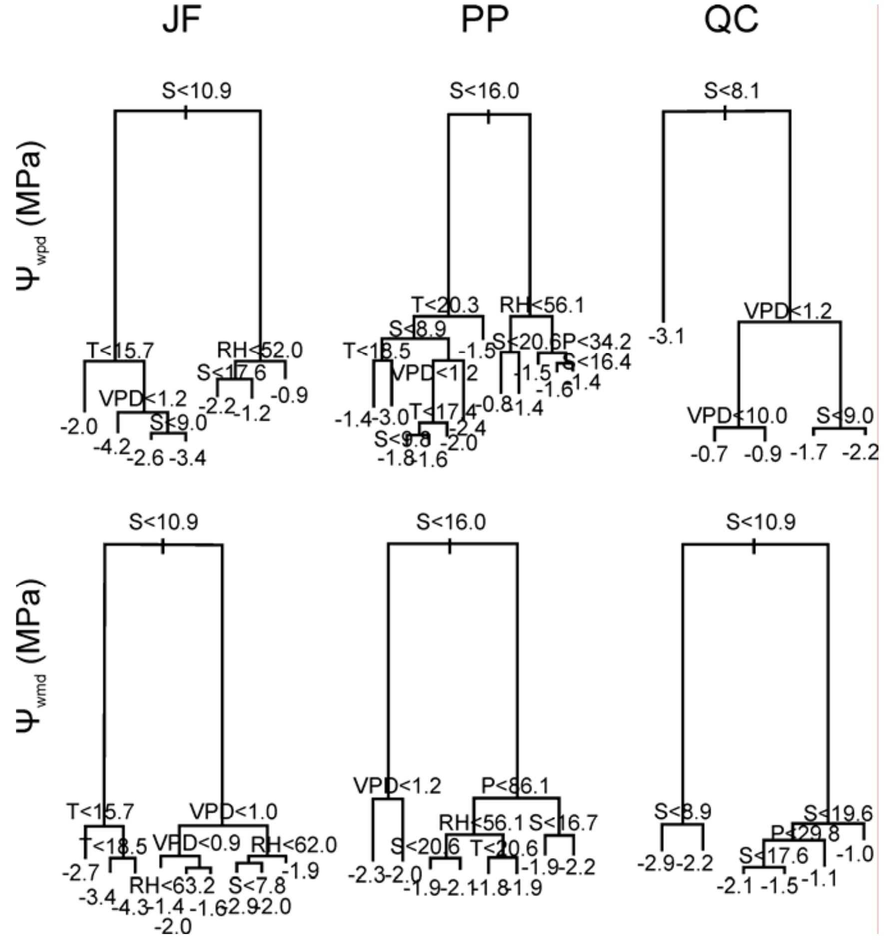

Fig.4.4 Plant water potentials (Ψ_{wpd} and Ψ_{wmd}) of three tree species (*J. flaccida*=JF, *P. pseudostrobus*=PP, *Q. canbyi*=QC) in relation to environmental variables on a seasonal base (observed data from the north (Plot-N) and south (Plot-S) aspect): monthly average soil moisture (S), air temperature (T), relative humidity (RH), vapour pressure deficit (VPD) and monthly sum of precipitation (P).

4.5 DISCUSSION

Diurnal water potential values studied in the course of eight months revealed significant differences between the two study sites and among species, owing to which species seem to employ different strategies to offset the harmful effect of drought periods. Also Rodríguez-Calcerrada et al. (2008) found a marked effect of site (comparing gap and understory) on pre-dawn leaf water potentials in *Q. petraea* and *Q. pyrenaica* seedlings. An understanding of these strategies is of importance for the selection of suited tree species for future reforestation of drought-affected areas (Gebrekirstos et al. 2006).

In the present study, Ψ_w showed a pattern of progressive decline during the dry season and recovered after the onset of precipitation. All species presented their highest values at predawn and their lowest during midday, the value that integrates and reflects all environmental influences on the plant's internal water balance, indicating the least favorable condition for the plant (Gebre et al. 1998). The water potential of a fully hydrated plant may be close to zero, while water potentials could reach as low as -8 MPa under extreme water stress conditions in the field (Mitlöhner 1990, González et al. 2004).

Minimum Ψ_{wmd} reached as low as -4.32±0.2 MPa for *J. flaccida* at Plot-S in May (Fig.4.2$_d$), while the highest values during this dry period were up to -2.07±0.1 MPa for *Q. canbyi* in Plot-N (Fig.4.2$_c$). Leaf water potentials of other hardwood species in upland oak forest in Tennessee ranged between -2.0 MPa (*Fagus glandifolia Ehrh.*) and -3.1 MPa (*Q. prinus L.*) in a dry treatment (Tschaplinski et al. 1998). This is comparable with results from Spain for *Q. petraea* (-1.8±0.1 MPa and -2.3±0.3 MPa) and *Q. pyrenaica* (-1.9±0.3 MPa and -2.3±0.2 MPa) in June and August 2003 (Rodríguez-Calcerrada et al. 2007, Rodríguez-Calcerrada et al. 2008), and our observations for *Q. canbyi*, -2.07±0.1 MPa and -3.09±0.1 MPa in Plot-N and Plot-S, respectively. Considerably more negative were the values of olive trees (-7.0 MPa; Dichio et al. 2005) and Tamaulipan thornscrub species with values even below -7.3 MPa (González et al. 2004).

A plant can make up its water deficit during the night, that is why many authors consider Ψ_{wpd} values as a true equilibrium of soil water potential (not measured in the present study) and plant water status, or rather use the plant water potential as an estimate for soil water potential (Mitlöhner 1997, González et al. 2004, Gebrekirstos et al. 2006). Minimum Ψ_{wpd} values among the studied species ranged during the dry period (May) between -1.39±0.03 MPa (*Q. canbyi*) in Plot-N (Fig.4.2$_a$) and -4.21±0.20 MPa (*J. flaccida*) in Plot-S (Fig.4.2$_b$). Concerning the wet period (August), minimum Ψ_{wpd} values varied between - 0.55±0.03 MPa (*Q. canbyi*) and -1.25±0.09 MPa (*P. pseudostrobus*), both in Plot-S (Fig.4.2$_b$). Consequently, Ψ_{wpd} reached values below the minimum of -2.5 MPa reported for trees growing in North Carolina (Roberts et al. 1980), but similar to the Ψ_{wpd} (-0.83 MPa to -3.02 MPa) reported for tree and shrub species in Ethiopia by Gebrekirstos et al. (2006).

A wide range ($\Delta\Psi_w$) in plant values between predawn and midday indicates a high degree of plasticity or high physiological capacity to use solutes to lower the osmotic potential and in this way to regulate water loss and maintain high Ψ_w (Mitlöhner 1998, Gebrehiwot et al. 2005). This was the case for *J. flaccida*, which had in Plot-S the widest $\Delta\Psi_w$ varying between 0.11 MPa and 0.22 MPa followed by *Q. canbyi* (-0.02 to 0.06 MPa), and *P. pseudostrobus* (-0.49 to 0.32 MPa) during severe drought conditions on Mar-31 and May-4 (Fig.4.2$_b$). Thus, species could hardly recover over night, why $\Delta\Psi_w$ even presented slightly negative values. Conversely, narrow diurnal range values during the dry period, as in the case of *P. pseudostrobus* and *Q. canbyi*, have been recognized as indicators for unfavorable environmental conditions and water deficiency, indicating a lack of the species' capacity to re-saturate during the night (González et al. 2004, Gebrehiwot et al. 2005, Gebrekirstos et al. 2006). So far, *J. flaccida* was identified as more drought tolerant due to its wide $\Delta\Psi_w$ during the dry period.

Furthermore, the Tukey test indicates that *Q. canbyi* maintained in general significantly (*p*-value<0.001) higher Ψ_{wpd} and Ψ_{wmd} values than

P. pseudostrobus and *J. flaccida* in Plot-N indicating relatively easy access to water sources (Gebrekirstos et al. 2006). In Plot-S Ψ_{wpd} and Ψ_{wmd} values were significantly different (*p*-value<0.001) between *Q. canbyi* and *P. pseudostrobus.* Kolb and Stone (2000), who compared the Ψ_{w} of old-growth *P. ponderosa* and *Q. gambelii* in Arizonian pine-oak forests, also confirmed this tendency, as well as Chung (1996) comparing Ψ_{w} of *P. densiflora* and *Q. variabilis* in Korea. Like in our case (Ψ_{wmd} of -2.34±0.11 MPa and -3.09±0.10 MPa for *P. pseudostrobus* and *Q. canbyi*, respectively on May-4), also in Arizona, *P. ponderosa* had higher Ψ_{wmd} values than *Q. gambelii* in the dry period of July (Kolb and Stone 2000).

J. flaccida, like *Q. canbyi,* showed a greater decrease in Ψ_{wpd} and Ψ_{wmd} values than *P. pseudostrobus* (Fig.4.2). However, at the end of the dry season both species recovered even higher Ψ_{w} values than *P. pseudostrobus*. This is in contrast to results of other studies, where it was identified that species of the genus *Juniperus* maintained the lowest predawn values under well-watered conditions (Bahari et al. 1985). In our case *J. flaccida* showed great capacity to recover higher values during the moist season, explaining the existence or rather frequent appearance of *J. flaccida* on generally xeric and degraded sites.

On a seasonal basis, gradual decrease in Ψ_{wpd} or Ψ_{wmd} (Fig.4.2) showed a considerable response to soil-water availability pattern (Fig.4.1) as highly significant correlations of Ψ_{wpd} and Ψ_{wmd} with soil water-content for all soil depths proof (Table 4.3). When soil water-content reached values approximately below 15%, the studied species responded with lower Ψ_{wpd} and Ψ_{wmd} values (<-1.00 MPa). This trend was also confirmed by studies about plant water relations of shrub species, where even lower Ψ_{w} values (<-5.0 MPa) were reached, when soil water-content fell below 0.15 kg kg^{-1} (Montagu and Woo 1999, González et al. 2004). Also Zobel et al. (2001) found strong correlations between Ψ_{w} and soil water-content in Himalayan forest types during the rainy season.

