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היבטים אקופיזיולוגיים ופנולוגיים של אורן ירושלים
(*Pinus halepensis* Mill.) בסביבה ים תיכונית – מדברית

Ecophysiological and phenological aspects of *Pinus halepensis* in an arid-Mediterranean environment

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This work is dedicated to my father, who has always encouraged me to do what I want and remains a source of inspiration.

Declaration

All group members associated with the Yatir Forest flux tower research site have been involved in the maintenance and operation of the flux tower, under the direction of Dr. Eyal Rotenberg. The database of flux and meteorological data is maintained by Dr. Ruth Meir. Interpretation and analysis of flux data is my own.

The irrigation experiment (Chapters 4 and 5) was established and maintained by Dr. Tongbao Lin. Dr. Lin performed the measurements and provided the data from the phenology, gas exchange and pigment analysis, and I performed the measurement, analysis and interpretation of the chlorophyll fluorescence data.

All other work presented in this thesis is my own.

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Abstract

A study has been conducted into pertinent physiological responses underlying survival and productivity of a 40 yr old *Pinus halepensis* Mill. forest growing in an arid-Mediterranean environment in Israel. Rates of carbon fixation and water loss were controlled mainly by stomatal conductance and were high in winter and spring and low in summer. Soil water content limited stomatal conductance in the summer months but the effect of vapour pressure deficit was apparent at any time of the year. Water use efficiency of photosynthesis was determined primarily by vapour pressure deficit and was therefore higher in the cool wet months than in the water limited period. The low rates of photosynthesis maintained through the summer were, however, seen to be sufficient to sustain the growth of new foliage. A highly constrained feature, dry summer leaf phenology also decoupled growth from nitrogen availability and relied on the reallocation of nitrogen from the mature and senescing needles. This phenological trait indicates off-season physiological performance is critical to tree survival. The capacity for photosynthetic activity through the summer was facilitated by effective photoprotection mechanisms. Both pre- and post-photochemical electron transport energy dissipation was employed to prevent any chronic reduction of photosystem efficiency under the conditions of high light and low stomatal conductance. The relative importance of the various protection mechanisms depended on the nature and extent of the drought stress, but pigment bed thermal dissipation was seen to be more important than sustaining electron flow through photorespiration. A down-regulation of foliage and stem dark respiration in summer minimized temperature induced respiratory CO₂ losses. Both stem and foliage displayed a continual acclimation of their respiration temperature response, but different metabolic drivers resulted in dissimilar seasonal patterns of respiration between the components. Foliage respiratory capacity was highly correlated with that of photosynthesis while stem respiration had a strong phenological influence. Evidence from the $\delta^{13}\text{C}$ composition of respired CO₂ indicates above-ground respiration also remained supported by current photosynthate during the dry summer. While the dry summer photosynthetic activity is a crucial feature of productivity in this ecosystem, reliance on off-season photosynthesis for growth and metabolism may also increase ecosystem vulnerability to more extreme conditions.

תקציר

המחקר התמקד בתגובות הפיסיולוגיות הקשורות באופן הישרדותו ומידת יצרנותו של יער בן 40 שנה הגדל בסביבה ים תיכונית – מדברית בישראל. קצבי קיבוע פחמן ואיבודי מים נשלטו בעיקר על ידי מידת הולכת הפיוניות והיו גבוהים בחורף ובאביב ונמוכים בקיץ. גרעון לחץ אדי המים באוויר (vapour pressure deficit - D) השפיע על קצבי הולכת הפיוניות לאורך כל השנה. במהלך הקיץ, הולכת הפיוניות הוגבלה גם על ידי תכולת המים בקרקע. יעילות ניצול המים (water use efficiency) בתהליך הפוטוסינטזה נקבע בעיקר על ידי D ולכן היה גבוה בחודשים הקרים והלחים ונמוך בחודשים היבשים. קצבי הפוטוסינטזה היו נמוכים לאורך כל עונת הקיץ ולמרות זאת הם איפשרו גדילה של עלווה חדשה. בדומה, זמינות נמוכה של חנקן בתקופת הקיץ היבשה גם היא לא הגבילה את הגדילה, מאחר ותוספת חנקן למערכת התקבלה על ידי ניצול חנקן ממחסים בוגרים טרם נשירתם. תכונה זו, של חוסר ההתאמה בין הגדילה ובין התנאים הסביבתיים לגדילה, מצביעה על הכרחיותה של פעילות פיסיולוגית גם בתקופות היבשות להשרדות היער. פעילות פוטוסינטטית במהלך הקיץ התאפשרה על ידי מנגנון הגנה יעיל, שאפשר ניצול מירבי של האנרגיה לפני ואחרי התהליך הפוטוכימי, בכדי למנוע ירידה כרונית ביעילות המערכת הפוטוסינטטית בתנאים של אור חזק והולכת פיוניות נמוכה. החשיבות היחסית של מנגנוני ההגנה השונים תלוי במידת העקת הבצורת, אך תהליך איבוד חום באמצעות פיגמנטים נמצא כבעל השפעה גדולה יותר מאשר השחיית זרימת האלקטרונים בתהליך הפוטו-נשימתית. ויסות העלווה ונשימת הלילה של הגזע בקיץ איפשר הקטנה איבודים של פחמן דו-חמצני כתוצאה מעליית הטמפרטורות. מנגנוני הנשימה של העלווה והגזע הראו שניהם תהליך רציף של התאקלמות לטמפרטורות, אך מאחר וגורמים שונים משפיעים על התהליכים המטבולים שלהם, דפוסי הנשימה העונתיים שלהם נבדלו זה מזה. נשימת העלווה נמצאה בתאימות גבוהה עם זו של הפוטוסינטזה, בעוד שנשימת הגזע הושפעה באופן משמעותי מהפנולוגיה. הרכב ה- $\delta^{13}\text{C}$ בפחמן הדו-חמצני שמקורו בנשימה הווה עדות לכך שגם הנשימה מעל הקרקע תלויה בקיומה של הפעילות הפוטוסינטטית בקיץ. בעוד שהפעילות הפוטוסינטטית בקיץ הכרחית לצורך יצרנותו של היער, היא עשויה בד בבד להגביר את מידת רגישותו של היער לתנאים קיצוניים, שכן המטבוליזם והגדילה של היער תלויים בפעילות במהלך התקופה היבשה

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Chapter 1

Introduction

1.1 Climate change and the biosphere

There is common acceptance amongst the scientific community that human-induced global environmental changes are in motion, a component of which includes changes to the climate system (Vitousek 1994, IPCC 2001). The potential for climate change is driven largely by the rapid alteration to atmospheric composition that has been underway for the last ~200 years of industrial activity. Through the combustion of fossil fuels, the addition of climate forcing gases, including (but not limited to) CO₂, CH₄ and N₂O, and aerosols to the atmosphere is considered to have increased Earth surface temperature by $0.6 \pm 0.2^{\circ}\text{C}$ during the 20th century (Folland *et al.* 2001). Irrespective of policy and future emission scenarios, further warming of about 0.6°C is expected due substantial time lags associated with the large heat capacity and thermal inertia of the ocean (Hansen *et al.* 2005).

The biosphere, upon which humanity is inextricably dependent, is a key element of global change processes. Many properties of the biosphere, including process rates, species distribution and diversity are a function at some level of the climate (Stenseth *et al.* 2002). There is increasing evidence that parts of biosphere show signs of a response to recent climate change (Walther *et al.* 2002, Parmesan and Yohe 2003, Root *et al.* 2003). Furthermore, as the biosphere is an important component of the global carbon cycle, it can feed back on the climate system (Falkowski *et al.* 2000). The terrestrial biosphere exchanges on the order of 60 Pg (10^{15} g) of C, or ~10 times that released through fossil fuel burning, with the atmosphere each year through photosynthetic uptake and respiratory release of CO₂ (Schimel *et al.* 2000). Therefore a mechanistic knowledge of the physiological processes affecting plant and ecosystem function is essential for understanding response to, and possibly effects on, short and long term climate variation (Hulme 2005).

1.2 Ecosystem research

The interest and need to study ecosystem level response to the climate and to determine the effect of the biosphere on atmospheric CO₂ concentrations provided the impetus in the last

decade to establish a number of research sites worldwide to investigate biosphere-atmosphere interactions. At the core of these sites is the continuous measurement of CO₂, H₂O and energy fluxes between the ecosystem and the atmosphere using the micrometeorological eddy covariance technique (Baldocchi *et al.* 1988, Aubinet 2000), complemented by finer scale physiological and ecological measurements and experimental manipulations. A central element of the flux site studies is to determine the underlying controls of ecosystem productivity; or the integrated result of photosynthetic, growth and respiration process (Buchmann and Schulze 1999).

The first, and only, flux site in Israel was established in the Yatir Forest in 2000 (Grünzweig *et al.* 2003), and is the site of this research. The forest is one of many forests planted in Israel over the last 50-80 years; it is the largest and most southern forest in Israel and probably the driest forest in the global Fluxnet network of flux tower sites. It is composed predominantly of *Pinus halepensis* Mill., most of which was planted between 1964 and 1969.

One of the key results to emerge from the flux measurements at this site is the high level of productivity, relative to the conditions, of the ecosystem. Despite receiving only ~300 mm precipitation a year, annual productivity in Yatir over the last 4-5 years has been in the order of 200-300 g C m⁻² (Grünzweig *et al.* 2003, E. Rotenberg, unpubli.data), which is similar to that observed in many temperate and boreal ecosystems (Law *et al.* 2002). Typical estimates of productivity in arid and semi-arid regions may be up to an order of magnitude lower than that observed in Yatir (Begon *et al.* 1990). This indicates the potential dryland regions may have for carbon sequestration and the extension of afforestation (Grünzweig *et al.* 2003).

1.3 Dryland productivity

Water shortage is the main factor limiting plant production worldwide (Boyer 1982, Flexas *et al.* 2004) and both episodic drought events and long term increases in aridity can have profound effects on crop, forest and natural ecosystem productivity (Hansen and Weltzin 2000, Peñuelas 2001, Angert *et al.* 2005, Ciais *et al.* 2005). Severe drought can lead to tree mortality and shifts in species distribution (Breshears *et al.* 2005, Mueller *et al.* 2005, Guarin and Taylor 2005). Drought is a normal, recurrent feature of climate, particularly in dryland regions, and can be defined in many ways depending on location and the activity or processes of concern. At the basis of drought is a deficit of precipitation over an extended period, resulting in a water shortage for the system of interest. Dryland areas include hyper-arid, arid,

semi-arid and dry sub-humid zones, in which the ratio of precipitation to potential evapotranspiration is less than 0.75 (le Hou  rou 1996)

One of the important features of a warming climate with particular relevance to plant and ecosystem productivity is the potential for increased aridity. There is evidence (Pin  l *et al.* 1998, Ziv *et al.* 2005) for warming trends in the Mediterranean region, and the latest IPCC projections were for a likely reduction in summer precipitation in the Mediterranean region and indications for an increase in the frequency of drought or dry spells for Europe, North America and Australia (Giorgi *et al.* 2001). Currently, dryland regions make up about 47% of the terrestrial earth's surface, of which up to 40% may be experiencing some form of degradation, or desertification (le Hou  rou 1996). Increasing human pressure on dryland areas and the possibility that reduced rainfall may expand water limited environments may place greater areas at risk of degradation. Agroforestry is one of the critical strategies for mitigating the effects of climate change, drought and desertification in dryland regions (le Hou  rou 1996). Therefore it is crucial that an understanding of ecosystem function under water limited conditions is developed. To this end, studying the response of ecosystems experiencing regular drought as opposed to episodic events will provide more relevant information on ecosystem function under long term exposure to water deficits.

1.4 Mediterranean pines and *Pinus halepensis*

Pinus halepensis (Aleppo pine) is one of 10 *Pinus* species in the Mediterranean Basin, and together with *P. brutia*, forms the *Halepenses* subsection (Klaus 1989). The group of Mediterranean pines are considered relics from the Tertiary Period, and are therefore pre-Mediterranean species. They have common ancestry with the pines of Central America and the Caribbean, emerging in the Lower Cretaceous (120 mya), but the American and Mediterranean species evolved separately. There are fossils of *P. halepensis* dating from the Miocene and Upper Oligocene (~25-30 mya), from which time the species has apparently changed little (Klaus 1989). Movement of Mediterranean pines into the present day Mediterranean region occurred during the Pliocene (~3.5 mya), after the isolation of the Mediterranean Basin and development of the Mediterranean-type climate (Barb  ro 1998).

Two important climatic events have characterised the development of the Mediterranean vegetation. First, at about 3.2 Mya, involved the appearance of the Mediterranean climate rhythm, with a shift from dominant summer rainfall to moderate summer drought, and is associated with the individualisation of modern Mediterranean flora.

The second was at about 2.3 Mya, in which the first xeric phase occurred, or the start of the Pleistocene glacial–interglacial cycles, and is when the oldest evidence of Mediterranean-type vegetation organisation appears (Suc 1984). Interglacial periods were characterised by a progression through deciduous oaks and subtropical vegetation, followed by coniferous forest leading to open steppe-meadows at the glacial transition. Typically it was steppe and *Pinus* species that persisted through the glacial periods, so pines, despite Arcto-Tertiary origins, have long been an integral and important component of the vegetation of the region (Raven 1973, de Beaulieu 2005).