The highly seasonal fluctuation in Ψ_{w} of *Q. canbyi* and *J. flaccida* in comparison to *P. pseudostrobus* indicates different rooting depths. *P. pseudostrobus* seems to be rooted in deeper soil layers than *Q. canbyi* and

J. flaccida. Therefore, water potentials did not fluctuate drastically during the study period, since *P. pseudostrobus* could still draw advantage of soil water stored in deeper horizons during the dry season. The remarkable seasonal fluctuation in water potentials and highly significant correlations with soil water content that was measured only in the upper soil layers (0-70 cm) indicate a relatively superficial rooting system of the species *J. flaccida* and *Q. canbyi* in comparison. These species took advantage of the surface water during the rainy season and are at a disadvantage during the dry season, when the upper soil layers were dried out. Comparing *J. flaccida* and *Q. canbyi*, the deeper rooting system of *Quercus* species in comparison to *Juniperus* (Jackson et al. 1999) may be one reason why *Q. canbyi* maintained higher Ψ_{wpd} values during the dry season than *J. flaccida*.

Furthermore, Ψ_{wpd} and Ψ_{wmd} of *J. flaccida* and *Q. canbyi* had highly significant correlations with climatic variables (Table 4.3). This is in accordance with other studies where plant Ψ_{w} were highly correlated with climatic variables (Kolb and Stone 1999, González et al. 2004). In the case of *P. pseudostrobus*, Ψ_{wpd} were significantly correlated only with VPD and RH, and Ψ_{wmd} just with VPD.

Stepwise regression including interaction terms resulted in very complex models difficult to interpret. Nevertheless, our models reflect the complex relationships between environmental factors and water potentials. Hence, Ψ_{wpd} and Ψ_{wmd} seem to depend on various combinations of several environmental variables or indicate great site diversity in even small areas (Fig.4). In the case of *J. flaccida* the final model explained very well with 95% accuracy (*p*-value=2.2e-16) and 93% (*p*-value=2.2e-16) the species' Ψ_{wpd} and $\Psi_{wmd,}$ respectively, while the results of *P. pseudostrobus* could be adjusted up to only 52% (*p*-value=8.41e-11) and 25% (*p*-value=5.15e-06). The final model of *Q. canbyi* explained about 91% (*p*-value=2.2e-16) and 83% (*p*-value=2.2e-16) for both, Ψ_{wpd} and Ψ_{wmd}, respectively. This confirms the theory that species use different strategies to withstand seasonal fluctuations of environmental conditions. *P. pseudostrobus* maintained rather equal potentials due to its deeper rooting system taking advantage of soil water in deeper soil layers where water availability was rather equal over the study period (Fig.4.1_d). Soil moisture content in layers deeper than 70 cm was not included in our study, possibly one explanation for the less fitted model in the case of *P. pseudostrobus*. In comparison, water potentials of *J. flaccida* and *Q. canbyi* reflect soil moisture availability in the topsoil, where fluctuations were much higher.

Osmotic potentials (Ψ_{spd} and Ψ_{smd}) decreased for all species parallel to the loss of soil moisture content in the course of the study until the onset of the summer precipitations. In general, seasonal declines in Ψ_s of mature leaves reported in several studies including various species have been interpreted as a response to either drought, shoot or leaf age (see Gebre et al. 1998 for references). Since Ψ_s were always measured on mature leaves or shoots of trees, the decline in Ψ_s is interpreted as water stress. Low osmotic potentials during the drought period indicate higher drought tolerance (Morgan 1984, Aranda et al. 1996, Gebre et al. 1998, Aranda and Pardos 2004). This was the case for the species *Q. canbyi* and *J. flaccida* (Ψ_{spd}=-3.48 MPa, Apr-5) (Fig.4.3_b) that had lower baselines than *P. pseudostrobus* at the southern aspect. Species with high baseline (*P. pseudostrobus*) may exhibit osmotic adjustment only under mild water stress being at a disadvantage under severe drought conditions in mixed forest ecosystems, where species with low baseline Ψ_s predominate (Gebre et al. 1998). This could be an explanation for the similar course of the species' Ψ_{spd} and Ψ_{smd} curves in Plot-N compared to the xeric Plot-S where *P. pseudostrobus* had definitively higher values (Fig.4.3). Also in the present study, the high Ψ_s baseline of *P. pseudostrobus* in Plot-S supports the strategy of a deeper rooting system the species uses to withstand drought periods. Furthermore, the Ψ_s of *P. pseudostrobus* was almost not correlated to soil moisture content (Table 4.3) and even less to the upper soil layers where electric conductivity was highest (Table 4.1). This in contrast explains the highly significant correlations of Ψ_s to the topsoil layer in the case of *J. flaccida* and

Q. canbyi, which in comparison to *P. pseudostrobus*, have superficial rooting systems, explaining why their Ψ_s reflect better topsoil qualities.

According to data from literature, American and European oak species in general have shown to be quite drought tolerant, due to their capacity of osmotic adjustment in response to water stress (Bahari et al. 1985, Abrams 1990, Epron and Dreyer 1993, Bréda et al. 1993, Aranda et al. 1996, Corcuera et al. 2002). So far and due to the highly significant difference in Ψ_s comparing a moist and dry sampling date, the higher capacity for osmotic adjustment could be one explanation for the remarkable seasonal fluctuation in Ψ_s of *Q. canbyi*. Comparing the osmotic adjustment of oak and other hardwood species in eastern Tennessee, also Tschaplinski et al. (1998) identified oak trees (*Q. alba* and *Q. prinus*) as most drought tolerant. Species of the genus *Juniperus* possess quite distinctive responses to drought compared with those of oaks (Bahari et al. 1985). According to Bahari et al. (1985) *J. virginiana* did not exhibit detectable osmotic adjustment. In fact, the inherent ability of *Juniperus* to sustain stomata opening at low water potentials combined with low capacity for water loss appear to promote the abundance of *Juniperus* in xeric environments.

4.6 CONCLUSIONS

Measurements of Ψ_w and Ψ_s resulted to be good indicators for a species' and a site's water status and our results are useful for species-site matching in restoration or even reforestation programs in forest areas where water is a limiting factor. The species studied are adapted to water stress and high concentrations of solutes in the soil, but apply different strategies. Therefore, their recommendation for restoration depends on the environmental condition in the area of improvement. When flat soils dominate, *J. flaccida* and *Q. canbyi* are a better choice for restoration than *P. pseudostrobus*, which has a deeper rooting system. Concerning the production of seedlings in tree nurseries, we recommend cultivating plants of *P. pseudostrobus* in containers where their roots can develop optimally for better survival success after transplantation. Our results also permit to optimize planting systems with respect to species' rooting characteristics. Finally, the different strategies of *J. flaccida*, *P. pseudostrobus* and *Q. canbyi* to deal with seasonal and environmental changes explain their successful co-occurrence in great parts of the Sierra Madre Oriental.

Acknowledgement

The study was funded in part by the Comisión Nacional Forestal and Consejo Nacional de Ciencia y Tecnología (CONAFOR-CONACYT, No. 2002-C01-5951) and the Universidad Autónoma de Nuevo León (UANL-PAICYT, No. CN 1133-05). We further would like to thank the Secretaría de Relaciónes Exteriores (SER) for the support by scholarship. We gratefully acknowledge the anonymous reviewers for their enriching comments and Marco Vinicio Gómez Meza, who gave us useful initial suggestions for the statistical analysis. Valuable technical assistance provided by Juan José González Álvarez and Claudia Platas Villanueva is gratefully recognized.

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CHAPTER 5

IMPACT OF WATER AVAILABILITY ON CHLOROPHYLL AND CAROTENOID CONCENTRATIONS IN LEAVES OF FIVE TREE SPECIES IN MEXICAN PINE-OAK FORESTS[1]

5.1 ABSTRACT

The allocation of limited photosynthate between growth and defence is a crucial component of tree survival during periods of resource stress, such as drought and high levels of light. Our objectives were to describe the seasonal dynamics of chlorophyll (a+b) and carotenoids content in comparison to seasonal fluctuations in predawn leaf water- (Ψ_{wpd}) and osmotic potentials (Ψ_{spd}) of six native species in mixed pine-oak forests, which undergo regular seasonal drought. All plant physiological variables, with exception of carotenoids content, were significantly different between two sites of different light environment and soil water availability and higher under shaded than light-intense conditions. Furthermore, all pigments were different between sampling dates and species, while *Q. canbyi* had the highest leaf pigment concentrations at both sites. This was surprising since *Leguminosae* like *A. rigidula* normally tend to reach higher chlorophyll concentrations than non-*Leguminosae*. Conifer species, such as *J. flaccida* and *P. pseudostrobus,* were at a disadvantage during periods of drought in comparison to broad-leaved species, as they showed lower leaf pigment concentrations and higher seasonal variation correlated with environmental site conditions. Thus, leaf chlorophyll (a+b) and carotenoids content has been related to water deficit and levels of irradiance, although the dimension of the response was different among species.

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[1] Sent for publication to the Indian international journal “International Journal of Agriculture, Environment and Biotechnology” (http://www.ndpublisher.com/).

Keywords: indicator, photosynthesis, Sierra Madre Oriental, water stress

5.2 INTRODUCTION

Leaf chlorophylls as dominant photosynthetic pigments in green plants provide an estimate of potential photosynthetic capacity of plant species (Carter 1998, Moran et al. 2000). They are together with carotenoids in effect essential pigments converting light energy into stored chemical energy (Sims and Gamon 2002, Gitelson et al. 2003). Moreover, chlorophyll is a direct indicator for the photosynthetic potential, the primary production of a plant and environmental quality, since the amount of solar radiation absorbed by a leaf is a function of the photosynthetic pigment content (Curran et al. 1990, Filella et al. 1995, Carter and Spiering 2002).

Plant growth depends generally on nutrients supplied by photosynthesis and transported by water between important plant organs (Kozlowski et al. 1991), why chlorophyll concentrations could be used as indicator for plant growth. Due to the high incorporation of leaf nitrogen in chlorophyll, also the nutrient status can be estimated indirectly by the chlorophyll content (Filella et al. 1995, Moran et al. 2000).

Water stress causes stomata closure, reduces cell enlargement and influences other plant physiological processes, which inhibits photosynthesis and affects finally plant growth (Kozlowski et al. 1991). Nonetheless, the effect of water stress on photosynthesis depends on the species. Drought tolerant species for instance carry on photosynthesis to a lower water potential than those native to humid areas (Kozlowski et al. 1991, Uvalle-Sauceda et al. 2008). Many drought tolerant plant species are able to keep functioning biochemical and physiological processes such as turgor maintenance, stomatal opening, leaf expansion, photosynthesis, root growth and water absorption at even lower plant water potentials than would otherwise be possible, due to their capacity of osmotic adjustment (Kozlowski et al. 1991).

There is a close relation between the ratios of leaf chlorophyll or rather its variation and plant physiological stress (Hendry et al. 1987, Merzlyak and Gitelson 1995, Merzlyak et al. 1999, Carter and Knapp 2001, Torres et al. 2002). Thus, various environmental stressors including water stress can result in decrease of leaf chlorophyll contents (Hendry et al. 1987, Carter and Knapp 2001).

Hence, the objective was to determine and compare chlorophyll and carotenoids concentrations in five native tree and shrub species of mixed pine-oak forests in Northeaster Mexico during periods of different water availability or rather different water stress conditions.

5.3 MATERIAL AND METHODS

5.3.1 Study area

This research was carried out at the Experimental Forest Research Station of the Faculty of Forest Science, Autonomous University of Nuevo Leon (UANL) in the Sierra Madre Oriental (24º42'N; 99º51'W), located 15 km southeast of Iturbide in the state of Nuevo Leon, Mexico. Two sites in different pine-oak forests were selected for the study: a) a major closed or rather shaded stand on a north-facing aspect (Plot-N) and b) an open fragmented stand on a south-facing aspect (Plot-S) with more incidence of light. Both sites are located very close from each other on the same mountain ridge at an elevation of about 1500 m. The mean annual air temperature is 13.9ºC and the average annual precipitation is 639 mm, which is concentrated from May to October. The dominant soils are rocky and comprise upper cretaceous lutite or siltstone (Cantú and González 2002).

5.3.2 Sampling procedures

The plant species considered for the determination of leaf pigments are: *Juniperus flaccida* (Schltdl., *Cupressaceae*), *Pinus pseudostrobus* (Lindl., *Pinaceae*), *Quercus canbyi* (Trel. *Fagaceae*) and *Rhus virens* (Lindh., *Anacardiaceae*) in both plots; *Arbutus xalapensis* (Kunth., *Ericaceae*) was further included in Plot-N and *Acacia rigidula* (Benth., *Leguminosae*) in Plot-S. Terminal shoots with fully expanded leaves from four different trees per species were randomly chosen from the regeneration in a 30 x 30 m plot located in each site. Collections were taken once a month from January until August 2006. Leaves were conserved in plastic bags and stored on ice under dark conditions for ensuing pigment analyses in the laboratory, which were performed within 12 h after collection.

5.3.3 Determination of chlorophylls and carotenoids

*Q*uadruplicate leaf tissue samples (1.0 g of fresh weight) of each tree species were used for analyses. The carotenoids and chlorophylls a and b were extracted in 80% (v v^{-1}) aqueous acetone, which is a mostly used solvent (Torres Netto et al. 2002), and vacuum filtered through a Whatman No. 1 filter paper. Pigment lectures were taken with a Perkin-Elmer Spectrophotometer (Model Lamda 1A) at wavelengths of 663, 646 and 470 nm for chlorophylls a and b and carotenoids, respectively. Pigment concentrations (mg g^{-1} f.wt.) were finally calculated using the equations of Lichtenthaler and Wellburn (1983).

5.3.4 Leaf water- and osmotic potential measurements

Six trees per species were selected from the natural regeneration in each plot for repeated leaf water- (Ψ_{wpd}, MPa) and osmotic potential (Ψ_{spd}, MPa) measurements. Measurements were taken at predawn (6:00 h) once a month, at the same day as pigments sampling in Plot-S or one day before in Plot-N, in the period of January until August 2006.

Plant water potentials were measured on mature leaves with a Scholander pressure chamber (Model 3005, Soil Moisture Equipment Corp., Santa Barbara, CA, USA) (Scholander et al. 1965), which is a widely used method (Sellin 1996).

For determination of the osmotic potential (Ψ_{spd}), plant tissue samples of fully developed leaves were taken in the field, saved immediately in small tubes and shock frozen in liquid nitrogen to conserve them for later laboratorial analysis. Once in the laboratory, the osmotic potential was determined using a Wescor HR 33T (Wescor Inc., Logan, UT) automatic scanning dew point microvoltmeter with three sample chambers (Wescor C-52) (Wilson et al. 1979).

R. virens was not included in leaf water- and osmotic potential measurements, since the used methodology was not optimal for the mentioned species.

5.3.5 Environmental variables

Simultaneously, microclimatic data of air temperature (ºC) and relative humidity (%) was registered in each plot on an hourly basis by using a thermo-hygrometer (HOBO® Pro Series Weatherproof Data Loggers) that was located between the sampling trees directly in the sampling site. Likewise, daily precipitation (mm) was obtained from self-emptying rain gauges (Onset® Data Logging Rain Gauge) installed in each plot. Vapour pressure deficit (VPD) was calculated on the basis of air temperature and relative air humidity (RH). On each sampling date, average soil moisture content (five replications at four depths: 0-10, 20-30, 40-50, 60-70 cm) was determined and expressed in percentage (%). Results are shown in Table 5.1.

Table 5.1. Average soil moisture content (ASMC, %) per sampling date, seasonal mean temperature (T; °C), relative humidity (RH; %), vapour pressure deficit (VPD; kPa) and monthly sum of precipitation (P; mm) at both study sites from January until August 2006.

Month	Plot-N					Plot-S				
	ASMC	T	RH	VPD	P	ASMC	T	RH	VPD	P
January	22.77	-	-	--	-	7.56	-	-	-	-
February	20.61	12.6	53	0.8	1	9.59	14.6	49	1.1	1
March	20.16	16.4	59	1.8	3	9.98	16.8	52	1.3	3
April	-	20.6	55	1.3	7	-	21.2	52	1.6	8
May	19.45	20.3	62	1.1	39	8.30	20.3	62	1.1	41
June	15.80	20.7	67	1.1	34	9.69	20.4	69	1.0	47
July	19.67	20.4	73	0.8	35	7.91	20.1	74	0.8	86
August	20.06	20.2	77	0.7	68	11.80	20.3	76	0.7	86

5.3.6 Statistical analysis

The desire was to test the null hypothesis of no effect of tree species, site and sampling date on the leaf pigment concentration (chlorophyll (a+b) and carotenoids) of common species in pine-oak forests in the Sierra Madre Oriental.

Differences between the two aspects (N and S), the species and the sampling dates (January to August 2006) were examined with a factorial analyses of variance (ANOVA) using the linear model procedure (aov) on R (R version 2.8.1, The R Foundation for Statistical Computing 2008) with the block variable “aspect”, two treatment variables (“sampling date” and “species”) and the interaction between the treatment variables up to two-way terms (Crawley 2007).

When significant variations were revealed, results were validated using the Tukey’s honestly significant difference (HSD) test ($p<0.05$), comparing the means of the two aspects (for each variable separately) and the means of species (or rather soil depths) for each aspect separately (Zar 1999, Crawley 2007).

For detailed information about differences between species per sampling date and vice versa one-way ANOVA was used (Zar 1999, Crawley 2007).

The relationship between pigment content and environmental variables (monthly mean air temperature, relative humidity, vapour pressure deficit, monthly sum of precipitation and average soil water content per sampling date) as well as the corresponding predawn water- and osmotic potential were analysed with the Spearman’s correlation coefficient (Zar 1999, Crawley 2007).

Linear regression analyses were used to express the relationship between leaf chlorophyll (a+b) and carotenoids content (Crawley 2007). All statistical methods were applied according to The R project for statistical computing (free statistic software, R version 2.8.1, The R Foundation for Statistical Computing 2008).

5.4 RESULTS

5.4.1 Climate and soils

Atmospheric conditions as well as soil moisture content, registered directly in the sampling plots, varied in the course of the study from January until August 2006. Fig.5.1 shows the seasonal course environmental conditions for Plot-N and Plot-S separately.

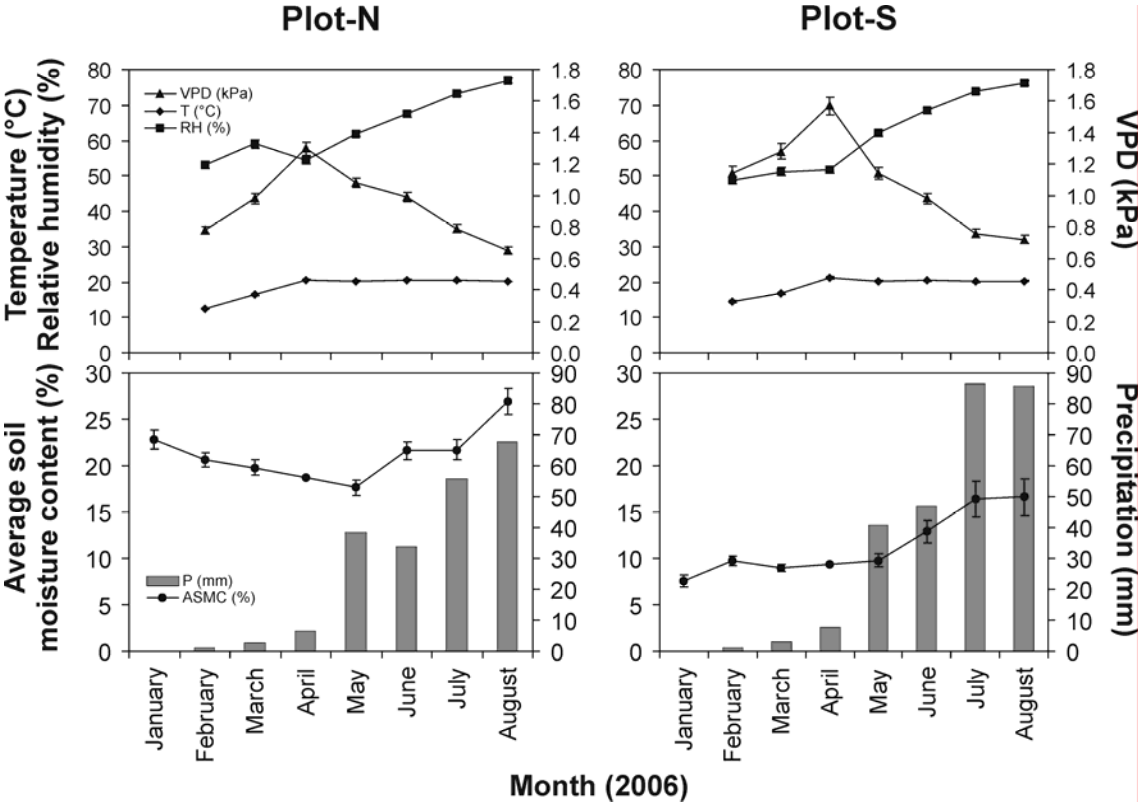

Fig.5.1. Seasonal variations in atmospheric site conditions (monthly mean vapour pressure deficit (VPD), air temperature (T), relative air humidity (RH) and precipitation (P)) and average soil moisture content (ASMC; 0-70 cm) at each sampling date. Error bars represent estimated standard errors, data are shown for Plot-N and Plot-S separately.