Despite its common name, *P. halepensis* is not native to the Aleppo region of Syria. It is predominantly a west Mediterranean species, but is also found in the Balkans, Libya and the Levant. *P. halepensis* is the only pine species native to Israel, although there is debate as to the extent of its original distribution (Weinstein-Evron and Lev-Yadun 2000, Liphshitz and Biger 2001). Temperature and precipitation requirements generally confine it to the Thermo- and Meso-Mediterranean altitudinal zones (minimum temperatures above 0°C) and sub-humid to arid bioclimatic regions (annual precipitation from 600-800 to 100-400 mm, summer drought from 2-3 to 7-11 months), with a preference for marl, limestone and dolomite soils (Barbéro 1998, Quézel 2000).

Although they rarely co-occur (but form natural hybrids where they do), the two species of *Halepenses* are the most common Mediterranean pine species, covering more than 7 million hectares (Quézel 2000). *P. halepensis* is often a coloniser species of abandoned land, accounting for much of its recent expansion in some areas (e.g. southern France), forming climax communities with *Quercus ilex* and *Q. rotundifolia* in more humid regions. In the arid regions, the *Halepenses* form climax communities with pre-steppe vegetation (Barbéro 1998). Due to its high level of drought tolerance, *P. halepensis* is the species that has been used most extensively in reforestation and afforestation efforts and is therefore one of the most important ecological and economic tree species in the region (Maestre and Cortina 2004, Quézel 2000).

1.5 Forestry in the Mediterranean and Israel

There is a long history of human interaction with forests in the Mediterranean and southern Europe. Wood is still an important economic resource that remains the basic source of energy in many countries and is a desired fuel source in many industrialised countries (Scarascia-Mugnozza *et al.* 2000). In addition to fuel wood, forests provide a range of traditional

products (e.g. food, resins, aromatic plants) and a range of environmental (e.g. soil and slope stabilisation, mitigation of desertification) and social (e.g. recreation and landscape aesthetics) benefits. Reforestation and afforestation programs and forest conservation and sustainable management policies are therefore quite developed and remain important economic and activities in southern Europe and west Asian countries (FAO 2001).

The dry climate of much of the region is an important factor affecting forest productivity, and one of the important roles of forests is in preventing desertification and protecting watersheds. However, the positive aspects of forestry need to be balanced by considerations for the potential effects on other species and on water resources downstream of forest regions in arid catchments (Maestre *et al.* 2004, Farley *et al.* 2005, Jackson *et al.* 2005).

Following many years of exploitation and degradation of the forest resources, reforestation and afforestation efforts were started in Israel in 1890, by independently operating settlers, and there is now widespread management and afforestation activity in Israel under the administration of the non-governmental Jewish National Fund (JNF, or *Keren Keyemet L'Israel*, KKL; Bonnef 2000). By 2000, there were 91000 ha of planted forest in Israel, being nearly 70% of the total (132000 ha) forested area (FAO 2001). The majority of the afforestation has been with Aleppo pine. The use of *P. halepensis* was predominant from the 1930s through to the 1970s, but the use of the closely related *P. brutia* has since increased due to the susceptibility of *P. halepensis* to the pine bast scale *Matsucoccus josephi* (Bonnef 2000).

1.6 Research goals

The importance of forestry and afforestation in many areas in the world, the role of forestry in dryland restoration, the potential need to expand forestry activity into drier regions and the possibility that dryland regions will expand or drought will become a more important feature in managed and natural ecosystems in the future all highlight the importance of understanding ecosystem function in dry environments. In this regard, it is important understand the physiological factors underlying the high level of productivity observed in the *P. halepensis* dominated Yatir Forest. Such information can provide important mechanistic information for predicting the response and estimating the potential of dryland ecosystems. Key features of plant and ecosystem productivity include photosynthetic and respiration responses, stress resistance and leaf phenology. Overall, productivity is the balance between the amount of carbon assimilated by the system during photosynthesis and the amount of this

carbon which is released through respiration during growth and maintenance metabolism. Vulnerability to stressful conditions can reduce photosynthetic capacity and a potentially stressful situation associated with Mediterranean environments is the combination of low soil water content and high radiation over summer. The timing and control of annual leaf production determines the extent of photosynthetic area and is crucial for maintaining productivity. An interesting observation in Yatir is that the trees continue to grow new needles each year over the hot and dry summer.

Specifically, the aims of this study were to:

- Determine leaf level rates of carbon fixation and identify the main factors that control seasonal and diurnal patterns of photosynthesis and affect the balance between carbon gain and water loss during photosynthesis;
- Investigate the timing and control of the dry summer leaf phenology and identify the carbon and nitrogen strategies involved in maintaining growth;
- Identify the photo-protection strategies employed in response to high radiation during the dry summer, determine the relative importance of the various responses and investigate any differential responses in relation to atmospheric and soil water deficits;
- Quantify the rates of foliage and stem respiration and determine the main factors influencing seasonal variation in respiration, investigate seasonal acclimation of the respiration temperature response and to analyse the $\delta^{13}\text{C}$ composition of respiratory CO_2 as a signal of the respiratory substrate.

Chapter 2

Materials and Methods

2.1 Site details

The research was conducted in Yatir Forest, Israel, at the location of a field research site in the centre of the forest (31°20'49" N, 35°03'07"E, 650m a.s.l.). The forest is an afforestation of predominantly *Pinus halepensis* Mill. mostly planted in the years 1964-69. The forest covers 2800 ha of undulating terrain, and current average tree density is ~300 trees ha⁻¹, tree height ~10m, diameter at breast height (DBH) ~17cm, leaf area index (LAI) ~1.5 and plant area index (PAI) ~2.0. Soil consists of a Rendzina layer about 0.2-1.0 m deep above chalk and limestone, and the water table is at a depth of ~300m, (Grünzweig *et al.* 2003, www.weizmann.ac.il/ESER/People/Yakir/YATIR).

The forest is located in the region where the Mediterranean climate zone of the Hebron Mountains gives way to the arid Negev Desert of the Beersheva plateau. Precipitation generally falls between the months of November – March, and is mostly concentrated over December – February. There is no summer precipitation and annual average rainfall for the hydrological year (Oct – Sept) for the last 35 years is 280 mm. Mean annual global radiation is ~7.5 GJ m⁻² y⁻¹, mean winter and summer daytime temperatures are 13°C and 31°C respectively, mean annual relative humidity is ~40% and site aridity index (precipitation / potential evapotranspiration) has been calculated as 0.18, a rating associated with hyper-aridity. Mean annual daytime maximum atmospheric vapour pressure deficit is ~1600 Pa, but ranges between <500 Pa in winter to >5000 Pa at times in summer (Fig. 2.1).

2.2 Ecosystem fluxes and environmental parameters

The research site is centred on a flux tower, established in 2000, for the continuous measurement of CO₂, H₂O and energy fluxes between the forest and the atmosphere, along with a number of meteorological variables on a scale of ca. 1x1 km (Grünzweig *et al.* 2003, Rotenberg *et al.* 2006, submitted). Research around the site is focussed on facilitating detailed understanding of ecosystem function as manifested in the ecosystem-scale flux tower measurements.

Canopy scale flux measurements were made using the eddy correlation technique, as used in the Euroflux network (Aubinet *et al.* 2000). Fluxes are calculated over a 30 min averaging period from 3D sonic anemometer measurements of wind speed and direction (Omnidirectional R3, Gill Instruments, UK; 21 Hz sampling rate) and measurements of canopy boundary layer CO₂ and H₂O concentrations, drawn from 9 m above the canopy (LI-7000 infra-red gas analyzer (IRGA); LI-COR Inc., Nebraska, USA; sampling rate 10Hz). Corrections for night-time low turbulent conditions (low frictional velocity, u^*) were done on a monthly basis.

Relevant meteorological measurements include net radiation (NR LITE, Kipp and Zonen, Delft, The Netherlands), photosynthetically active radiation (PAR, LI190SB quantum sensor, LI-COR Inc.), air temperature and relative humidity (HMP45C Vaisala, MA, USA) and precipitation (tipping bucket, Campbell Scientific). Soil moisture content was initially measured gravimetrically from soil cores taken every 1-3 weeks in winter and 2-6 weeks in summer from 2000 – 2003 (J. Grünzweig) and continuously (30 min averaging period) since October 2003 using three time domain reflectometry sensors (N. Raz-Yaseef, pers. comm.). Soil water content (SWC) data is for the 0-30cm depth, in which the majority of fine root biomass has been observed to occur (J. Grünzweig, N. Raz-Yaseef, pers. comm.).

As a more relevant parameter for plant available water, root Relative Extractable Water (REW) was calculated from the measurements of volumetric SWC from (Granier *et al* 1999):

$$REW = (W - W_{\min}) / (W_f - W_{\min}) \quad (2.1)$$

where W is volumetric SWC, W_{\min} is the lower limit of available water (8%) and W_f is SWC at field capacity (35%).

2.2.1 Ecosystem and flux carbon budget parameters

Parameters calculated from the flux measurements are presented from the atmospheric point of view, so a negative CO₂ flux, F_C , indicates CO₂ uptake from the atmosphere. The measurement of F_C at any point in time (i.e. 30 min period) is a measure of the Net Ecosystem Exchange (NEE) of carbon, or the net effect both canopy photosynthetic CO₂ uptake (Gross Primary Productivity, GPP) and ecosystem CO₂ respiratory release (R_e). NEE integrated over any period of time determines Net Ecosystem Productivity (NEP), giving:

$$NEE = NEP = GPP - R_e \quad (2.2)$$

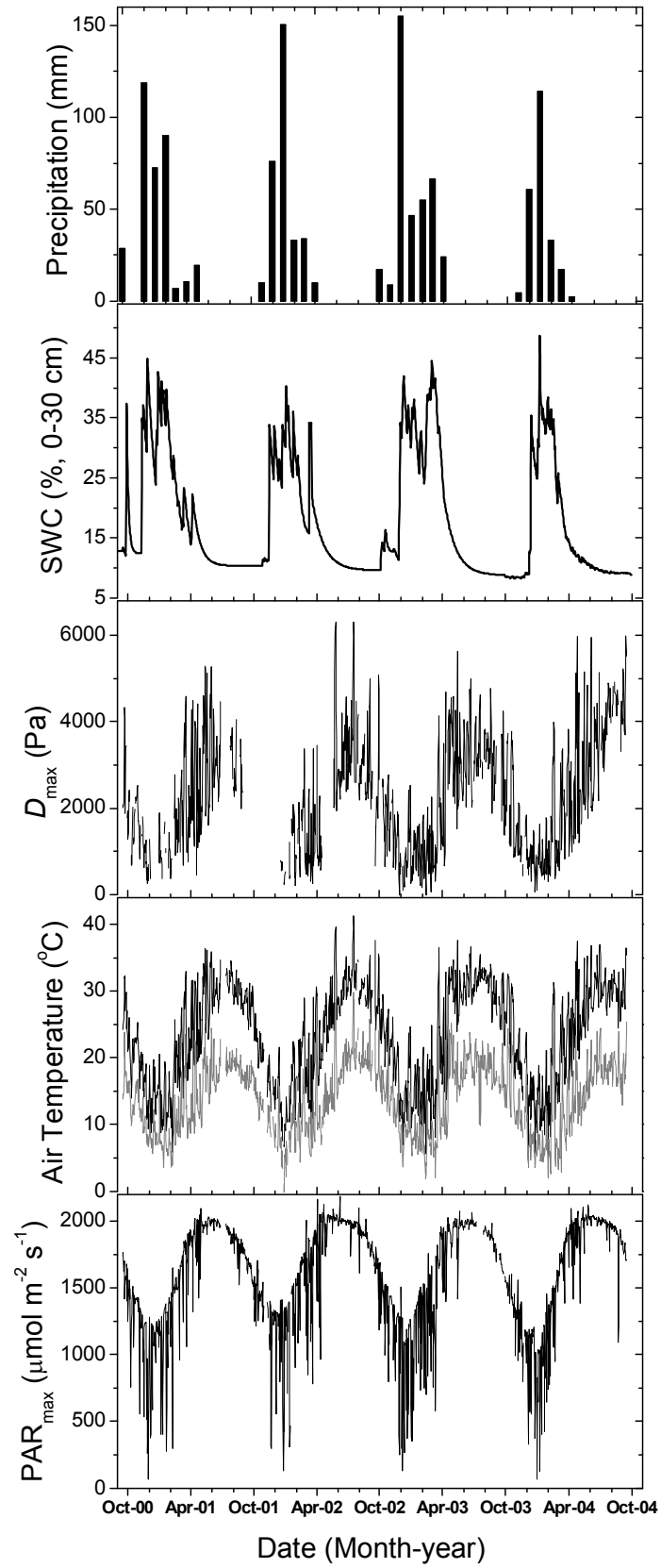


Figure 2.1 Measurements of monthly precipitation, daily soil water content (SWC), daily maximum vapour pressure deficit (D_{\max}), average air temperature (black line – daytime; grey line – night time) and daily maximal PAR measured at the Yatir tower site over 4 years.

R_e is composed of both autotrophic (R_a , i.e. plant derived) and heterotrophic (R_h , i.e. soil microbial) respiration.