April was identified as the driest month, whereas the wettest conditions were found in August. Comparing the two sites, air temperature and vapour pressure deficit were not significantly (*p*-value>0.05) different, but relative humidity and soil moisture content were significantly (*p*-value<0.001) higher in Plot-N compared to Plot-S (Fig.5.1).

5.4.2 Water potentials

Furthermore, predawn leaf water- (Ψ_{wpd}) and osmotic potentials (Ψ_{spd}) differed significantly comparing the two plots, sampling dates and species. For both variables the interaction term “species x sampling date” was highly significant (*p*-value<0.0001) (Table 5.2).

Table 5.2. *F*-statistics of analyses of variance for factors effects on content of chlorophyll (a+b), carotenoids, predawn leaf water- (Ψ_{wpd}) and osmotic potential (Ψ_{spd}) during the study period from January until August 2006. *F*- and *p*-values are provided; “sd” stands for sampling date.

		Plant physiological variable								
Factors		Chlorophyll (a+b)		Carotenoids			Ψ_{wpd}		Ψ_{spd}	
	DF	*F*	*p*-value	*F*	*p*-value	DF	*F*	*p*-value	*F*	*p*-value
aspect	1	6.05	0.016	0.04	0.841	1	132.82	<0.0001	3.95	0.048
species	5	32.62	<0.0001	42.93	<0.0001	4	60.83	<0.0001	10.98	<0.0001
sd	6	6.04	<0.0001	8.96	<0.0001	5	105.48	<0.0001	22.62	<0.0001
species x sd	30	1.61	0.029	3.11	<0.0001	20	7.49	<0.0001	2.63	<0.0001

Seasonal fluctuations were much higher in water potentials (Ψ_{wpd} and Ψ_{spd}) than in leaf pigment concentrations (Fig.5.2). Especially Ψ_{wpd} showed clear response to average soil moisture availability and evaporative demand components (Fig.5.1), indicating least favourable site conditions for plant growth in April and May (Fig.5.2). Seasonal variation in leaf water potentials was highest for the species *Q. canbyi* and *J. flaccida*. The same species had also the lowest seasonal values (-3.5 MPa in April) with regard to the osmotic potential.

5.4.3 Total chlorophyll content

In general, chlorophyll (a+b) content was higher at the beginning of the study (Jan-20 to May-4) and decreased, when water stress increased in April and May (Fig.5.2).

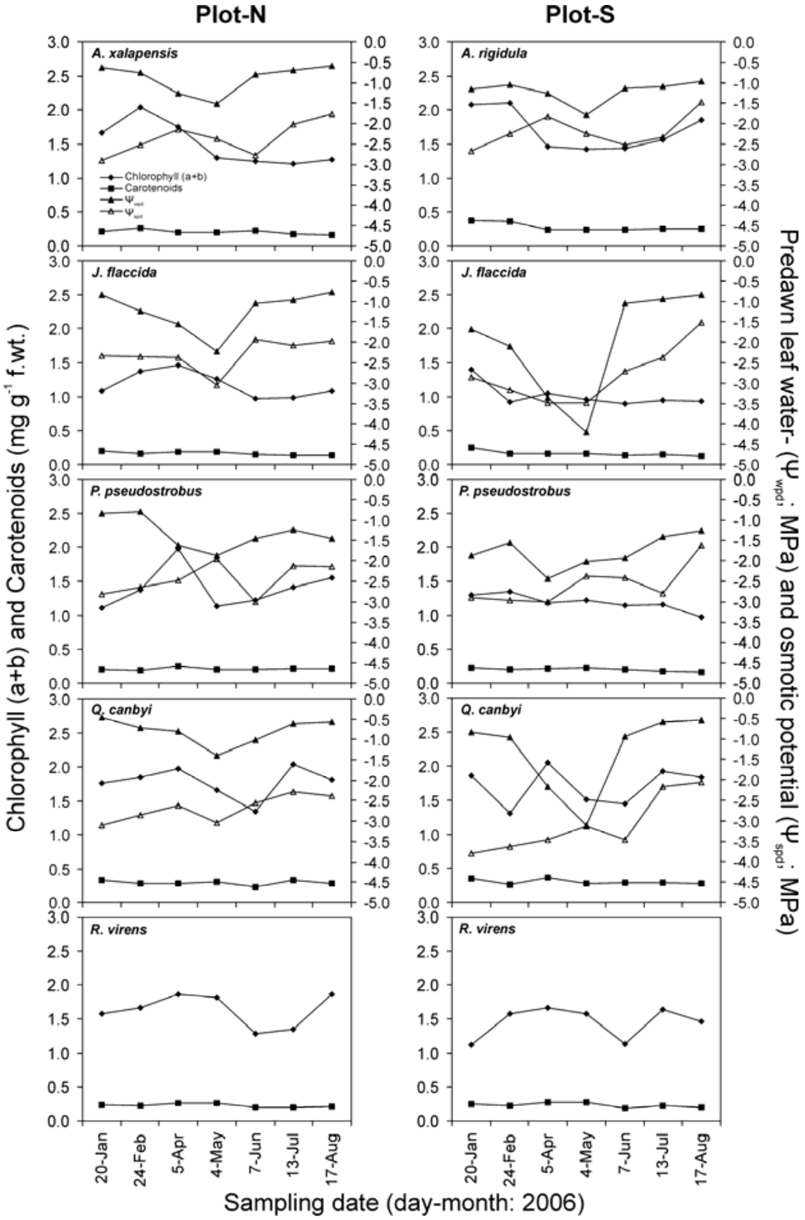

Fig.5.2. Time courses of leaf chlorophyll (a+b) and carotenoids concentration in comparison to seasonal predawn leaf water- (Ψ_{wpd}) and osmotic potential (Ψ_{spd}) at two different aspects (Plot-N and Plot-S) from January to August 2006.

At the northern aspect, values recovered again until Aug-17 for most species, apart from *Q. canbyi*, while no clear increase in chlorophyll content could be found at the southern aspect, except for *A. rigidula* (Fig. 2). The factorial ANOVA revealed that chlorophyll (a+b) concentration was significantly (*p*-value<0.05) different between sampling plots, months and plant species (Table 5.2).

Also the interaction term was found significant (*p*-value<0.05), which means that differences in chlorophyll (a+b) content between species depended on the sampling date. The ensuing Tukey HSD test revealed higher chlorophyll (a+b) concentrations in the shaded Plot-N than in the sunny Plot-S.

Analysing (Tukey HSD test) the differences between species at each aspect, chlorophyll (a+b) contents were significantly (*p*-value<0.05) higher for *Q. canbyi* or rather *R. virens* in comparison to the two conifers in Plot-N. Furthermore, *A. xalapensis* had significantly higher chlorophyll contents than *J. flaccida*. In Plot-S, the two conifers *J. flaccida* and *P. pseudostrobus* had significantly lower (*p*-value<0.05) chlorophyll (a+b) contents compared to any other species. Thus, the broad-leaved species had similar (*p*-value>0.05) chlorophyll (a+b) contents. Moreover, seasonal differences in chlorophyll (a+b) content were found only in Plot-N. Hence, total chlorophyll, including data from all species, was higher on Apr-5, the driest sampling date (see Fig. 1), than on Jun-7 and/or Jul-13. Also on Feb-24 higher chlorophyll (a+b) concentrations were found than on Jun-7. In Plot-S, no significant (*p*-value>0.05) differences between sampling dates were revealed.

Means per species and sampling date and the results of the one-way ANOVA comparing each, differences between species per sampling date as well as differences between sampling dates per species are shown in Table 5.3.

Mean chlorophyll (a+b) content varied between 1.17 mg g^{-1} (*J. flaccida*) and 1.78 mg g^{-1} (*Q. canbyi*) in Plot-N, and between 1.01 mg g^{-1} (*J. flaccida*) and 1.71 mg g^{-1} fresh weight (*Q. canbyi*) in Plot-S. Furthermore, differences between species were mainly found on Jan-20, Jul-13 and Aug-17 at the northern aspect, whereas at the southern aspect, chlorophyll contents were significantly (*p*-value<0.05) different comparing the species at each sampling date (Table 5.3). With regard to the seasonal variations in contents of chlorophyll (a+b) were significant (*p*-value<0.05) only for the species *J. flaccida* and *R. virens* in Plot-N and *A. rigidula* and *P. pseudostrobus* in Plot-S (Table 5.3).

Table 5.3. Seasonal contents (mg g^{-1} f.wt.) of chlorophyll (a+b) in six native tree and shrub species (*A. xalapensis*: AX, *A. rigidula*: AR, *J. flaccida*: JF, *P. pseudostrobus*: PP, *Q. canbyi*: QC and *R. virens*: RV). Means and *F*-statistics of one-way analysis of variance per sampling date (SD) and species are provided.

Plot	SD	Plant species					Mean	*F*-value	*p*-value
		AX/AR	JF	PP	QC	RV			
North	Jan-20	1.67	1.08	1.11	1.76	1.57	1.44	5.00	0.0092
	Feb-24	2.04	1.38	1.37	1.85	1.66	1.66	0.78	0.5538
	Apr-5	1.75	1.46	1.98	1.97	1.87	1.81	2.73	0.0691
	May-4	1.30	1.25	1.14	1.66	1.81	1.43	2.25	0.1118
	Jun-7	1.25	0.97	1.22	1.34	1.28	1.21	1.16	0.3844
	Jul-13	1.21	0.98	1.41	2.04	1.35	1.40	4.65	0.0121
	Aug-17	1.27	1.09	1.55	1.81	1.87	1.52	5.86	0.0048
	Mean	1.50	1.17	1.40	1.78	1.63	1.50		
	F-value	1.77	3.26	2.45	1.11	3.77			
	p-value	0.1612	0.0201	0.0588	0.3895	0.0122			
South	Jan-20	2.08	1.40	1.30	1.87	1.13	1.55	4.23	0.0188
	Feb-24	2.11	0.91	1.35	1.31	1.58	1.45	7.52	0.0016
	Apr-5	1.46	1.05	1.19	2.06	1.67	1.49	6.19	0.0038
	May-4	1.43	0.95	1.22	1.51	1.57	1.34	3.80	0.0251
	Jun-7	1.43	0.89	1.15	1.45	1.14	1.21	3.27	0.0407
	Jul-13	1.57	0.95	1.16	1.93	1.64	1.45	9.90	<0.0001
	Aug-17	1.85	0.93	0.97	1.84	1.47	1.41	16.54	<0.0001
	Mean	1.70	1.01	1.19	1.71	1.46	1.41		
	F-value	3.33	1.46	2.92	2.09	2.16			
	p-value	0.0184	0.2470	0.0314	0.1000	0.0893			

5.4.4 Carotenoids content

In the case of carotenoids concentrations, there was no significant (*p*-value>0.05) difference between the shaded Plot-N and sunny Plot-S (factorial ANOVA). Nevertheless, differences between sampling dates and species as well as the interaction term were highly significant (*p*-value<0.0001). Consequently, also the differences between species in carotenoids content depended considerably on the sampling date or rather season (Table 5.2).

Analysing (Tukey HSD test) the differences between species at each aspect, *Q. canbyi* had significantly higher (*p*-value<0.05) values than any other species in comparison under shaded conditions in Plot-N as well as *R. virens* compared to *J. flaccida*. At the sunny spot Plot-S, both conifers had significantly lower carotenoids contents than any other species, as well as *R. virens* compared to the other two broad-leaved species, *Q. canbyi* and *A. rigidula*.

The results for the seasonal variation in carotenoids content were much more convincing than the ones of total chlorophyll content. Thus, carotenoids content was significantly higher on May-4 than on the humid sampling dates Feb-24, Jun-7 and Jul-13 in Plot-N (see also Fig.5.2). Also in Plot-S, significantly higher carotenoids content was found for the dry and cold date Jan-20 compared to the more humid dates Jun-7 and Aug-17.

Table 5.4 shows the means per species and sampling date, and additionally the results of the one-way ANOVA comparing each, differences between species per sampling date as well as differences between sampling dates per species. Mean carotenoids content varied between 0.14 mg g^{-1} (*J. flaccida*) and 0.29 mg g^{-1} (*Q. canbyi*) in Plot-N, and between 0.17 mg g^{-1} (*J. flaccida*) and 0.30 mg g^{-1} fresh weight (*Q. canbyi*) in Plot-S (Table 5.4).

Table 5.4. Seasonal contents (mg g^{-1} f.wt.) of carotenoids in six native tree and shrub species (*A. xalapensis*: AX, *A. rigidula*: AR, *J. flaccida*: JF, *P. pseudostrobus*: PP, *Q. canbyi*: QC and *R. virens*: RV). Means and *F*-statistics of one-way analysis of variance per sampling date (SD) and species are provided.

Plot	SD	Plant species							
		AX/AR	JF	PP	QC	RV	Mean	*F*-value	*p*-value
North	Jan-20	0.21	0.20	0.20	0.32	0.24	0.23	5.90	0.0047
	Feb-24	0.27	0.16	0.18	0.28	0.22	0.22	1.41	0.2786
	Apr-5	0.21	0.19	0.26	0.28	0.26	0.24	7.17	0.0020
	May-4	0.20	0.19	0.20	0.30	0.27	0.23	105.73	<0.0001
	Jun-7	0.22	0.15	0.20	0.23	0.20	0.20	2.35	0.1251
	Jul-13	0.18	0.14	0.21	0.33	0.20	0.21	9.52	0.0005
	Aug-17	0.17	0.14	0.22	0.28	0.22	0.21	10.88	0.0002
	Mean	0.21	0.17	0.21	0.29	0.23	0.22		
	F-value	0.70	3.59	1.11	41.96	3.01			
	p-value	0.6565	0.0131	0.3876	<0.0001	0.0308			
South	Jan-20	0.38	0.25	0.23	0.35	0.25	0.29	3.34	0.0404
	Feb-24	0.37	0.16	0.20	0.26	0.23	0.24	20.14	<0.0001
	Apr-5	0.24	0.16	0.22	0.37	0.28	0.25	11.95	<0.0001
	May-4	0.24	0.17	0.22	0.28	0.28	0.24	6.46	0.0031
	Jun-7	0.24	0.14	0.21	0.28	0.19	0.21	16.38	<0.0001
	Jul-13	0.25	0.15	0.18	0.29	0.23	0.22	10.64	0.0003
	Aug-17	0.26	0.13	0.17	0.28	0.20	0.21	11.05	0.0002
	Mean	0.28	0.17	0.20	0.30	0.24	0.24		
	F-value	7.12	2.27	2.94	2.33	2.61			
	p-value	0.0003	0.0759	0.0303	0.0720	0.0473			

Comparing the species' carotenoids concentrations at each sampling date, differences were significant (p-value<0.05) at almost each sampling date and with this much higher than for chlorophyll (a+b) content. Only on Feb-24 and Jun-7 no significant (*p*-value>0.05) differences were found between species in Plot-N. Seasonal variations in carotenoids content were highest for the species *J. flaccida, Q. canbyi* and *R. virens* in Plot-N, and for *A. rigidula, P. pseudostrobus* and *R. virens* in Plot-S (Table 5.4).

5.4.5 Chlorophyll (a+b) versus carotenoids

Moreover, a significant positive linear relationship was found between chlorophyll (a+b) and carotenoids content (Fig.5.3), although this relationship was very different depending on the aspect. So, chlorophyll (a+b) content explained about 77% (*p*-value<0.001) of the total seasonal variation in carotenoids at the light intense Plot-S, whereas only 24% (*p*-value<0.001) under shaded conditions in Plot-N. Ensuing divided analyses per species resulted in following percentages: 81% for *A. rigidula*, 67% for *J. flaccida* (here, data had to be log-transformed), 66% for *Q. canbyi*, 54% for *R. virens* and 48% for *P. pseudostrobus* (*p*-values<0.001).

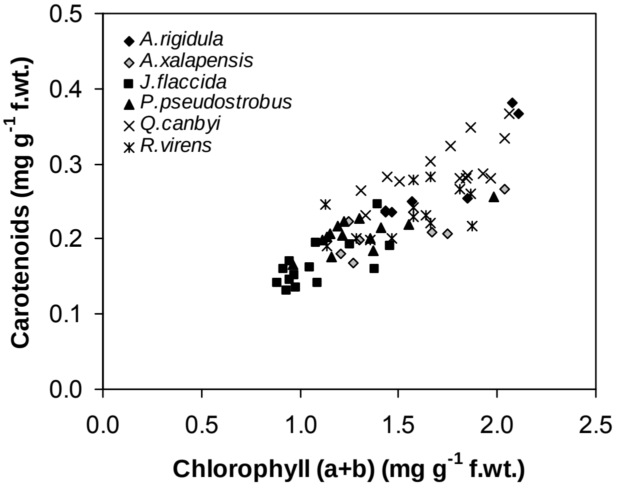

Fig.5.3. Relationship between leaf carotenoids and chlorophyll (a+b) for six studied species. Mean data per sampling date are plotted including results from both study sites (Plot-N and Plot-S).

5.4.6 Relationship of leaf pigments to environmental site conditions

Due to significant differences in leaf pigments and water potentials comparing the two different light environments, correlations per species were calculated for each plot separately. In general, total chlorophyll was mainly correlated under shaded and carotenoids under sunny conditions (Table 5.5).

Table 5.5. Relationship of carotenoids- and chlorophyll (a+b) content to environmental site conditions (air temperature: T, relative humidity: RH, vapour pressure deficit: VPD, precipitation: P, average soil moisture content: ASMC) as well as water potentials (Ψ_{wpd} and Ψ_{spd}) of six species (*A. xalapensis*: AX, *A. rigidula*: AR, *J. flaccida*: JF, *P. pseudostrobus*: PP, *Q. canbyi*: QC and *R. virens*: RV). Correlations were calculated on pooled individual data.

Plot	Variable	Species				
		AX/AR	JF	PP	QC	RV
		Chlorophyll (a+b) content				
North (shade)	T	-0.50 *	-0.70 ***	-0.34	-0.21	-0.48 *
	RH	-0.45 *	-0.59 **	-0.02	0.00	-0.18
	VPD	-0.02	-0.05	-0.30	-0.21	0.00
	P	-0.45	-0.33	-0.12	-0.02	0.14
	ASMC	-0.04	-0.28	0.00	0.09	-0.22
	Ψ_{wpd}	-0.04	-0.41 *	-0.12	0.18	
	Ψ_{spd}	-0.32	-0.58 **	0.12	0.10	
South (sun)	T	-0.31	-0.06	-0.53 **	-0.03	-0.38
	RH	-0.15	-0.06	-0.67 ***	0.26	-0.11
	VPD	-0.10	0.17	0.61 **	-0.09	0.14
	P	-0.23	-0.08	-0.55 **	0.28	-0.07
	ASMC	-0.04	-0.36	-0.40	0.10	0.31
	Ψ_{wpd}	0.40 *	-0.13	-0.36	0.23	
	Ψ_{spd}	0.06	-0.15	-0.53 **	0.11	
		Carotenoids content				
North (shade)	T	-0.19	-0.28	-0.11	0.03	-0.25
	RH	-0.48 *	-0.49 *	-0.01	-0.01	-0.35
	VPD	0.27	0.41 *	0.03	0.31	0.45 *
	P	-0.47 *	-0.19	-0.01	0.33	-0.03
	ASMC	-0.27	-0.35	-0.14	-0.22	-0.46 *
	Ψ_{wpd}	-0.27	-0.38 *	-0.29	-0.13	
	Ψ_{spd}	-0.61 *	-0.55 **	0.12	-0.30	
South (sun)	T	-0.42 *	-0.42 *	-0.19	-0.08	-0.40
	RH	-0.27	-0.55 **	-0.45 *	-0.06	-0.35
	VPD	0.01	0.60 **	0.51 *	0.26	0.48 *
	P	-0.28	-0.48 *	-0.44 *	-0.06	-0.29
	ASMC	-0.24	-0.58 **	-0.63 ***	-0.28	-0.42 *
	Ψ_{wpd}	0.25	-0.53 **	-0.43 *	-0.06	
	Ψ_{spd}	-0.18	-0.53 **	-0.27	-0.24	

In Plot-N, chlorophyll (a+b) had negative correlations (p-value<0.05) with air temperature and RH, whereas only the chlorophyll content of

P. pseudostrobus was significantly (*p*-value<0.01) correlated with each (!) climatic variable in Plot-S (Table 5.5).

Concerning the carotenoids concentrations, significantly (*p*-value<0.05) negative correlations were mainly found with RH and precipitation, as well as positive ones with VPD under light-limited conditions, while at the sunny spot correlations were significant (*p*-value<0.05) with all environmental variables mainly for *J. flaccida* and *P. pseudostrobus* (Table 5.5). Both, total chlorophyll and carotenoids content of *R. virens* was significantly (*p*-value<0.05) correlated with VPD and soil moisture at both sites. Correlations between leaf pigments and water potentials were only significant (*p*-value<0.05) in some cases (Table 5.5).