2.3 Gas exchange measurements

2.3.1 Needle photosynthetic gas exchange

Needle daytime gas exchange measurements were made in the field using a portable, open-flow infra-red gas analyzer photosynthesis system (LI-6400, Li-Cor Inc.). Light saturated photosynthesis (A_{sat}) and transpiration (E) was measured on 6 fascicles (12 needles) from 8 – 12 trees on branches from the lower portion of the canopy exposed to full sunlight. Measurements were made under saturating light conditions ($PAR > 1000 \mu\text{mol m}^{-2} \text{s}^{-1}$) at ambient temperatures and vapour pressure deficit (D), using the standard clear-top leaf chamber, during the period of maximal activity (generally between 9:00 and 11:00 in winter and 08:00 and 10:00 in summer). When cloud prevented measurements being made under saturating or stable PAR, the 6400-02B LED light source was used to provide a constant, saturating light intensity of $1200 \mu\text{mol m}^{-2} \text{s}^{-1}$. Stable inlet CO_2 concentrations were provided by using the 6400-01 CO_2 Mixer and were adjusted accordingly to account for seasonal changes in atmospheric CO_2 concentration and photosynthetic activity to maintain ambient concentrations in the sample cuvette. The LI-6400 IRGAs were periodically calibrated using a calibration gas of known CO_2 concentration (Gordon Gas and Chemicals) and an LI-610 Dew Point Generator (LI-COR Inc.).

Measurements were made on average once every 2 – 4 weeks between March 2002 and April 2005 to record seasonal scale changes in photosynthetic gas exchange. In addition to the seasonal scale measurements (i.e. once in the day), occasionally repeat measurements were made of A_{sat} and E through the diurnal cycle (i.e. photoperiod). On most occasions, the two most recent age classes of needles were measured (*P. halepensis* generally retains 2 - 4 age cohorts of needles on the tree). Needles that developed in the current year are termed y0 needles and the age class from the previous year are y1 needles. The change in age class labelling occurred from the point of the first measurement of the newly developing needles (in about May or June of each year). Therefore needles that grew in the 2002 season for example are considered y0 until May 2003, when the first measurements of the 2003 needles were made. Photosynthesis, transpiration and stomatal conductance (g_s) were calculated online by the LI-6400 software according to the equations of von Caemmerer and Farquhar

(1981), and expressed on a projected needle surface area basis. Needle temperature was determined by energy balance.

2.3.2 Foliage respiration

Foliage (needle) respiration (R_F) rates were determined using the 6400-05 Conifer Chamber with the LI-6400. Measurements were made less frequently than for photosynthesis, but R_F was measured on 36 occasions between March 2002 and October 2004. Measurements were started at least 30 min after sundown in the dark. An entire age class cohort of needles was enclosed in the cuvette in order to improve the signal strength under the generally low rates of respiration. The needles were collected after the measurement for leaf area determination using a LI-3000A Leaf Area Meter (LI-COR Inc.). For the periodic determination of R_F response to temperature, the temperature in the cuvette was increased or decreased in 3°C increments from ambient within the limits of the system, covering a temperature range of between 7 – 19°C.

2.3.3 Stem respiration

Stem (trunk) respiration (R_T) was measured using a purpose built chamber connected to the LI-6400 analyzer head using a specific manifold (provided by LI-COR) that connected to the lower half of the sensor head in place of the standard leaf chamber. To isolate the upper half of the flow line, a strip of CO₂-impermeable plastic was placed over the holes between the upper and lower halves of the sensor head and held in place by firmly closing the head. The chamber consisted of a piece of Perspex tubing 10 cm in diameter and 5cm in length, open at one end and sealed at the other with a flat piece of Perspex, into which the manifold was inserted. The open end was inserted into PVC collars permanently attached to 12 trees at the field site at a height of about 1.5 m. The PVC collars were cut from tubing to ~ 5cm in length, cut away on one side to accommodate the curvature of the stem and sealed to the stem using silicon sealant. The sealant required periodic (~yearly) replacement due to weathering. A foam ring (as used with the LI-COR LI-6900 soil chamber) fitted into a groove on the outside of the chamber near the open-end pressed against the PVC collar, creating an airtight chamber with the tree stem as the base. The system was maintained in a stable position using a tripod to support the chamber head and an adjustable strap that wrapped around the tree stem and chamber. Stem temperature was determined using a type E thermocouple connected to the LI-6400 leaf thermocouple port and inserted ~5mm into the

stem. Stem respiration was measured in the afternoon (generally between 14:00 and 16:00) on 46 occasions between March 2002 and April 2005. Rates were determined on an area basis after individual correction for stem curvature within the enclosed chamber space. Additional measurements were made on some days that involved repeat measurements over the diel (day-night period) cycle.

To determine the R_T response to temperature, three types of data sets were used. Firstly those from the diel campaigns in which R_T was measured at least three times in the diel period and that covered a temperature range of at least 7° C (Diel). Secondly, by combining data from measurements that were made close to each other in the same season, where the maximum time period covering the measurements was 69 days and the temperature range was a minimum of 8°C (Seas.). Thirdly, by combining data from different years but made at a similar time of the season (MultiSeas.), within a maximum time frame (in day of the year) of 50 days and minimum temperature range of 9°C.

Quantifying the temperature response of respiration

To quantify the temperature response of R_F and R_T , the data were fitted with a standard exponential function:

$$R = Ae^{bt_a} \quad (2.3)$$

where R is the respiration rate (either R_F or R_T), t_a is stem or leaf temperature and A and b are empirically determined, from which the Q_{10} value (proportional increase in R with 10°C temperature increase) is used as the sensitivity parameter, where:

$$Q_{10} = e^{10b} \quad (2.1)$$

2.4 Chlorophyll a fluorescence measurements

Chlorophyll a fluorescence measurements were made *in situ* on trees in the irrigation experiment, using a pulse modulated excitation and detection fluorometer (PAM-2000 Portable Fluorometer, Heinz Walz GmbH, Germany). Measurements were made on both y0 and y1 needles of three trees each from both the irrigated and control (non-irrigated) plots at intervals through the diurnal period at different times in the season. The Pulse Amplitude saturation pulse technique was used to determine the maximal quantum efficiency of electron transport of photosystem II (PSII) under dark adapted conditions, known as F_v/F_m , and the actual quantum efficiency of PSII under illumination (Φ_{PSII}). The saturation pulse technique

involves recording the stable baseline fluorescence signal (F_o or F , in the dark or light adapted state, respectively) using a very weak actinic light source, then applying a saturation pulse of white light to completely saturate the photosystems and reduce the electron transport chain, giving a measure of the maximal fluorescence signal (F_m or F_m' , in the dark and light respectively). From these parameters, the variable fluorescence (F_v), F_v/F_m and Φ_{PSII} are calculated, where (Genty *et al.* 1989)

$$F_v / F_m = \frac{(F_m - F_o)}{F_m} \quad (2.5)$$

and

$$\Phi_{PSII} = \frac{(F_m' - F)}{F_m'} = \frac{\Delta F}{F_m'} \quad (2.6)$$

Additional parameters were determined in the light, namely the efficiency of open PSII reaction centres (F_v'/F_m'), and the quenching of maximal fluorescence due to non-photochemical processes (NPQ). In order to determine F_v'/F_m' it is necessary to determine the baseline or minimal fluorescence yield (in the dark) of a pre-illuminated sample (F_o'). This was done by excluding the ambient light (i.e. briefly shading with a black cloth), followed by a brief application of far-red light (peak of about 735 nm) to excite photosystem I only, causing the reoxidation of photosystem II acceptors. The steady state F_o' is then recorded while the far-red light is on. F_v'/F_m' and NPQ are calculated from:

$$F_v' / F_m' = \frac{F_m' - F_o'}{F_m'} \quad (2.7)$$

$$NPQ = \frac{F_m - F_m'}{F_m'} \quad (2.8)$$

From measured fluorescence parameters it is possible to determine the fraction of absorbed light energy that is used in photochemistry (P), the fraction that is dissipated thermally by non-photochemical processes (TD) and any potential 'excess' (E), i.e. not going towards electron transport or being dissipated thermally, and therefore having the potential to cause oxidative damage. These parameters are determined as (Demmig-Adams *et al.* 1996):

$$P = F_v' / F_m' \cdot qP = \Phi_{PSII} \quad (2.9)$$

$$TD = 1 - F_v' / F_m' \quad (2.10)$$

$$E = (1 - qP) \cdot F_v' / F_m' \quad (2.11)$$

where $qP = (F_m' - F) / (F_m' - F_o')$. Note that the use of $1 - F_v' / F_m'$ for the measure of D , as opposed to $F_v / F_m - F_v' / F_m'$, accounts for the fact that the maximal level of F_v / F_m measured is always less than 1.0 (usually $\sim 0.8 - 0.9$ in healthy leaves). This difference is also due to some thermal loss of energy (unrelated to the dynamic processes that operate in the light) so is included in the TD parameter.

Measurements of chlorophyll fluorescence parameters were made over the diurnal period on different dates between December 2001 (low stress period) and October 2002 (end of the drought period). Measurements from May through to October 2002 were used for quantifying the allocation of absorbed light between the various pathways. Measurements were made on a group of needles aligned and held together to form a continuous flat surface, using instrument functions to provide the measuring, saturating and far-red light beams. The fluorescence signals were monitored graphically in real-time on a laptop computer using flurometer software in order to determine steady-state levels of F_o , F and F_o' . When F_m' was reduced to levels that were so low that it became difficult to separate from the signal noise of F (e.g. at midday under high light), the measuring beam frequency was increased from the standard setting of 600 Hz to 20 kHz just prior to applying the saturation pulse.

2.5 Phenology measurements

Needle and stem growth were monitored on the same trees that were used for the stem respiration measurements. Three branch ends on each tree were marked on each tree and the average needle length of the cohort of developing needles on each branch end was measured periodically through the growing season with a ruler (1 mm resolution). Metal dendrometer bands were fixed to the stem at breast height (~ 1.3 m) and stem circumference was measured at approximately monthly intervals through the study period using digital callipers (0.1 mm resolution).

2.6 Leaf analyses

Following measurements of assimilation and respiration, samples were collected for determination of chlorophyll and total carotenoids content, carbon and nitrogen concentration, and the stable carbon isotope ratios of $^{13}\text{C}/^{12}\text{C}$.

2.6.1 Pigment analysis

Pigment concentrations were determined following extraction in *N,N'*-dimethylformamide (DMF) according to Porra *et al.* (1989) and Wellburn (1994). Approximately 10mm sections from the middle of 5 fascicles per sample were immersed in 4 ml DMF and kept in the dark at 5°C for 5 days. Samples were then centrifuged and the supernatant absorbance measured (Carey 100 Bio UV-visible spectrophotometer) between 720 and 380 nm at 0.1nm resolution. Concentration of Chl *a*, Chl *b* and total carotenoids were calculated according to the equations of Porra *et al.* (1989) and Wellburn (1994). Samples were expressed on a dry weight (DW) basis following the drying of the extraction tissue at 60°C for 24 hrs and converted to an area basis using specific leaf area (SLA, cm² g⁻¹DW of leaf) values determined from remaining needles in the sample.

2.6.2 Carbon and Nitrogen content and $\delta^{13}\text{C}$ composition

Needle carbon and nitrogen concentration and ^{13}C composition were determined on needles that were oven dried (60°C for 48hrs) and mechanically ground (40 mesh). Two replicates from each sample of ~0.3 – 0.4 mg were weighed into 3x5mm tin foil capsules (Elemental Microanalysis Ltd., UK) and combusted in a elemental analyzer (Carlo Erba 1108; Carlo Erba, Italy, precision $\pm 0.5\%$) connected on-line to a continuous-flow isotope ratio mass spectrometer (Optima; Micromass Ltd., UK). Stable isotope ratios are expressed in the conventional delta (δ) notation in ‰ relative to an international standard:

$$\delta = \left(\frac{R_{sam}}{R_{std}} - 1 \right) \cdot 1000 \quad (2.12)$$

where δ is $\delta^{13}\text{C}$, and R_{sam} and R_{std} are the isotope ratios ($^{13}\text{C}/^{12}\text{C}$) of the sample and standard, respectively. The standard for $\delta^{13}\text{C}$ measurements is Vienna Pee Dee Belemnite (VPDB). Calibration of each batch run was done by measuring four samples of the Acetanilide (Elemental Microanalysis Ltd.) international standard at the start of each run and two samples of a cellulose laboratory working standard for every 12 sample capsules, making a correction for a blank cup. Precision is $\pm 0.1\text{‰}$.

2.7 $\delta^{13}\text{C}$ composition of respired CO_2

To enable the collection of foliage respired CO_2 from a reasonable number of samples over a relatively short period in the field, a novel chamber incubation method was developed. Collections were made after dark from entire detached cohorts from 5-6 branches per tree. Collections were made over three seasons, in the first season no separation was made between new (y0) and (y1) needles, in the following two seasons, collections were made from y0 and y1 age classes separately. Six samples (trees) were usually sampled at each collection date.

2.7.1 Foliage incubation chambers

Chambers were constructed from Perspex tubes of 63 mm i.d. and 150 mm in length (volume 470 cm^3). Each end was threaded and fitted with Perspex caps. A 2 mm O-ring embedded in the wall at each end of the chamber provided for an air-tight fitting when the caps were screwed on. A Swagelock[®] fitting was imbedded into the center of each cap and was connected by a short piece of Bev-a-line[®] tubing to a push fit valve. The valves were connected to a 3-way tap within a manifold system that connected a series of four chambers in line. The manifold system was connected up stream to a CO_2 scrub tube (sodalime) and pump. Downstream, the manifold outlet was connected to a drying tube containing DRIERITE[®] (WA Hammond Drierite Co., OH, USA) and a 200 mL glass flask. The flask system was the same as that which was developed for the NETCARB European-wide air sampling project (Hemming *et al.* 2005). It consists of a two-layer inlet tube attachment to a single stopcock that enables airflow into the flask through a small inner tube and out via the external air space and a T-junction in the attachment. The outlet of the flask was connected to an IRGA (LI-6262; LI-COR Inc.). All gas lines were Bev-a-line[®] tubing (Fig. 2.2).

2.7.2 Sampling routine

Samples were placed into the chambers and sealed. The manifold tap configuration was set to flow through the first chamber only. Air was pumped at $\sim 1 \text{ L min}^{-1}$ through the soda lime scrub and chamber to purge the chamber of ambient background air with CO_2 -free air, monitoring the CO_2 concentration on the IRGA until a low steady-state CO_2 concentration is reached (~ 3 -5 mins). This represents the foliage respired CO_2 release only. The chamber was then isolated (commencing incubation) and the flow directed through the adjacent chamber.

This was repeated until all chambers were flushed and isolated. The chambers were left to incubate for between 15-30 min depending on season. During incubation, or for a sufficient period prior to collection, CO₂ free air was pumped through the entire system (excluding the isolated chambers) to maintain all lines and the flask free of ambient CO₂. With the [CO₂] on the IRGA steady at zero, the 3-way taps of the first chamber were switched to flush the first chamber with the CO₂-free air stream, hence carrying the CO₂ that had built up within the chamber to the flask, while monitoring the [CO₂] on the IRGA. When the [CO₂] reached a peak the pump was turned off and the flask immediately isolated. Therefore the flask now contained a high concentration of CO₂ (in the order of 300 – 800ppm or more, depending on collection conditions) that is purely of respiratory origin. Flasks were transported to the laboratory for analysis of the CO₂ directly from the flasks. A schematic of the collection system is shown in Fig. 2.2.

2.7.3 System tests

Leak tests

Empty chambers were filled with CO₂ free air and left for periods of time of up to 20 hours (in room air with a [CO₂] of about 500 ppm) before flushing as per the collection routine and measuring the CO₂ concentration. It was determined from these tests that the leak rate into the chambers was ~2.1 ppm hr⁻¹ (SD = 0.4 ppm, n = 5). Therefore leaks into the system over a 30 min incubation period are in the order of less than 0.5% of the sample CO₂.

Fractionation tests

The system was checked to ensure that the transfer of the gas air from the chamber to the flask did not induce some kinetic fractionation effects. This was done by filling the chambers with air with of a known $\delta^{13}\text{C}$ composition at various concentrations and transferring them to the flasks as per the collection routine. This was compared with the values derived from directly filling flasks from the cylinder and from filling the flask by flushing through the entire chamber system. Two gasses of differing $\delta^{13}\text{C}$ composition were used (28.4 ‰ and 49.3 ‰). The difference between all the samples of each the two gasses was 0.1‰ or less.

Collection into CO₂ free air

To determine whether temporarily reducing the partial pressure of CO₂ around the respiring tissue to zero has an effect on the isotopic composition of the subsequent respiratory CO₂

release, a comparison was done of the chamber method with values determined by the two end member mixture model often referred to as the Keeling plot method (Pataki *et al.* 2003). By plotting the $\delta^{13}\text{C}$ value of the mixture against the inverse of the CO_2 concentration, the $\delta^{13}\text{C}$ of the component being added can be determined from the intercept of the linear regression between the $\delta^{13}\text{C}$ and inverse of the $[\text{CO}_2]$.

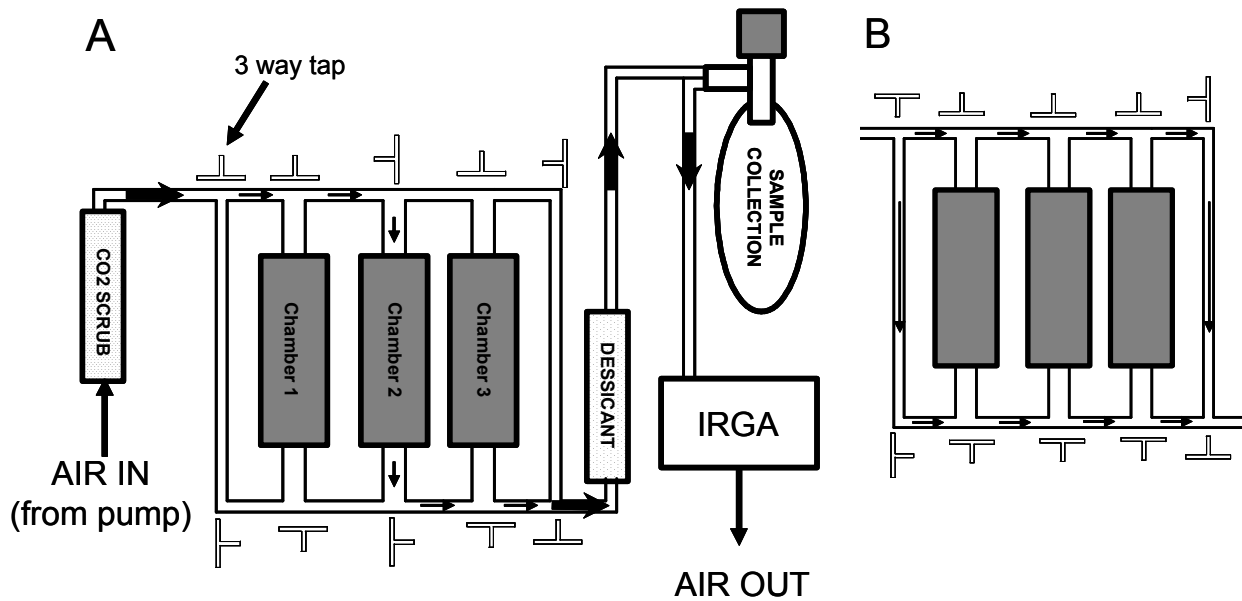


Figure 2.2 Schematic of the incubation system used for collecting CO_2 respired from needles, as described in the text. **A:** the complete system, with the 3-way taps configured for flushing chamber 2 (pre-incubation or during collection). **B:** the 3-way tap configuration for flushing all lines with CO_2 -free air prior to collection.

Respiratory CO_2 $\delta^{13}\text{C}$ composition in the chambers was determined using Keeling plots in two ways. Firstly, the system was used in an open flow manner in which air at a constant (ambient) $[\text{CO}_2]$ and $\delta^{13}\text{C}$ was supplied to the chamber. By altering the flow rate of the air through the chamber, a range of mixing ratios due to modification of the air by the respired CO_2 could be obtained. From this a Keeling plot could be used to determine the isotopic composition of the respired CO_2 . This was followed by 1-3 collections into CO_2 free air using the standard procedure. This method was used on a number of samples including: (1) a sample of detached cohorts as in the field collections; (2) a potted seedling sample, with the end of one chamber modified to enclose the stem of the seedling. This was to enable repeat measurements on a sample without introducing artefacts associated with using detached

samples over the prolonged period required for the Keeling plots collections; (3) an uncut branch sample in the field in the same manner as for (2).

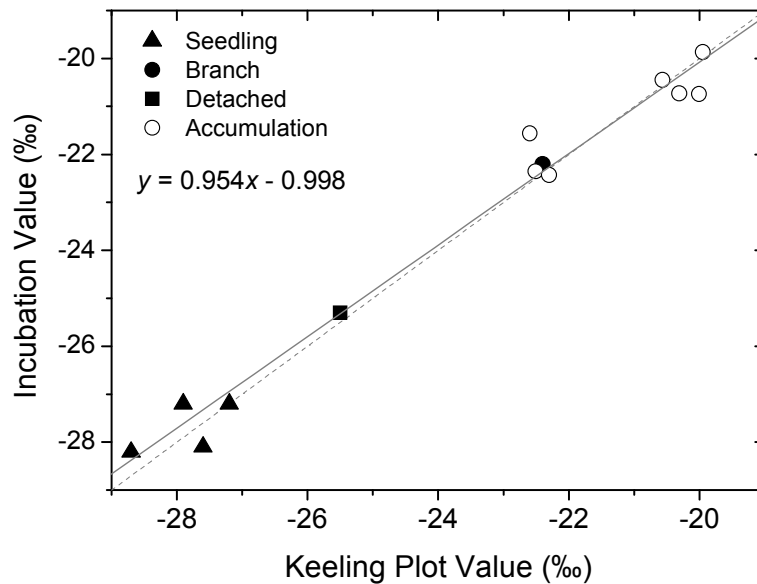


Figure 2.3 The results from the comparison of $\delta^{13}\text{C}$ of respired CO_2 from determined by the Keeling plot approach with the incubation chambers. Solid symbols are using the variable flow rate method and the open symbols the accumulation method (see text for details). The solid grey line is the regression for all samples together ($R = 0.989$), and the dashed line the 1:1 relationship.

The second method involved flushing the chamber and sample with a background gas of constant $[\text{CO}_2]$ and ^{13}C composition. The chamber was then isolated and the $[\text{CO}_2]$ allowed to accumulate due to respiratory release before being collected in the usual manner. The process is the same as ‘branch-bag’ techniques that have been employed in the field. By varying the incubation time, a range of mixing ratios of respired CO_2 in a constant background was obtained that were then used to determine the ^{13}C composition of the respired CO_2 by Keeling Plot. This was followed by collections into CO_2 -free air in the usual manner.

The results of these tests are shown in Fig. 2.3. The average difference between the Keeling plot values and the incubation values from the variable flow rate method was -0.18 ‰ ($\text{SD} = 0.42$, $n = 6$) and for the accumulation method was 0.02 ‰ ($\text{SD} = 0.56$, $n = 7$). Therefore, the accuracy of this method is considered to be $\pm \sim 0.2 \text{ ‰}$, which is well within the range of natural variability and the magnitude of change observed over time, and compensated for by the ability to take a number of replicates simultaneously in the space of less than 60 min.

2.7.4 Collection of stem respired CO₂

Due to the porous nature of wood and the inability to isolate sections of the tree stem, a different approach was used for collecting respiratory CO₂ from the stem for $\delta^{13}\text{C}$ analysis. The system used for the stem respiration rate measurements was used with modification, namely the flask unit described above was connected to the outlet tube (match-tube) of the respiration chamber, forming an open flow system with the LI-6400 providing the pump and IRGA. Ambient air (i.e. not using the CO₂ mixer) was flushed through the system for ~5mins to get a stable reading in the cuvette, taking care not to breath close to the setup when attending to the system. The pump was then turned off and the [CO₂] left to build up in the chamber due to the stem efflux. In this period there is the potential for ambient air to leak into or cuvette air to leak out of the system through the open-ended gas lines, but the rate of this diffusion is very slow relative to the build up in the chamber. The introduction of ambient air into the system does not introduce an error to the measurements as it is of the same composition as the initial background. After leaving the chamber for a period of time the pump was turned on, pumping the mix of ambient and respiratory CO₂ to the flask, which was then isolated when the concentration in the cuvette dropped to about half the pre-flushing concentration. By leaving the chamber for different lengths of time, a range of CO₂ concentrations representing different levels of respiratory CO₂ in a constant background were obtained. The total range of [CO₂] was usually ~100 ppm, from 5 flasks per tree. From these samples the $\delta^{13}\text{C}$ of the respired CO₂ was determined using the Keeling plot method. As this method was more time consuming and flask intensive, usually only 2-3 trees were sampled each time.

2.7.5 Air sample analysis

The $\delta^{13}\text{C}$ of the CO₂ in the flasks was measured as described in Hemming *et al.* (2005). An aliquot of 1.5 mL was removed from each flask into a sampling loop and the CO₂ cryogenically trapped using helium as a carrier gas. It was then passed through a Carbosieve G packed column at 70°C to remove N₂O and analyzed on a Europa 20-20 continuous-flow isotope ratio mass spectrometer (Crewe, UK). Batches of 15 flasks were measured at time from a manifold system, with five flasks of a standard gas being measured for every 10

samples. Precision of the measurements was $\pm 0.1\%$, and are presented relative to the VPDB standard.

The $[\text{CO}_2]$ of the flasks was determined by removing an additional 40 mL from the flask into a mechanical bellows and then passing the sub-sample through an IRGA (LI-6262, LI-COR Inc.). Two sub-samples were taken in series from each flask, with the value from the second sample being used, to reduce memory effects between samples. Precision was $\pm 0.1\text{ppm}$.