Thus total chlorophyll was negatively correlated with both water potentials for *J. flaccida* in Plot-N, for *P. pseudostrobus* with the osmotic potential under the same conditions and for *A. rigidula* a positive correlation was found with the leaf water potential. Concerning the carotenoids, correlations with both, Ψ_{wpd} and Ψ_{spd}, were only significant for *J. flaccida* at both sites, for *P. pseudostrobus* with Ψ_{wpd} in Plot-S and for *A. xalapensis* with Ψ_{spd}. Remarkably is that leaf pigments of *P. pseudostrobus* were only correlated under sunny conditions and in the case of *Q. canbyi* not at any site.

5.5 DISCUSSION

The allocation of limited photosynthate between growth and defence is a crucial component of tree survival during periods of resource stress, such as drought and high levels of light (Aranda et al. 2005, Gaylord et al. 2007). Leaf pigments are integrally related to leaf physiological functions. Chlorophylls absorb and transfer light energy into the photosynthetic apparatus needed for plant growth. Yellow pigments or rather carotenoids also contribute energy to the photosynthetic system. Nevertheless, they gain importance, when incident light energy exceeds the needs for photosynthesis, and protect the photosynthetic system from light damage, composing the xanthophylls cycle dissipate excess energy (Sims and Gamon 2002, Aranda et al. 2005).

In general, temporal variation in chlorophyll concentration differed among sites and species. Comparing two different light environments, total chlorophyll content was higher at the shaded than at the light intense site. Hence, species were able to invest more energy into growth than defence under shaded conditions. Or rather, species at the sunny spot had to invest more energy in defence in order to avoid damage from light. Also Aranda et al. (2005) confirm higher investment of nitrogen in chlorophyll of light-limited oak seedlings than of sun exposed ones, which showed a lower photosynthetic capacity in terms of maximum net photosynthesis at saturating light. This has been frequently interpreted as higher nitrogen allocation for light harvesting under shaded environments (Niinemets and Tenhunen 1997, Evans and Poorter 2001, Aranda et al. 2005). Numerous studies reported higher chlorophyll concentrations in shade leaves than in sun exposed ones (Moran et al. 2000, Vallardes et al. 2000), since decreased solar irradiance enhances chlorophyll synthesis (Niinemets 1997).

Under shaded conditions, total chlorophyll content was higher at dry and light-intense sampling dates, while no significant differences in seasonal chlorophyll (a+b) concentration were found at the sunny site. Thus, light must be temporarily a limiting factor at the closed and light-limited stand.

Chlorophylls tend to decline more rapidly than carotenoids when plants are stressed or during leaf senescence (Gitelson and Merzlyak 1994a, 1994b, Merzlyak et al. 1999). This is in accordance to results of the present study, where chlorophyll (a+b) content declined rapidly during April and May, when stress factors, such as high evaporative demand and low soil moisture availability, were highest. The seasonal fluctuations of the predawn water potentials (Ψ_{wpd} and Ψ_{spd}) emphasize further the period of main water stress. However, correlations between leaf pigments and water potentials were only significant (p-value<0.05) in some cases (Table 5.5). A common tendency was that total chlorophyll was mainly correlated with environmental variables under light-limited conditions, with the exception of *P. pseudostrobus*, whereas carotenoids content was rather influenced by environmental condition under high light intensity, especially for the two conifers. Leaf pigments of *Q. canbyi* were not correlated to any environmental variable at both sites, even though the species showed the greatest seasonal variations in both leaf pigment concentrations. Hence, depending on the species, seasonal variations in leaf pigment concentration seem to be drought induced, at least for the conifers *J. flaccida* and *P. pseudostrobus* under the light-intense conditions of Plot-S.

Thus, *Q. canbyi* seems to possess plant physiological capacities to avoid water stress and keep on photosynthesis even under drought conditions. According to Groom and Lamont (1997), water stress and shade are significant for the development of mechanisms, such as osmotic adjustment, to overcome periods of water scarcity. Osmotic adjustment is generally limited to sunny sites, where the negative effects of drought are usually more intense (Kloeppel et al. 1993) even though not always (Valladares and Pearcy 2002). In these cases, high irradiance allows plants to accumulate osmolytes, such as soluble sugars (Ellsworth and Reich 1992). Thus, the ability to set in osmotic adjustment may be conditioned by the development of a higher photosynthetic capacity in sun lighted areas (Abrams 1988, Kloeppel et al. 1993, Mendes et al. 2001). This was evident for *Q. canbyi*, supported by numerous studies, where American and European oak species were identified as quite drought tolerant in comparison to other species, due to their capacity of osmotic adjustment in response to water stress (Bahari et al. 1985, Aranda et al. 1996, Tschaplinski et al. 1998, Corcuera et al. 2002). Both, *Q. canbyi* and *J. flaccida* had lowest baselines (-3.5 MPa in Plot-S) during the dry period and a wide range between dry and moist sampling dates, indicating higher drought tolerance (Aranda et al. 1996, Gebre et al. 1998, Aranda and Pardos 2004). However, Bahari et al. (1985) reported that species of the genus *Juniperus* possess distinctive responses to water stress compared with those of oaks. According to them, *Juniperus* is water loss resistant and has the ability to sustain stomata opening even at low water potentials (Bahari et al. 1985). The throughout high correlations of carotenoids content with environmental variables for *J. flaccida* under light-intense conditions, explains the species' capacity to protect itself from light damage and keep on with photosynthesis even under extreme conditions. *A. rigidula* for instance was less affected by water stress keeping high water- (>-2.0 Mpa) and osmotic potentials (>-2.5 Mpa) throughout the study period.

As expected, carotenoids concent was significantly higher on comparatively dry sampling dates. Highest concentrations were found in leaves of *Q. canbyi*, whereas the lowest ones in *J. flaccida* leaves at both sites. Hence, *Q. canbyi* and also *A. rigidula* showed better capability to protect itself from light damage and water stress than the other species maintaining photosynthetic processes even under less favourable conditions. The conifers were more disadvantaged.

No clear relationship between leaf pigment concentrations of eleven species and seasonal variations in atmospheric conditions and soil moisture content were found in north Mexican thornscrub species (Uvalle-Sauceda et al. 2008). In this case, studied species did not reduce leaf pigments as a result from severe photoinhibitory damage; instead, it may be an adaptive response against the adverse conditions of local winter and summer as it was found in comparable studies under Mediterranean field conditions (Kyparissis et al. 1995).

Carotenoids content did not vary significantly between the two light environments. Valladares et al. (2000) in comparison reported an increase of carotenoids content and non-photochemical quenching with light. However, carotenoids content was closely related (77%) to total chlorophyll content under light-intense conditions, which is comparable to results (87%) found by Sims and Gamon (2002) and much more than in north-eastern thornscrub species; where carotenoids content was explained between 32% and 45% by chlorophyll a content (Uvalle-Sauceda et al. 2008). Moreover, as a result of the regression analysis per species, chlorophyll (a+b) explained about 81% of the variation in carotenoids content in the case of *A. rigidula*, followed by *J. flaccida*, *Q. canbyi, R. virens* and finally *P. pseudostrobu*s.

In general, total chlorophyll content was lowest (<1.5 mg g^{-1}) in *J. flaccida* at both sites, and in *P. pseudostrobus* in the sun lighted area, where its leaf pigment contents were significantly correlated with environmental site conditions as well. It might be that N-deficient plants, which have generally reduced photosynthetic rates, may tend toward greater photoprotection (Gamon et al. 1997).

Mean chlorophyll (a+b) contents are slightly higher in Mexican mixed pine-oak forest species than in thornscrub species of the same region (Uvalle-Sauceda et al. 2008) that are exposed to even extremer site conditions. *Q. canbyi* could be identified as the species with highest mean chlorophyll (a+b) and carotenoids content in comparison to the other species studied. This is surprising, since species of the family of *Leguminosae*, such as *A. rigidula*, are capable to fix symbiotic nitrogen (Zitzer et al. 1996) and, in this way achieve higher leaf nitrogen content (Uvalle-Sauceda et al. 2008). Since nitrogen is an essential element of the chlorophyll structure (Goodwin and Mercer 1988), *A. rigidula* was expected to have higher chlorophyll contents than the other species in comparison. Thus, the results of the present study are in discordance to comparable studies, where *Leguminosae* showed higher content of chlorophyll than non *Leguminosae* (Northup et al. 2005, Hughes et al. 2007, Uvalle-Sauceda et al. 2008).

5.6 CONCLUSION

Finally, the leaf pigment concentrations varied among species and within each species in time and space. So, the impact of drought was much higher at the light-intense site. Depending on the species, chlorophyll (a+b) and carotenoids content showed clear response to drought periods as it was true for *J. flaccida* and *P. pseudostrobus*. Thus, the photosynthetic activity of conifers was affected much earlier by water stress than of broad-leaved species, which maintained generally higher carotenoids and chlorophyll (a+b) contents at the sunny and more drought affected site. Nevertheless, *J. flaccida* as well as *R. virens* seemed to suffer under light-limited conditions, where seasonal variation in light-intensity was higher due to foliar development in spring (not measured in the present study), a possible explanation for the significant seasonal fluctuations in total chlorophyll and carotenoids content under shaded conditions.

Acknowledgement

Valuable technical assistance provided by Juan José González Álvarez in the field and Elsa Dolores González at the laboratory is gratefully recognized. This research was funded in part by the Comisión Nacional Forestal and the Consejo Nacional de Ciencia y Tecnología (CONAFOR-CONACYT, No. 2002-C01-5951) and the Universidad Autónoma de Nuevo León (UANL-PAICYT, No. CN 1133-05). We further would like to thank the Secretaría de Relaciones Exteriores (SRE) for the support by scholarship and acknowledge gratefully the anonymous reviewers for their enriching comments.

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CHAPTER 6

THE RELATIONSHIP OF WATER POTENTIAL TO AIR AND SOIL WATER STATUS[1]

6.1 RELATIONSHIP OF DIURNAL LEAF WATER POTENTIALS AND VAPOUR PRESSURE DEFICIT

Relative humidity and temperature data were used to calculate vapour pressure deficit of the atmosphere (VPD) as a measure of the atmospheric evaporative demand. Diurnal leaf Ψ_w were related to VPD for three sampling dates characterized by different soil- and air water status. Depending on the combination of air and soil water availability, different patterns in the daily dynamics of the leaf water potentials were observed (Fig.6.1).