2.8 Manipulation experiments

2.8.1 Defoliation Experiment

During the growth season of 2003, a defoliation experiment was conducted to investigate the influence of local foliage on the development of the new needles. Soon after needle flushing, three 2nd order branches (containing multiple branch ends) on each of six trees were defoliated. This involved either removing all the older age class needles and leaving only the developing (y_0) needles (Defoliation), or removing all new y_0 buds and leaving the older needles (Debudding). Three more branches were labelled as controls. Leaf growth was measured on the control and Defoliation y_0 needles through the season, and gas exchange (A_{sat} and E) measurements were made on the all treatments once in the summer (late June) and again during the peak of the activity in the following spring (March).

2.8.2 Irrigation Experiment

As part of the research into drought responses in the ecosystem, an experiment led by Dr. Tongbao Lin was conducted over a dry-wet-dry period from May 2001–October 2002 that involved providing supplementary summer-time irrigation. Drip-irrigation was provided daily around the base of ~ 10 trees together in one plot to maintain soil water content at close to field capacity through the rain-free periods. Irrigation was reduced once natural precipitation resumed in autumn and suspended over winter once non-irrigated soil had reached field capacity. Measurements of chlorophyll a fluorescence were made on both the irrigated and control (adjacent non-irrigated) trees at this site by me, additional measurements (phenology and gas exchange) were made by Dr. Lin and are introduced here where relevant for the interpretation.

2.9 Statistical analysis

Statistical analysis and linear and linear non-linear curve fitting were done using the built-in statistical and curve fitting functions of Origin 7.5 graphing and analysis software (OriginLab Corp., MA, USA). Errors shown in all figures are \pm standard errors of the mean, or \pm standard deviation where $n < 3$.

Chapter 3

The control of carbon gain and water loss in *P. halepensis* in an arid Mediterranean climate

3.1 Introduction

Pinus halepensis is a tree species favoured for forestry use in dry regions of the Mediterranean due to its ability to withstand long periods of water shortage and therefore its water relations have been of interest to physiologists for sometime (see Schiller 2000). Early studies identified morphological features, such as sunken stomata and a thick waxy cuticle (Oppenheimer 1947), physiological responses such stomatal closure to reduce transpiration rates under adverse conditions (Oppenheimer 1947, Oppenheimer and Shomer-Ilan 1963, Whiteman and Koller 1964) and the ability to endure low soil water contents (Waisel 1958) as being important aspects of drought tolerance in *P. halepensis*.

Field measurements of daily and seasonal patterns of transpiration have found that transpiration rates may be inversely related to evaporation intensity and that the ratio of transpiration to potential transpiration declines as soil water content and leaf water potentials decline (Gindel 1964, Schiller and Cohen 1995, 1998). These studies suggested that stomatal regulation of water loss is a crucial feature for maintaining water balance in *P. halepensis*. Although there are intra-species (provenance or ecotype) differences in relative drought tolerance (Tognetti *et al.* 1997, Atzmon *et al.* 2004), it is clear that *P. halepensis* is a highly drought tolerant species, apparently employing a drought avoidance strategy that minimizes water loss during periods of low supply and high evaporative demand.

In addition to physiological and morphological adaptations, the ability to endure long dry summers is also facilitated to some extent by the nature of the substrate that *P. halepensis* is commonly found on. Following observations of Aleppo pine and other species (Oppenheimer 1933, see Oppenheimer 1956) growing on and with root penetration into bare rock, the water retention capacity of calcareous rocks (in addition to the soil) was discussed by Oppenheimer (1951). The soft and porous nature of chalk, limestone and marls lend them to being both vulnerable to dissolution by acidic root secretions and to being able to store quantities of water that could then become available for use by the plants over the summer. Differences in productivity between Aleppo stands in similar climates in Israel have been

attributed to the degree of water holding capacity of the soil-bedrock matrix, with highest growth seen on marl and chalk substrates (Schiller 1982).

While a few studies have addressed carbon relations of *P. halepensis*, most work has been done on seedlings in controlled environments or open top chambers, with little or no information available from field measurements under natural conditions. The more recent physiological investigations into drought responses in *P. halepensis* have been as part of interactive studies investigating the effects of drought and ozone on *P. halepensis*. These have been prompted by observations of declines in tree vitality in the Mediterranean region in recent years, along with indications of needle chlorosis characteristic of oxidation (Vivelissariou *et al.* 1992).

Photosynthesis was found to decline, and stomata close, in response to increasing vapour pressure deficit (D) and decreasing water potential (Whiteman and Koller 1964, Melzack *et al.* 1985). CO_2 assimilation rates, carboxylation efficiencies, growth rates and biomass accumulation are all lower in droughted seedlings (Wellburn *et al.* 1996, Manes *et al.* 2001, Le Thiec and Manninen 2003, Inclán *et al.* 2005). Reductions in photosynthetic activity may also be related to biochemical adjustments in photosynthetic components. Reductions in the levels of soluble protein (Wellburn *et al.* 1996) and Rubisco polypeptides (Pelloux *et al.* 2001), Rubisco activity (Gerant *et al.* 1996) and chlorophyll contents (Alonso *et al.* 2001, Manes *et al.* 2001) have also been reported under drought. However, others found an absence of drought effects on Rubisco activity (Pelloux *et al.* 2001, Inclán *et al.* 2005), chlorophyll levels (Le Thiec and Manninen 2003) and phosphoenolpyruvate carboxylase activity (Fontaine *et al.* 2003, Inclán *et al.* 2005), indicating the difficulty of extrapolating short term studies of differing duration and intensity on potted seedlings to the natural environment.

Despite the economic and ecological importance of *P. halepensis* in the Mediterranean region, there is paucity of data on leaf scale carbon and water relations measured *in situ* on mature trees. It is clear that water conservation is an important feature for survival, but there has been little attempt to quantify water loss and carbon gain responses to environmental drivers under ecologically relevant climate conditions (Schiller and Cohen 1995, 1998). An improved mechanistic knowledge of the carbon-water relations is essential for understanding the basis of both current levels of forest productivity (Grünzweig *et al.* 2003) and how *P. halepensis*, and dryland species in general, may respond to climate fluctuations or long term changes in climate conditions (Rathgeber *et al.* 2000).

The carbon-water relations become even more pertinent when considering the requirements for growth of new tissue. The needle phenology of *P. halepensis* is characteristic

of conifers, displaying spring flushing and summer growth (Weinstein 1989a, 1989b), resulting in a growth pattern that overlaps the stressful drought period. The production of new biomass requires carbon for both the construction of new tissue and to provide metabolic energy for the synthesis of the new tissue. Growth of new tissue also requires high cellular relative water content, continual supply of water to the low water potential regions of the dividing cells and the maintenance of cellular turgor in the new cells to exert pressure for cell wall extension (Bradford and Hsiao 1982, Boyer 1988). Reductions in cell turgor can also affect normal metabolism as metabolic processes may be more sensitive to turgor and volume than absolute tissue water potential due to the need to maintain distances between cellular components (Jones and Corlett 1992). Therefore it is commonly observed that drought has a negative impact on growth through both the reduction in water and carbon availability (Boyer 1988, Gholz *et al.* 1990, Larcher 2003).

To gain a better understanding of the successful drought tolerant strategies of *P. halepensis*, measurements of leaf level carbon and water relations were made on trees growing in the field under the extensive drought conditions in Yatir Forest. Measurements were made over diurnal, seasonal and annual time frames and are combined with analysis of ecosystem level carbon fluxes with the aim of quantifying responses to both soil and atmospheric water deficit and the controls on the relationship between water loss and carbon gain. Measurements were also made on the trees in the defoliation experiment to investigate the influence that leaf phenology may have on the carbon and water relations. The results are discussed in terms of ecosystem productivity and the strategies that are relevant for the success of *P. halepensis*, and ecosystem function under water limiting conditions in general, are identified.

3.2 Results and Discussion

3.2.1 Seasonal and diurnal gas exchange responses and ecosystem productivity

The seasonal trends in CO₂ assimilation rate (A_{sat}), transpiration rate (E) and stomatal conductance (g_s) measured under light saturated conditions during mid-morning (peak activity) are shown in Fig. 3.1. There was a large, qualitatively similar, inter-seasonal pattern in all parameters. Highest rates of CO₂ uptake and water loss occurred in the winter-spring period, generally between December and April, and were associated with high stomatal conductance. Low stomatal conductance reduced both A_{sat} and E in the summer months,

despite the increase in atmospheric water demand. Maximal A_{sat} rates reached $15 - 18 \mu\text{mol m}^{-2} \text{s}^{-1}$, and dropped close to zero at times during the drought period. The first gas exchange measurements of the new season's needles (y0) were made in late May or early June, when they were about 40 – 50% of their final length and already photosynthetically active. There was little difference in the gas exchange parameters between the y0 needles and the previous year (y1) needles from about July.

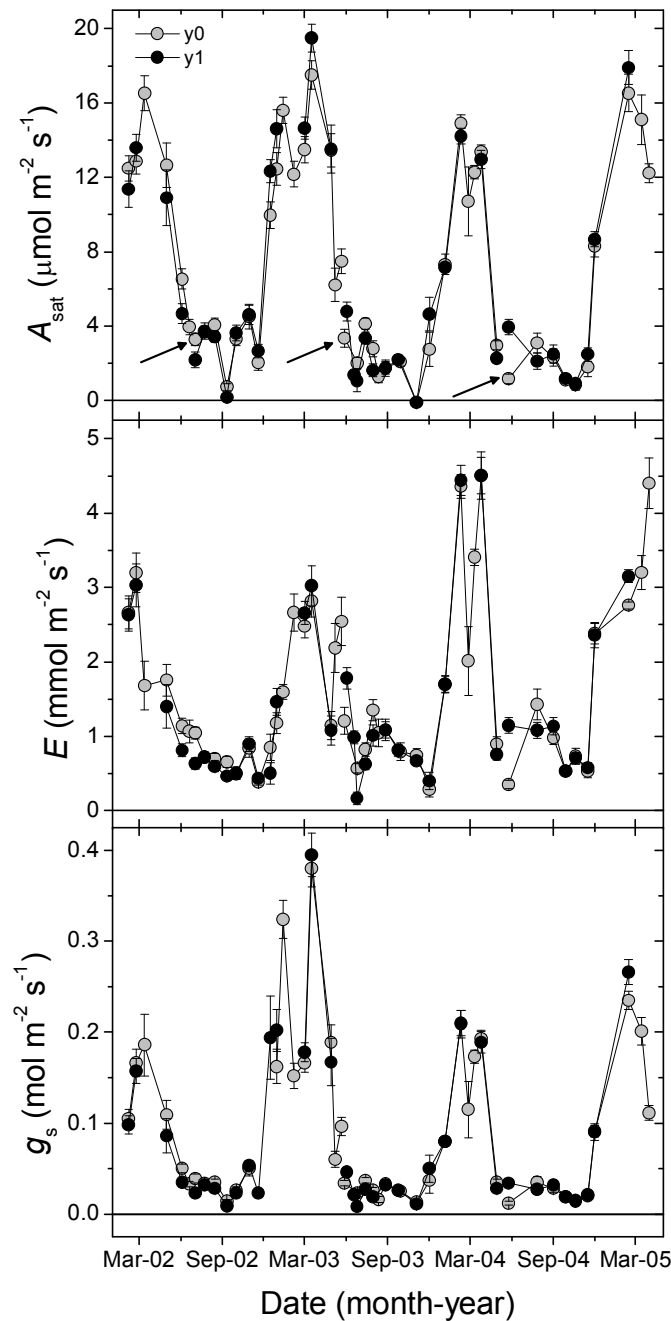


Figure 3.1. Rates of CO_2 assimilation (A_{sat}), transpiration (E) and stomatal conductance (g_s) measured under light saturated conditions during peak activity (mid-morning) over 3 years. Symbols represent the current years (y0) and previous years (y1) needles. Arrows indicate the first measurement of the y0 needles in that year, and the change of age class from y0 to y1 for the previous years' needles.

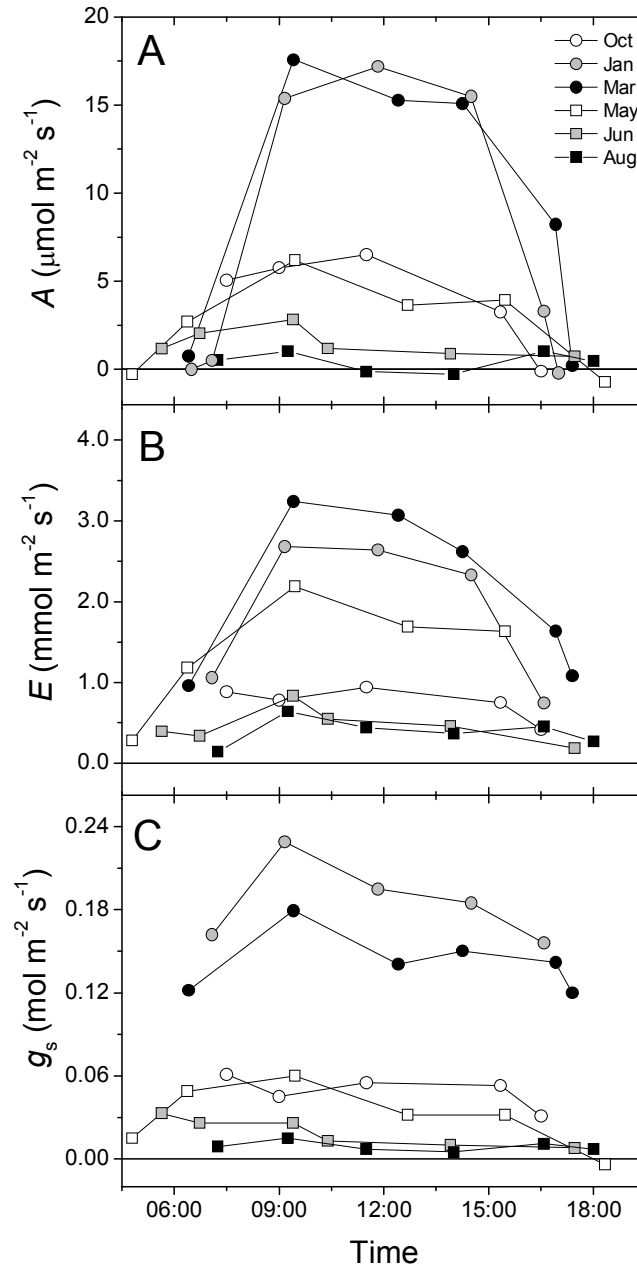


Figure 3.2. Diurnal patterns of assimilation, transpiration and stomatal conductance measured at different times of the year. Symbols are the same for each panel, as shown in the legend at the top.

Representative examples of diurnal patterns of instantaneous assimilation rate (A), E and g_s from different times in the year are shown in Fig. 3.2. In addition to the overall seasonal reduction in CO_2 assimilation, there were also qualitative changes in the daily gas exchange patterns. There were pronounced midday depressions of photosynthesis (relative to the absolute daily rates) in the warmer months (Fig. 3.2A). This is exemplified in the response observed during the peak of summer (August) when midday net CO_2 assimilation rates became negative (due to respiratory CO_2 efflux exceeding uptake). However, even though

assimilation was highly suppressed for much of the day in mid-summer, a certain degree of activity was evident through the entire drought period. Likewise, there were seasonal differences in the daily patterns of E (Fig. 3.2B). Transpiration rate were maximal around midday in winter or spring but either remained relatively stable or even decreased at midday in summer. The reductions in assimilation and transpiration were associated with reductions in g_s (Fig. 3.2C), indicating the important role that stomatal regulation has on gas exchange at the diurnal as well as the seasonal scale.

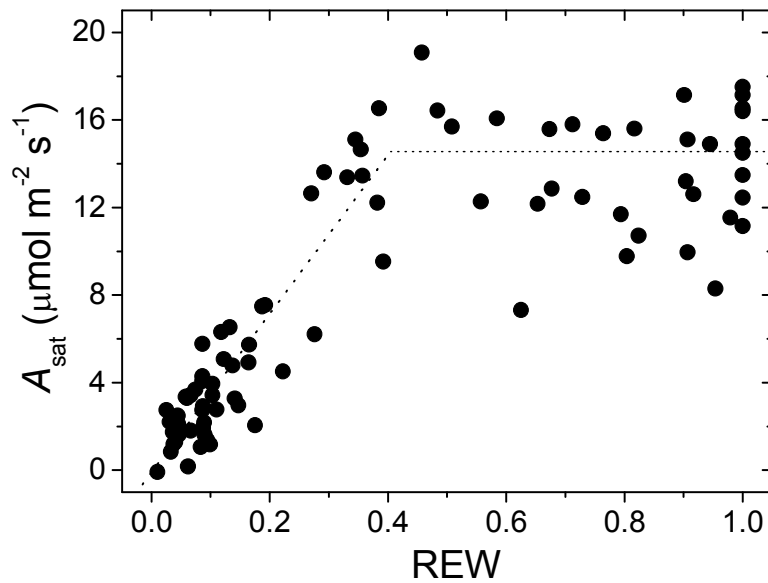


Figure 3.3. The relationship between light saturated assimilation rate and relative extractable water.

The relationship between A_{sat} and g_s is shown in Fig. 3.4, and includes both data from the seasonal and diurnal scale measurements. The relationship between g_s and A_{sat} is common for the different needle age classes, time scales and measurement year. A_{sat} shows an asymptotic exponential relationship with g_s , with relatively little increase in A_{sat} above a g_s of about $0.2 \text{ mol m}^{-2} \text{s}^{-1}$, and a sharp decrease in A_{sat} below this value.

Maximal photosynthetic rates occurred during the winter-spring period, when soil water content was high and temperatures were mild (see Fig. 2.1). Fig. 3.3 shows the relationship between A_{sat} and soil water content, expressed as relative extractable water (REW), from the seasonal changes (wetting up and drying down) of soil water over multiple years. Photosynthesis appeared limited by soil water content when REW was in the range between 0 and 0.4, but was relatively insensitive to higher values of REW.

Photosynthetic rates and seasonal variation

The rates of CO₂ assimilation from the field measurements in *P. halepensis* presented here are higher than those measured on seedlings of *P. halepensis* in the laboratory (Melzack *et al.* 1985) or in open top chambers (Inclán *et al.* 2005), but similar to those reported by Winner *et al.* (1989) and Le Thiec and Manninen (2003). The maximal A_{sat} rates of *P. halepensis* are similar to or higher than those of another drought tolerant species, *Pinus ponderosa*, measured on well watered seedlings (up to $\sim 12 \mu\text{mol m}^{-2} \text{s}^{-1}$; Zhang *et al.* 1997) or on mature trees in the field (up to $\sim 16 \mu\text{mol m}^{-2} \text{s}^{-1}$; Panek and Goldstein 2001). Overall, the maximal values of A_{sat} recorded for *P. halepensis* in this study are higher than most reported values for other species of *Pinus* (between ~ 5 - $25 \mu\text{mol m}^{-2} \text{s}^{-1}$; Rundel and Yoder 1998). Therefore it appears that part of the success of *P. halepensis* in dry environments lies in being able to achieve high rates of carbon gain when the environmental conditions are favourable.

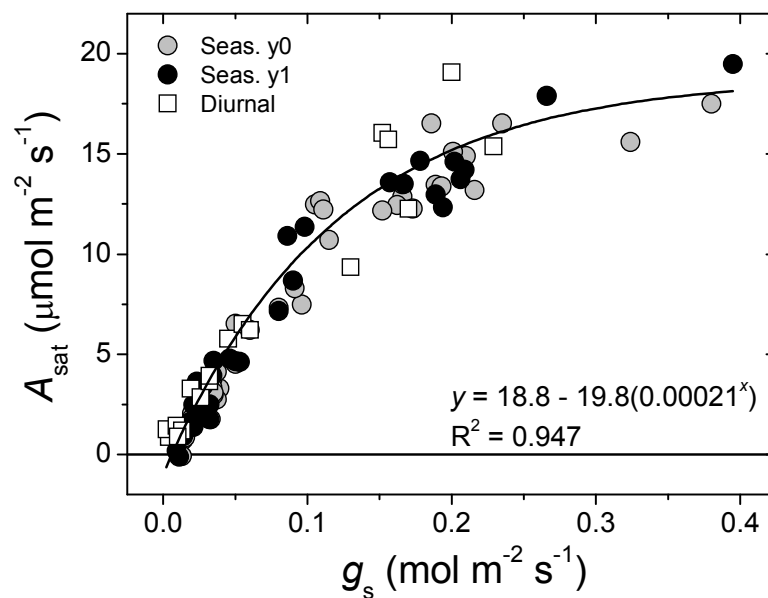


Figure 3.4. The relationship between light saturated assimilation rate and stomatal conductance across age classes and seasonal and diurnal time scales. The data is that from Figures 3.1 (Seas. y0 and y1) and 3.2 (Diurnal).

The period of maximal photosynthetic activity in this system, in late winter or early spring, differs from conifers in more temperate regions, where maximal rates of photosynthesis tend to occur over spring and summer (Teskey *et al.* 1994, Rundel and Yoder 1998), but is characteristic of other species in the Mediterranean. Depending on the extent of winter cold stress the and timing and duration of summer drought (Mitrakos 1980), maximal activity in Mediterranean regions may be in winter or spring, but minimal rates of activity are usually

observed in mid or late summer (Mooney *et al.* 1974, Harley *et al.* 1987, Flexas *et al.* 2001). These results indicate that photosynthetic activity in *P. halepensis* is not constrained by inherent features aimed to maximise photosynthetic rates over summer, or is decoupled from any phenological processes that might be associated with summer activity.

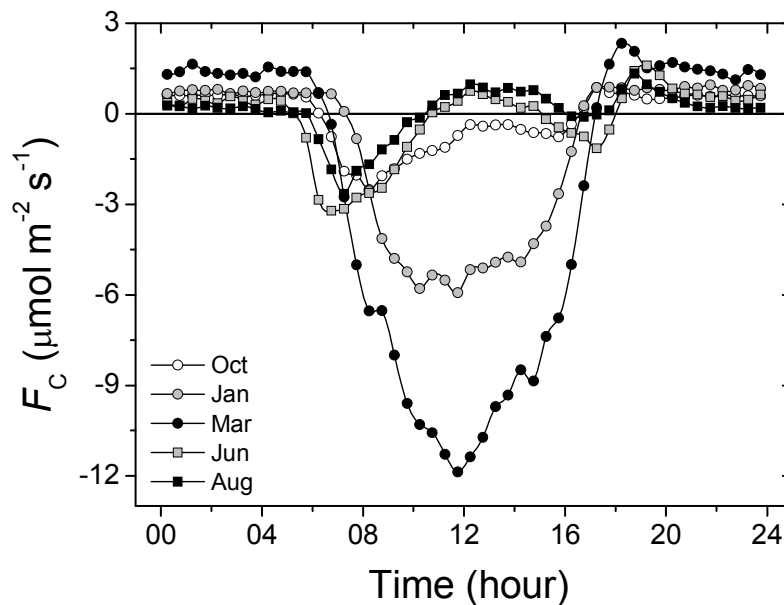


Figure 3.5. Diel patterns of ecosystem CO₂ fluxes (F_C) from different periods in the year. Each point is the average value across the month for that half-hour period.

Influence of soil water content on seasonal variation of photosynthesis

The shift in maximal photosynthetic activity to winter-spring was related to soil water content. Maximal photosynthetic rates were reached when REW was above 0.4, but A_{sat} declined with soil water content below this value. The dependence on soil water also resulted in the inter-annual variability in the timing of photosynthetic activity due to variations in precipitation. The 2003-04 season, for example, was dry relative to the previous years (total rainfall of 230 mm compared with 368 mm in 2002-03), and the rain both arrived later and ended earlier. In the wetter years photosynthetic activity started earlier, reached maximal rates faster and lasted for longer than in the dry year of 2003-04 (Fig. 3.1). For example, near maximal rates were measured in January in 2003 while in January 2004 the rates were still only about 50% of the maximum. While other factors also affect assimilation rates, it is clear that *P. halepensis* exhibits a high degree of plasticity in its physiological activity and responds accordingly to a highly seasonal environment. This represents an important aspect of its adaptation to the Mediterranean climate and underlies the indeterminate growth patterns that have been observed in *P. halepensis* (Lipshitz and Lev-Yadun 1986, Ferrio *et al.* 2003).

The role of stomatal conductance at diurnal and seasonal scales

The summertime diurnal patterns of gas exchange, with midday depressions of CO₂ fixation, are also characteristic of species of the Mediterranean (Harley *et al.* 1987, Epron *et al.* 1992, Faria *et al.* 1998) or other dry environments (Franco and Lüttge 2002, MacFarlane *et al.* 2004). The midday reduction in photosynthesis is generally attributed to stomatal regulation to limit water loss and is also considered an important feature of plant adaptation to Mediterranean and other seasonally dry environments (Harley *et al.* 1987, Damesin and Rambal 1995, Flexas *et al.* 2001, Ogaya and Peñuelas 2003). However, the sensitivity of the response (rapidity and extent of closure) may differ between and even within a species (Epron and Dryer 1993, Dang *et al.* 1997, Zhang *et al.* 1997, Fillela *et al.* 1998). The significant effect that stomatal regulation has on both seasonal and diurnal variation of CO₂ fixation rates (when PAR is above saturation) can be seen in the relationship between A_{sat} and g_s (Fig. 3.4). The changes in g_s at both diurnal and seasonal time scales and in different years explain 95% of the variation in light saturated photosynthetic rate. Therefore the control and sensitivity of stomatal regulation is a key aspect of plant performance in the water limited environment.

Relationship between leaf physiology and ecosystem productivity

The physiological information from the leaf scale gas exchange measurements can be compared with total ecosystem carbon relations determined from the flux tower measurements. Fig. 3.5 shows the average diel (24 hour period) pattern of total ecosystem carbon flux (F_C) from different times in the year. Each data point is the average for the 30 min period across all days in the month. Negative F_C values indicate CO₂ uptake, as per convention in flux data presentation. A comparison with the diurnal curves of leaf level assimilation (Fig. 3.2) shows that the behaviour is qualitatively very similar, for example with high rates of uptake in March, intermediate in January and lowest in August. Seasonal changes in the diurnal dynamics, such as the shift in the timing of peak activity from around midday in winter to early morning in summer is clearly seen. Furthermore, the afternoon recovery of assimilation during periods experiencing midday depressions is also evident.