Under high soil moisture availability (mean soil moisture content (MSMC>21%) and low atmospheric evaporative demand (VPD<0.8 kPa) on Jul-25, the dynamics of Ψ_w were obviously controlled by VPD, since Ψ_w followed nearly the current level of VPD throughout the day (Fig.6.1).

When soil moisture contend was lowest on May-3 (MSMC<16%) accompanied by quite dry air conditions (VPD>1.7 kPa), Ψ_w was coupled with the evolution of VPD only in the morning. After midday Ψ_w did not match VPD and no noticeable recovery of Ψ_w could be observed before 18:00 hr (Fig.6.1).

On Feb-2, under high soil water availability (MSMC>20%) and high atmospheric evaporative demand (VPD>1.5 kPa), Ψ_w decreased with increasing VPD reaching its minimum value around midday, while a further increase in VPD

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[1] As the previous articles show, leaf water potentials are principally correlated with soil moisture availability, mainly at predawn and evaporative demand components after noon. Thus additional results emphasising mentioned relationship are provided.

after midday was accompanied by an increase in $\Psi_{w.}$ In the afternoon Ψ_w matched the changes in VPD again but demonstrated a marked hysteresis in the case of *A. xalapensis*. Ψ_w of *P. pseudostrobus* could not recover completely predawn values before night fall. In comparison to the other species studied, Ψ_w of *J. flaccida* and *Q. canbyi* were still controlled by VPD under mentioned environmental conditions (Fig.6.1).

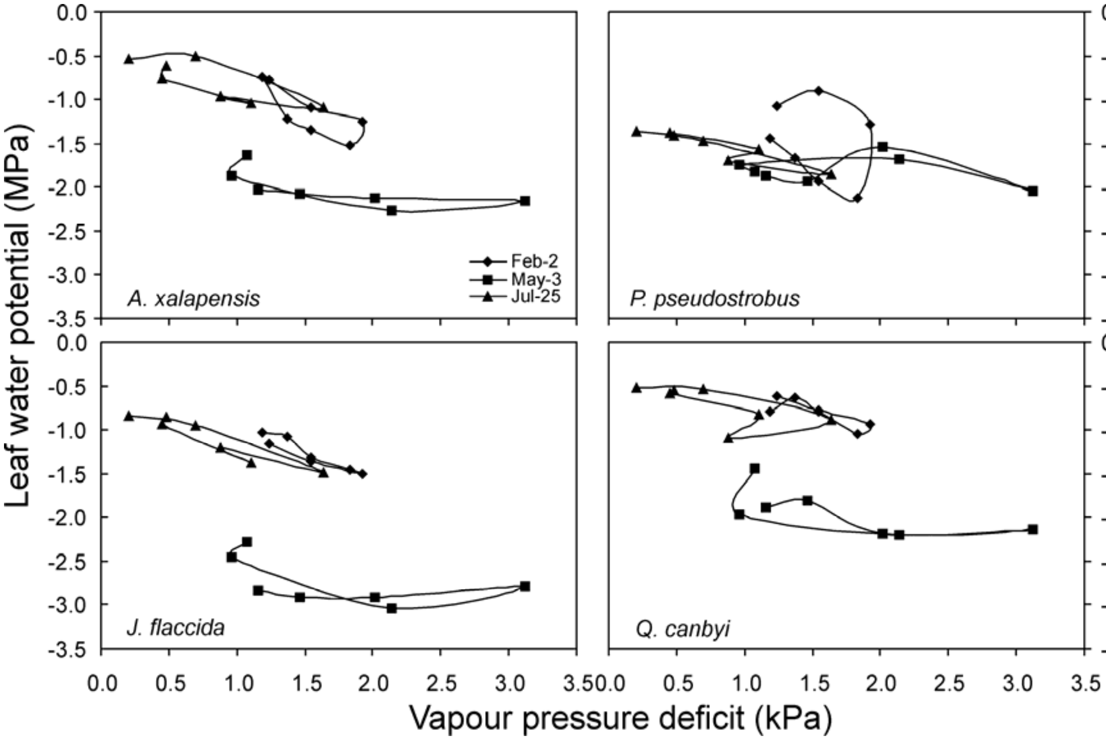

Fig.6.1. Daily patterns of leaf water potentials depending on the combination of air and soil conditions for four tree species at Plot-N. Feb-2: high soil water content and high atmospheric evaporative demand; May-3: low soil water content and very high atmospheric evaporative demand; Jul-25: high soil water content and low atmospheric demand conditions.

Thus, the impact of atmospheric evaporative demand and plant water supply on diurnal Ψ_w is different comparing the four species studied. *P. pseudostrobus* reacted less to water availability than the other species that had very similar curves. This confirms the previously discussed idea of different rooting systems (Chapter 3), since there is a high temporal and spatial variability in soil moisture content of the upper horizons, while deeper layers are more homogenous in this respect (Sellin 1998). *P. pseudostrobus* as a deep rooter shows less variability than *J. flaccida*, which was identified as a very superficial rooter. The rooting depth of *Q. canbyi* and *A. xalapensis* is to be categorized somehow between the one of the two conifers.

Hence, daily dynamics of leaf water potentials depend on the combination of air and soil humidity (Table 6.1). Under wet soil conditions and low atmospheric evaporative demand, Ψ_w decrease rapidly with increasing VPD, while the daily pattern of Ψ_w is coupled with the current level of VPD (Jul-25). Under high soil water availability and high VPD levels, minimum Ψ_w can be reached already at low VPD values. When Ψ_w fall to a certain level (-1.0 to -2.0, depending on the species), the stomata seem to narrow significantly (Sellin 1998) and a further rise in VPD after midday was accompanied by an increase in Ψ_w again (Feb-2). In contrast to that, Ψ_w declined slowly, due to the plants' intent to weaken the impact of VPD on leaf water supply by more efficient stomata- and with that transpiration control (Sellin 1998).

Table 6.1. Average soil moisture content (ASMC, %) per sampling date, seasonal mean temperature (T; °C), relative humidity (RH; %), vapour pressure deficit (VPD; kPa) and monthly sum of precipitation (P; mm) at both study sites from January until August 2006.

Month	Plot-N					Plot-S				
	ASMC	T	RH	VPD	P	ASMC	T	RH	VPD	P
January	22.77	-	-	-	-	7.56	-	-	-	-
February	20.61	12.6	53	0.8	1	9.59	14.6	49	1.1	1
March	20.16	16.4	59	1.8	3	9.98	16.8	52	1.3	3
April	-	20.6	55	1.3	7	-	21.2	52	1.6	8
May	19.45	20.3	62	1.1	39	8.30	20.3	62	1.1	41
June	15.80	20.7	67	1.1	34	9.69	20.4	69	1.0	47
July	19.67	20.4	73	0.8	35	7.91	20.1	74	0.8	86
August	20.06	20.2	77	0.7	68	11.80	20.3	76	0.7	86

At low soil moisture content, VPD is only coupled with Ψ_w in the morning and values did not recover after they have reached a threshold value around midday (May-3). In the afternoon, the variation in Ψ_w is not related to the one in VPD. Similar patterns of variation in Ψ_w, depending on soil moisture availability and evaporative demand conditions, were observed in other conifer and broad-leaved tree species (Bréda et al. 1995, Sellin 1998 and references within).

Hence, if there is sufficient water stored in the soil, leaf Ψ_w are strongly governed by VPD of the atmosphere. As soil dries, plants prevent water loss more efficiently by stomata closure, why leaf Ψ_w become less sensitive to atmospheric evaporative demand. This is in accordance to other studies where trees' responsiveness to atmospheric conditions is determined by soil moisture availability (Sellin 1998).

Described tendencies are confirmed by the calculated Spearman's correlations comparing the three sampling dates Feb-2, May-3 and Jul-25 (Table 6.2). On May-3, the driest sampling date, leaf Ψ_w were less correlated to atmospheric evaporative demand components than on the two sampling dates in comparison, when soil moisture availability was high.

Table 6.2. Spearman's correlation coefficient values for leaf water potential in relation to atmospheric evaporative demand components (air temperature: T, relative humidity: RH, vapour pressure deficit: VPD) in four tree species on three sampling dates (SD) characterized by different soil and air water status. Correlations were performed per species on pooled individual data of the seven sampling hours (6:00, 8:00, 10:00, 12:00, 14:00, 16:00 and 18:00 hr).

SD	AEDC	*A. xalapensis*		*J. flaccida*		*P. pseudostrobus*		*Q. canbyi*	
		rho	*p*-value	*rho*	*p*-value	*rho*	*p*-value	*rho*	*p*-value
Feb-2	T	-0.58	<0.001	-0.89	<0.001	-0.01	0.949	-0.51	0.002
	RH	0.71	<0.001	0.28	0.106	0.61	<0.001	0.32	0.062
	VPD	-0.68	<0.001	-0.86	<0.001	-0.11	0.541	-0.52	0.001
May-3	T	-0.56	<0.001	-0.61	<0.001	0.01	0.9315	-0.49	<0.001
	RH	0.18	0.266	0.13	0.397	-0.09	0.590	0.24	0.119
	VPD	-0.56	<0.001	-0.63	<0.001	0.00	0.985	-0.43	0.004
Jul-25	T	-0.65	<0.001	-0.81	<0.001	-0.65	<0.001	-0.56	<0.001
	RH	0.72	<0.001	0.84	<0.001	0.64	<0.001	0.58	<0.001
	VPD	-0.72	<0.001	-0.84	<0.001	-0.64	<0.001	-0.58	<0.001

Thus, under sufficient soil moisture availability, leaf Ψ_w are strongly related to VPD of the atmosphere. Soil water scarcity in contrast implicates plant physiological processes preventing severe water loss and leaf Ψ_w are less correlated to atmospheric demand components (Table 6.1). Comparing the species studied, the Ψ_w of *P. pseudostrobus* were less correlated than the potentials of *J. flaccida, A. xalapensis* and *Q. canbyi*. This is in accordance to the results obtained by relating diurnal leaf Ψ_w and VPD (Fig.6.1).

6.2 PREDAWN WATER POTENTIALS IN RELATION TO AVERAGE SOIL MOISTURE CONTENT

The impact of soil water content on the predawn water potential ($\Psi_{6:00\ \mathrm{hr}}$) is discussed widely among plant physiologists. Hence, some authors report no coincidence of $\Psi_{6:00\ \mathrm{hr}}$ values with soil water potential under well-watered conditions (Richter 1997, Donovan et al. 2001, Donovan et al. 2003), while others consider $\Psi_{6:00\ \mathrm{hr}}$ as an indicator for soil water potential and actual water availability during a plant's night time recovery from daily minimum values (Hinckley et al. 1978, Bréda et al. 1995, Sellin 1996, Mitlöhner 1998, Zobel et al. 2001).