Net ecosystem fluxes of CO₂ are the sum of the photosynthetic uptake and respiratory release so the extent to which the rates of photosynthetic activity are meaningful in terms of whole tree carbon balance depends also on the concurrent rates of respiration. Aspects of respiration are investigated in Chapter 6, but some important features relating to overall forest productivity are now apparent. The stomatal closure during midday hours in the hottest

months reduces assimilation rates to the point where total ecosystem respiratory CO₂ release exceeds photosynthetic gain and there is a net loss of carbon by the ecosystem. However, the dynamic nature of the stomata enables periods of photosynthetic activity in both the morning and afternoon as conditions permit. This contrasts with the situation that would occur if the stomata were not as responsive, and remained stable at the minimum conductance (for that time of year) through the day. The extent of the photosynthetic activity that is sustained translates into net ecosystem carbon gain for at least parts of the day throughout the whole year. This is an important point because the balance between carbon loss and carbon gain ultimately determines ecosystem productivity and survival. The sensitivity of stomata can therefore be seen as a critical feature of the success of this species under very dry conditions, and may indicate a feature that is of importance in general for understanding how ecosystem productivity may be affected under various climatic scenarios.

3.2.2 Control of stomatal conductance

Stomatal conductance is influenced by various factors, including soil water content and the vapour pressure difference between the leaf and the atmosphere (D_L), factors which are obviously important in the environment of this study. The balance between water availability and evaporative demand determines stomatal responses to a large extent due to the role of stomata in regulating plant water balance (Whitehead 1998). The dependence of g_s on REW and D_L is shown in Fig. 3.6. When REW was very low (less than about 0.18 in this data), g_s remained below 0.05 mol m⁻² s⁻¹ (Fig. 3.6A) but maintained a degree of sensitivity to D_L within the restricted range of g_s , decreasing as D_L increased (Fig. 3.6B). The range of REW from 0.18 to about 0.4 defined a region in which g_s showed an overall increase in response to increasing soil water content, but also increasing variability of g_s as REW increased (Fig. 3.6A). The increase in variability was associated with an increase in responsiveness to D_L (Fig. 3.6B). When REW was above about 0.4, g_s was again relatively insensitive to soil water content, but still quite variable, generally in the range of 0.1 to 0.3 mol m⁻² s⁻¹ (Fig. 3.6A). A large part of the variability in the high soil water content region was related to sensitivity to D_L (Fig. 3.6B), with an exponential decline in g_s with increasing D_L (Dang *et al.* 1997, Oren *et al.* 1999).

To investigate the effects of soil and atmospheric water deficits on carbon fluxes at the canopy level, a unique approach was taken that made use of particular climatic events that involve rapid changes in D over a short period. Known locally as *sharav* or *hamseen*, these

events tend to occur in the spring or autumn and are associated with hot, dry air masses from the Arabian or Saharan Deserts. Over a period of 2-3 days, D may increase by up to 4000 Pa, while soil water content remains constant. A typical example of ecosystem carbon flux (F_C) in response to D during one of these events is shown in Fig. 3.7. Each set of lines and symbols represents the mean half-hour value of F_C through the daylight hours of each day. There was a sharp decline in F_C with increasing D through the progression of the event due to stomatal closure. However, the day immediately following the peak in D (and F_C was minimal), D had returned to pre-event levels and F_C had fully recovered to the pre-event rates, with no negative impacts due to the intense stress period evident.

By comparing the response to these events at different periods, the effect of soil water content on the sensitivity of F_C to D can be determined. The slope of the line of a linear fit to the F_C - D relationship, as shown in Fig. 3.7, indicates the rate at which F_C decreases (as stomata close) as D increases. This slope was determined from a number of events that covered a range of soil water contents up to a REW of about 0.4, and is shown in relation to REW in Fig. 3.8. As soil water content decreased the sensitivity of canopy carbon gain to increasing D also decreased. The pre-event F_C rate also decreased with soil water content (data not shown) but in all cases the post-event F_C rates were the same as the pre-event rates, and therefore no evidence was seen of a soil water dependence on vulnerability to damage under the extreme events.

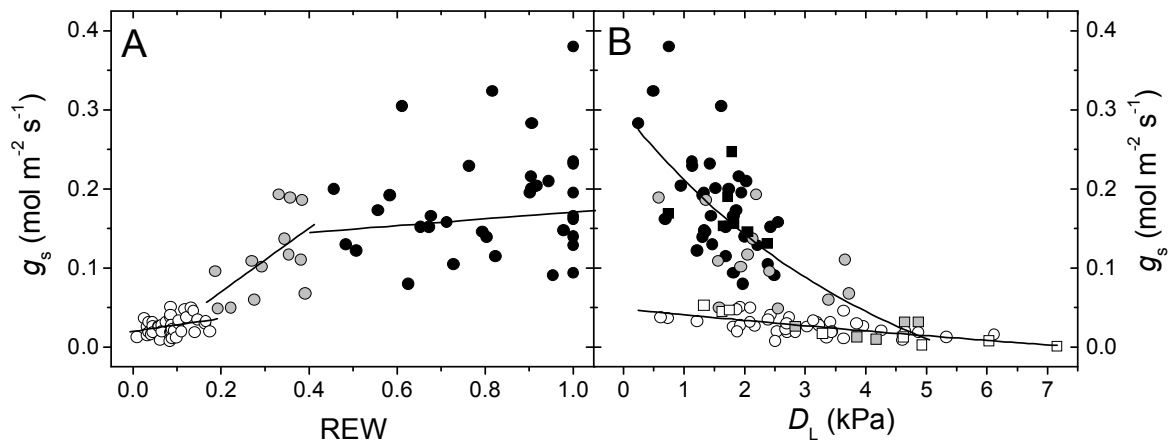


Figure 3.6. The relationship between g_s and REW (A) and D_L (B). Data in A is from the seasonal scale gas exchange measurements of the y1 needles (see Fig. 3.1). The circles in B are the same points as those in A, with the different shading representing the three soil water content divisions. The squares in B are from diurnal scale measurements (i.e. response to changing D during the day), also divided on the basis of soil water content. The fits in B are to the low soil water content (open symbols) and to the transition and high soil water contents (grey and black symbols combined).

To investigate the influence the drought-period needle phenology trait has on leaf level gas exchange, measurements were made on the branches in the defoliation experiment in June (see Chapter 2). In Fig. 3.9 the relationship between g_s and A_{sat} in the y0 needles in the defoliated branches (i.e. all previous years mature needles removed), in the y1 needles in the debudded branches (i.e. all the current years new needles removed at bud-burst) and in the y0 and y1 needles on the control branches (no removal) are shown. The range of g_s that were measured was due to the range of D_L that the measurements were made under (1.5 - 3 kPa). For the y1 needles, the data had a similar $A_{\text{sat}} - g_s$ relationship between control and debudded branches, but the debudded samples maintained higher g_s than the control samples, and therefore had higher A_{sat} rates under similar conditions (see inset). In the defoliated samples (mature needles removed, leaving only the growing needles), the A_{sat} rates in the y0 needles were also higher than the corresponding control needles on the same tree and under similar D_L (inset), but the range of g_s was similar between control and treatment samples.

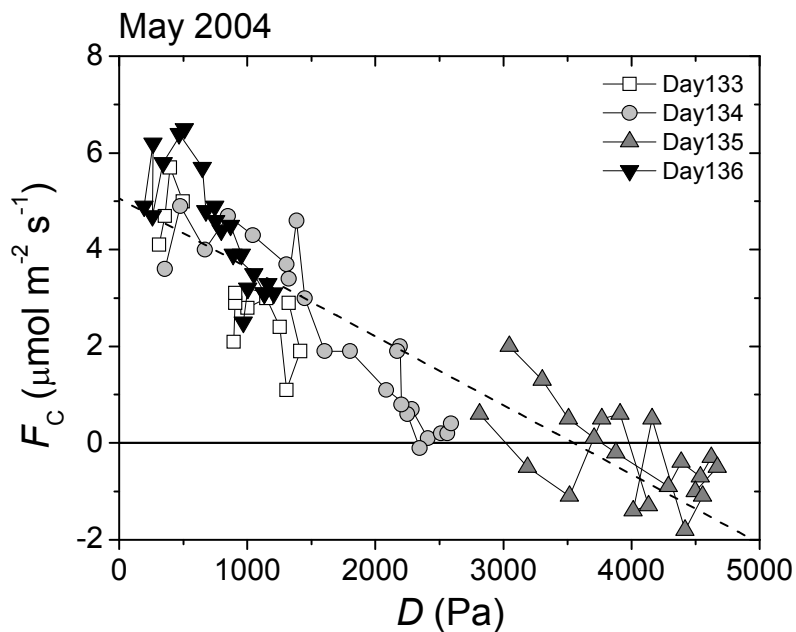


Figure 3.7. The response of net ecosystem CO_2 flux (F_C , expressed as positive NEE so positive values indicate carbon uptake) to D over a period of rapidly changing D in May, 2004. Each point represents a half hour F_C value, with the lines connecting the points through the course of F_C through the daylight hours of each day. The linear fit to determine the sensitivity of F_C to D is to all data combined.

The influence of soil and atmospheric water deficits on stomatal conductance

The reduction in g_s below a REW of about 0.4 indicates the point at which soil water deficits start to limit transpiration and photosynthesis (see Fig. 3.3), defined as REW_c (Granier *et al.* 1999). The value of 0.4 is similar to the value which has been observed in other forests

(Granier *et al.* 1999), but it is also clear in this system that vapour pressure deficit also has a strong influence on g_s . The decrease in g_s as REW declined below REW_c was also associated with seasonal increases in D_L , hence g_s also remained quite variable. The dynamic regulation of stomata at the diurnal scale may also depend on the stomatal response to changes in D , but the range of potential g_s that can be realised depends on soil water deficit. Variability in the response of g_s to soil water decreased at lower soil water contents and below a REW of ~ 0.2 g_s remained below $0.05 \mu\text{mol m}^{-2} \text{s}^{-1}$, regardless of D_L . As a result, from mid-summer to autumn, transpiration rates were much lower, and water conservation higher, than in the winter or spring period regardless of whether evaporative demand was high or low (see Figs. 2.1 and 3.1, Gindell 1964, Schiller and Cohen 1995, 1998). However, concurrent with the reductions in maximal g_s as soil water content declined was also a reduction in the sensitivity of g_s to short term increases in D under constant soil water content. This enabled stomata to operate over a large range of D within the potential g_s range determined by the soil water deficit limits, and therefore maintain a degree of carbon uptake at high D and low soil water content.

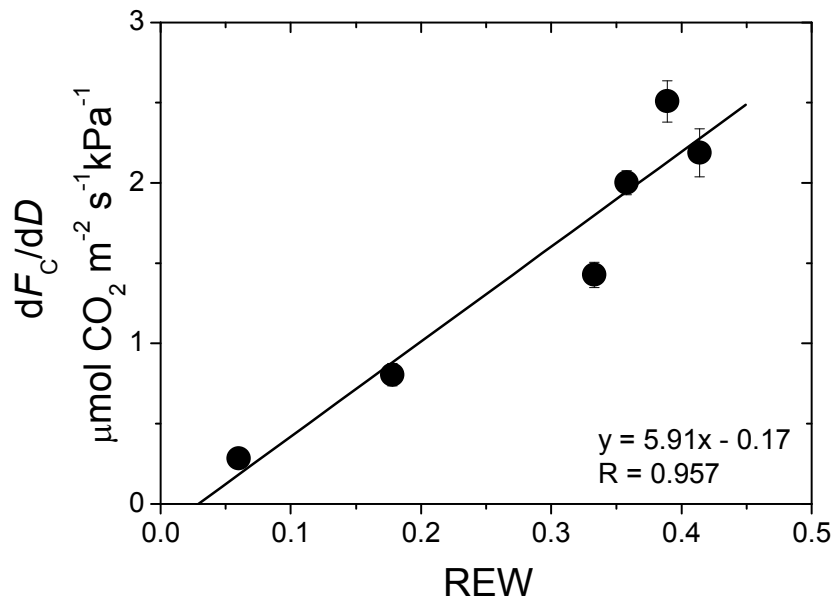


Figure 3.8. The sensitivity of F_C to D (dF_C/dD) determined from a number of *sharav* events as depicted in Fig. 3.6 in relation to the soil water content at each of the events.

Stomatal regulation and hydraulic conductance

The results presented here are consistent with the notion that stomata operate as the first response to prevent transpiration rates exceeding the capacity of the hydraulic architecture of the trees, minimising the risk of xylem cavitation and dysfunction (Tyree and Sperry 1988,

1989, Whitehead 1998). This involves both short term (the diurnal dynamic response of g_s to changes in D) and long term (the reduction of maximal g_s in response to soil water content) responses. Other long term responses may include adjusting the ratio of conducting (e.g. sapwood) to transpiring tissue, thus preventing excessive demand on the conducting system (Mencuccini and Grace 1994). Concurrent with the long term changes in maximal g_s were adjustments in the sensitivity of stomata to D due to changes in hydraulic conductance associated with the changes in soil water content (Oren *et al.* 1999, Addington *et al.* 2004).