On a seasonal bases, regression analyses was performed between $\Psi_{6:00\ \mathrm{hr}}$ and average soil moisture content (ASMC) in four soil depths (0-10, 20-30, 40-50 and 60-70 cm) considering the individual and mean values, respectively. The ASMC in the 0-70 cm soil depth profile explained between 4% (*P. pseudostrobus*) and 71% (*J. flaccida*) of the variation in $\Psi_{6:00\ \mathrm{hr}}$ in Plot-N and between 14% (*A. rigidula* and *P. pseudostrobus*) and 78% (*J. flaccida*) in Plot-S (Table 6.3).

Table 6.3. Least-squares coefficients for predawn leaf water potential ($\Psi_{6:00\ \mathrm{hr}}$) in relation to average soil moisture content (ASMC) in five tree species (n=55).

Plot	Tree species	Least-squares statistics						Adjusted r^2
		y-axis intercept			Slope of regression model			
		$\hat{\beta}_0$	E.S.E.	p-value	$\hat{\beta}_1$	E.S.E.	p-value	
Plot-N	*A. xalapensis**	5.44	0.57	<0.0001	-1.87	0.19	<0.0001	0.65
	*J. flaccida**	5.99	0.51	<2e-16	-1.94	0.17	<0.0001	0.71
	P. pseudostrobus	-2.01	0.33	<0.0001	0.03	0.02	0.0783	0.04
	*Q. canbyi**	4.70	0.68	<0.0001	-1.65	0.22	<0.0001	0.50
Plot-S	*A. rigidula**	0.93	0.26	0.0009	-0.32	0.11	0.0045	0.14
	*J. flaccida**	5.22	0.37	<2e-16	-1.95	0.15	<2.2e-16	0.78
	P. pseudostrobus	-2.37	0.23	<0.0001	0.06	0.02	0.0039	0.14
	*Q. canbyi**	4.16	0.45	<0.0001	-1.70	0.18	<0.0001	0.63

*Least-squares estimates have indicated that the best fitted model to relate $\Psi_{6:00\ \mathrm{hr}}$ as a function of different ASMC corresponded to a power ($\hat{\Psi}_w = \hat{\beta}_0 \times ASMC^{\hat{\beta}_1}$; where $\hat{\Psi}_w = -\Psi_w$) mathematical function. $\hat{\beta}_0$ and $\hat{\beta}_1$ are the y-axis intercept and slope of regression model, respectively. Estimated standard errors (E.S.E.'s), p-values and adjusted coefficient of determination (r^2) values are provided. In the case of *P. pseudostrobus*, simple linear regression without log transformation was the best fitted model.

Furthermore, it was found that ($\Psi_{6:00\ hr}$ values were high and mainly constant at soil moisture content values above 15% as in Plot-N (Fig.6.2) Below this value, water potentials declined gradually, see Plot-S (Fig.6.2).

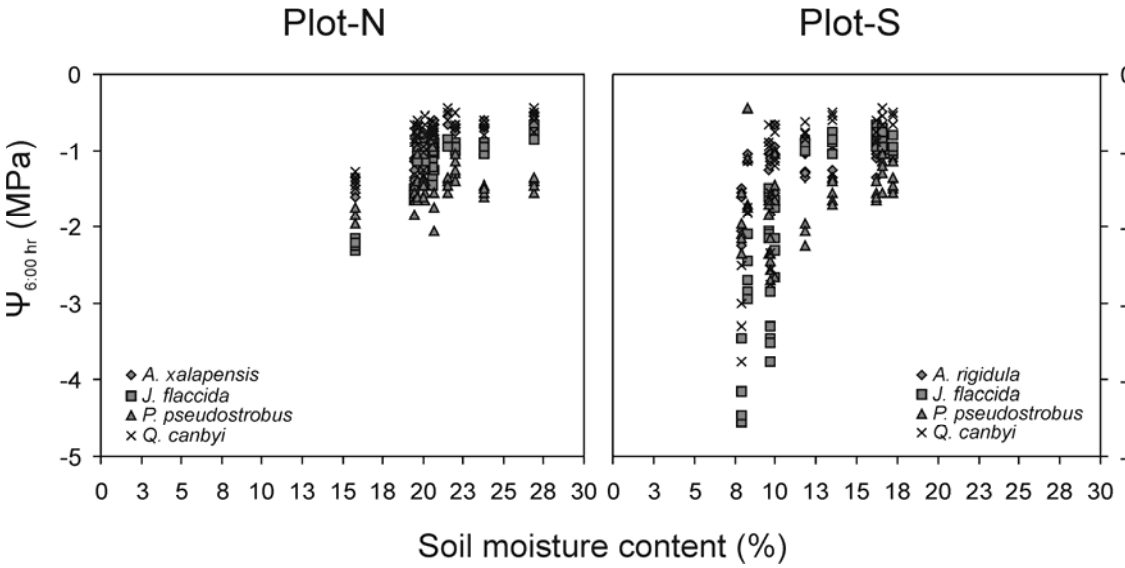

Fig.6.2. Predawn leaf water potential ($\Psi_{6:00\ hr}$) of the tree species as a function of the average soil moisture content in the 0-70 cm soil layer. Relationships are presented for Plot-N and Plot-S separately.

These results are partly different from comparative studies, where average soil water content explained between 70% (*E. texana*) and 87% (*A. rigidula*) of the variation in predawn water potentials of six different shrub species (González et al. 2004). Surprising was the result of 14% in the case of *A. rigidula*, which had highest correlations between soil moisture content and predawn water potential in Mexican thornscrub ecosystems (González et al. 2004). *J. flaccida* was identified as the species, whose predawn water potential was strongly influenced with 78% (Plot-S) by soil water availability followed by *A. xalapensis* (65% in Plot-N) and *Q. canbyi* (63% in Plot-S). The weak relationship of $\Psi_{6:00\ hr}$ and soil moisture content in the case of *P. pseudostrobus* confirms again (see Chapter 3 and 4) the theory of a deeper rooting system and thus another strategy to avoid water stress.

Comparing north and south aspect, soil moisture availability had higher correlations with $\Psi_{6:00\ hr}$ in Plot-S, which was comparatively much, drier, than Plot-N. Hence, these results confirm the importance of soil water availability as a limiting factor, especially in fragmented Plot-S.

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CHAPTER 7

GENERAL CONCLUSION

The measurement of leaf water- and osmotic potentials in combination with the determination of leaf pigment concentrations was a useful method to identify the adaptation of natural regeneration of different tree species to seasonal fluctuations in water availability in mixed pine-oak forests of the Sierra Madre Oriental. All species showed capacities of adaptation to water stress and some grade of soil salinity, but applied quite different strategies. Thus, also the micro-site conditions were reflected quite differently by the species of interest, and according to their functional features, species site-matching can be optimized, and the aim, to contribute to the improvement of species selection in reforestation programs, achieved.

Q. canbyi seemed to overcome periods of drought by osmotic adjustment, *P. pseudostrobus* as a deep-rooter took advantage of soil water stored in deeper soil layers, *J. flaccida* recovered rapidly from water stress taking advantage of summer precipitation in the upper soil layers due to its superficial rooting system. The latter one resulted furthermore as the best site-indicator, reflecting best the environmental conditions measured at each study site, while *P. pseudostrobus* was the worst one. At the light-intense south aspect, *A. rigidula* was less affected by water stress than other species in comparison, and *A. xalapensis* showed similar capacities as *Q. canbyi* at the shaded, north-facing aspect.

Water stress had in general an effect on photosynthetic activity, measured indirectly by total chlorophyll and carotenoids concentrations, decreasing with some delay after periods of drought. Especially in the case of *J. flaccida* and *P. pseudostrobus*, photosynthesis showed clear response to drought. Photosynthetic activity of *Q. canbyi* in comparison was not at all affected by water stress. Hence, *Q. canbyi* was identified as the species with greatest flexibility and capacity to react to environmental changes.

Comparing the two study sites, trees suffered less water stress and photosynthesis was enhanced under shaded conditions in comparison to the fragmented stand, where drought had a greater impact on the tree regeneration, which was subject of the present study.

Depending on the site conditions in areas considered for reforestation or ecological restoration, species should be recommended according to their characteristics. Thus, *J. flaccida* and *A. rigidula* are good candidates for flat, sandy or rocky soils, while *P. pseudostrobus* promises better reforestation success on deeper soils. *A. xalapensis* in comparison has difficulties to grow in very light-intense areas and needs already some protection by forest canopy that is in contrast to *A. rigidula*, which is not found under shaded conditions. *Q. canbyi* fits in between the extremes, possessing plant physiological capacities of adaptation to different environments. In this way, *Q. canbyi* as a slowly growing species can compete or rather co-exist with fast growing species such as *P. pseudostrobus*. Thus, an explanation for the successful co-occurrence of the studied species in Mexican mixed pine-oak forests in the Sierra Madre Oriental.

Curriculum vitae

Born in Offenburg, southwest Germany, the 27th of July 1976, Wibke Himmelsbach started her studies in Forest Sciences at the Faculty of Forest Sciences and Forest Ecology at the Georg-August University Göttingen in 1997, after half year working experience as an in the Carpathian Large Carnivore Project of the Wildbiologische Gesellschaft München e.V. In 2003 she graduated successfully with the Master of Sciences with the specialization in Tropical and International Forestry. Within this study program, she could gain international experience in Ecuador analysing the costs and benefits of organic coffee plantations, and during half a year in Burma, where she was introduced to the Burmese Selective Timber System and traditional timber harvest using domesticated elephants. After her graduation she started working in several institutions such as ECO Consulting Group in Oberaula and the Tropenzentrum in Göttingen. Later on in 2005 she was offered a grant by the Mexican Government through the SRE (Secretaría de Relaciones Exteriores) to continue her academic career as Ph.D.-Student at the Autonomous University of Nuevo León (UANL) in Mexico. In January 2006, she finally started her research within the Ph.D.-Program "Natural Resource Management" at the Faculty of Forest Sciences in Linares, Nuevo León. The following four years, Mrs. Wibke Himmelsbach was concerning herself with the effect of water stress and salt excess on native tree species in natural pine-oak forests in the Sierra Madre Oriental. In December 2009, she graduated her doctorate successfully.

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