The coordinated changes in maximal g_s and sensitivity of g_s to D with decreasing soil water content ensure that leaf water potentials remain above the level that prevents xylem cavitation and hydraulic failure (Tyree and Sperry 1989, Oren *et al.* 1999). Diurnal measurements of branch water potential in *P. halepensis* in Yatir and elsewhere in Israel (see Appendix 1) and by others (Borghetti *et al.* 1998) have shown that water potentials do not drop below about -2.5 to -3.0 MPa during the day, even in mid-summer. These minimum water potentials are well above the point at which a 50% loss of xylem conductivity (-4.9 MPa) occurs in this species (Froux *et al.* 2002). By the time water potentials drop to the point that marks the start of a loss in xylem conductivity (i.e. due to embolism development), stomata are near to being fully closed (Froux *et al.* 2005).

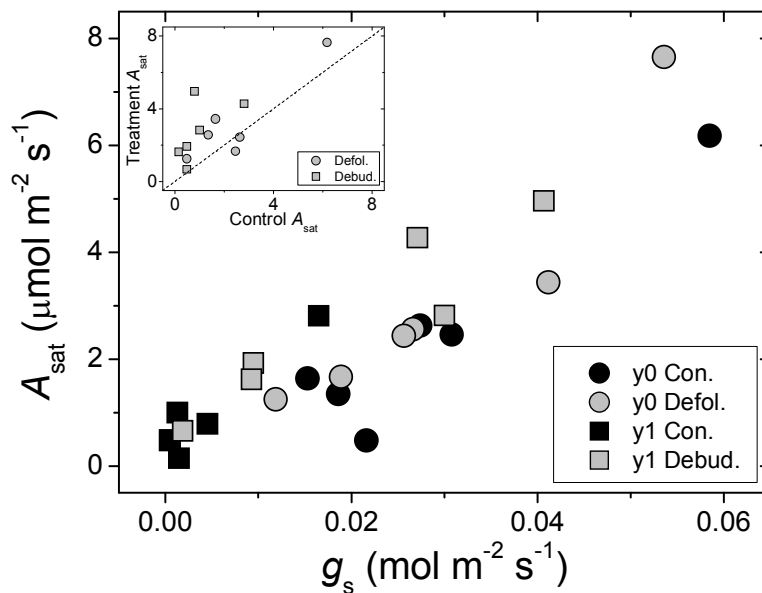


Figure 3.9. A_{sat} rates in needles from the control (Con.), defoliated (Defol.) and debudded (Debud.) branches in relation to g_s , measured in June. The inset shows the relationship between control and treatment A_{sat} from branches of the same tree.

Seasonal changes in xylem water content were, however, observed in *P. halepensis* by Borghetti *et al.* (1998) and interpreted as indicating the occurrence of xylem cavitation and

refilling. To what extent these fractional water volume changes affect tree hydraulic capacity is not clear, and no evidence for runaway cavitation and irreversible loss of hydraulic conductance was found (Borghetti *et al.* 1998). The diurnal measurements of leaf gas exchange (Fig. 3.2) showed that even in mid-summer (August), there can be afternoon recovery of CO₂ assimilation, transpiration and stomatal conductance as conditions became milder. These diurnal patterns indicate that any reduction in hydraulic capacity is rapidly restored or does not impact significantly on the critical water transport pathways.

Particularly dramatic examples of the resilience of the trees to potentially hydraulically stressful situations are seen during the *sharav* climatic events, in which there was always full recovery of physiological activity immediately after the extreme events. This resilience contrasts with the response observed in a *P. ponderosa* forest to a 3-day heat wave, during which D increased to above 3000 Pa (Goldstein *et al.* 2000). In that case the heat wave induced a reduction in H₂O and CO₂ fluxes that persisted through the rest of the season, including a rain event, which was attributed to the effects of xylem cavitation. However, similar events the following year, in which the soil water content was much higher, did not result in similar dysfunction (Goldstein *et al.* 2000). However, we observed that there was no soil water content dependence on either vulnerability to or rate of recovery from the extreme events. Evapotranspiration rates tended to remain relatively constant through the high D episodes (data not shown), indicating that stomatal closure was maintaining water flux through the trees constant and within the hydraulic capacity of the system. Therefore stomatal regulation operates to maintain hydraulic integrity and physiological function over the full range of soil water contents experienced seasonally, and highlights how adaptation and acclimation to regular drought results in different responses from those observed to episodic events.

The need to maintain the integrity of the water transport system over summer is made even more critical considering that this is the period of needle growth. Growth of new tissue requires that full turgor is maintained in the new cells, exerting pressure on and extending growing cell walls (Bradford and Hsiao 1982, Boyer 1988). Loss of hydraulic conductivity due to xylem embolism will not only affect productivity potential, but will also reduce the pressure gradient required to maintain water supply to the elongating needles. Therefore the highly conservative water use may suggest that carbon gain is compromised on the short term, but in the long term it maintains conditions suitable for cell expansion.

The gas exchange measurements made on the branches in the defoliation experiment showed that higher g_s was maintained in the branches in which the growing needles had been

removed. A possible explanation for this result is that the removal of the growing needles removed the low pressure region exerting control over branch water relations and water flow, enabling a higher water flux through the remaining needles and therefore higher stomatal conductance. The y0 needles on the defoliated branches also had higher A_{sat} rates than the corresponding control needles on the same tree and under similar conditions, but the range of g_s was similar. Therefore the removal of the extra evaporating surface did not induce a higher transpiration flux through the remaining needles, but did possibly improved the water relations (relative water content) of the remaining needles, resulting in the higher A_{sat} rates. This result also indicates that perhaps a (hormonal) signal from growing needles is responsible for maintaining a lower branch level g_s in order to facilitate their growth. It does appear that the growing needles do influence the water relations of the trees during summer, in addition to the overall requirements to minimize water loss and maintain hydraulic integrity.

3.2.3 The relationship between carbon gain and water loss

As the trees operate on the basis of limiting their water loss, the incremental rate at which carbon gain is limited due to restricting water loss becomes an important determinant of plant performance and success in water limited environments. At the physiological level this is commonly expressed as A/E and A/g_s , termed instantaneous and intrinsic water use efficiency (WUE), respectively. However, these ‘efficiencies’ differ from conventional efficiency parameters in that they do not vary between 0 – 1 and do not have a theoretical upper limit. Instantaneous WUE (WUE_{ph}), also known as transpiration efficiency, is an absolute measure of the carbon-water economics, in mol carbon assimilated per moles of water lost, during photosynthetic activity and is dependant on the physiological state of the plant and current environmental conditions. Intrinsic WUE (WUE_{in}) is the rate of carbon gain at a particular stomatal conductance. WUE_{in} is representative of photosynthetic capacity relative to the stomatal resistance and is a useful measure of plant performance between species and across different conditions as it does not include the direct effects of ambient conditions such as D .

To determine to what extent the data from single, mid-morning (peak activity) measurements in the day can be used as indicative of the diurnal balance of carbon uptake and water loss, a comparison of the whole day (light hours) total carbon gain to total water loss ($[A/E]_{\text{tot}}$) was made with that of the instantaneous ratio (A/E) measured during peak activity on the same day. Included in the analysis was data from diurnal gas exchange measurements

made at the irrigation site. The absolute values were slightly higher in the data from the mid-morning measurements, but there was a good correlation between A/E determined at the two temporal scales ($y = 0.777x + 0.24$, $R = 0.968$, where x is the peak activity and y the daily total values), giving confidence in the use of peak activity measurements to indicate relative seasonal changes in whole day carbon-water relations.

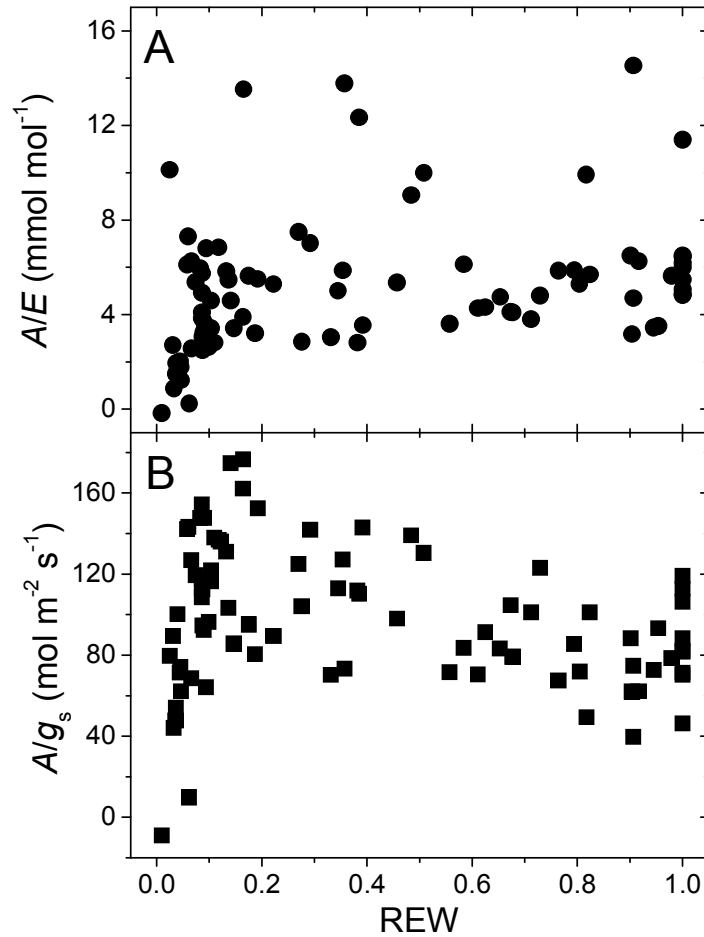


Figure 3.10. Water use efficiency values in relation to soil water content from seasonal scale gas exchange measurements. **A:** $\text{WUE}_{\text{ph}} (A/E)$ and **B:** $\text{WUE}_{\text{in}} (A/g_s)$.

The two water use efficiency parameters (A/E and A/g_s) are shown in relation to plant available soil water in Fig. 3.10. Over much of the seasonal variation in soil water content, including periods below REW_c , A/E was between 3 – 6 $\text{mmol CO}_2 \text{ mol H}_2\text{O}^{-1}$, with an average value of 5.0, but was at times be much higher and on occasion dropped to values close to zero (Fig. 3.10A). The low A/E values were associated with the very low REW values, but high values of A/E were also seen when soil water content was low, so overall there appeared little dependence of A/E on soil water content. The intrinsic water use

efficiency, on the other hand, shows a general increase from values of around 80 up to about 150 $\mu\text{mol CO}_2 \text{ mol H}_2\text{O}^{-1}$ as soil water content declines (Fig. 3.10B), but with a high degree of variability around these values. Again, values approaching zero are seen at times when the soil water content is very low.

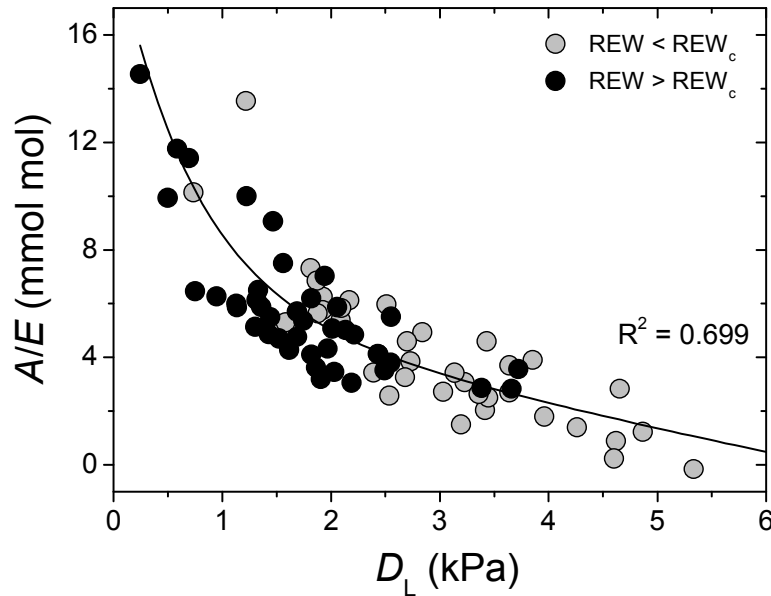


Figure 3.11. The dependence of WUE_{ph} during peak activity (A/E) on D_L from seasonal scale gas exchange measurements. The data is separated on the basis of whether soil water content at the time of measurement was above (black symbols) or below (grey symbols) REW_c (see Fig. 3.5A).

The dependence of A/E on D_L is shown in Fig. 3.11 and demonstrates that D is critical in determining the water economics of carbon gain. The extreme high and low A/E values were associated with high and low values of D_L , respectively. There was an exponential decline in A/E as D increased, independent of whether the trees are in the high or low REW regime. The lack of a dependence of A/E on soil water content but importance of D is further illustrated by the relationship between $[A/E]_{tot}$ and daytime average D_L in the samples from both irrigated and non-irrigated trees (Fig. 3.12). A common relationship exists for both the irrigated and non-irrigated trees with a clear decline in the ratio of carbon fixed to water lost as average D_L increased, irrespective of soil water content. While the absolute amount of carbon gained was higher in the irrigated trees (TB Lin, unpubl. data), the transpiration water loss was proportionally higher as a consequence, and the ratio of carbon gain to water loss remains highly coordinated.

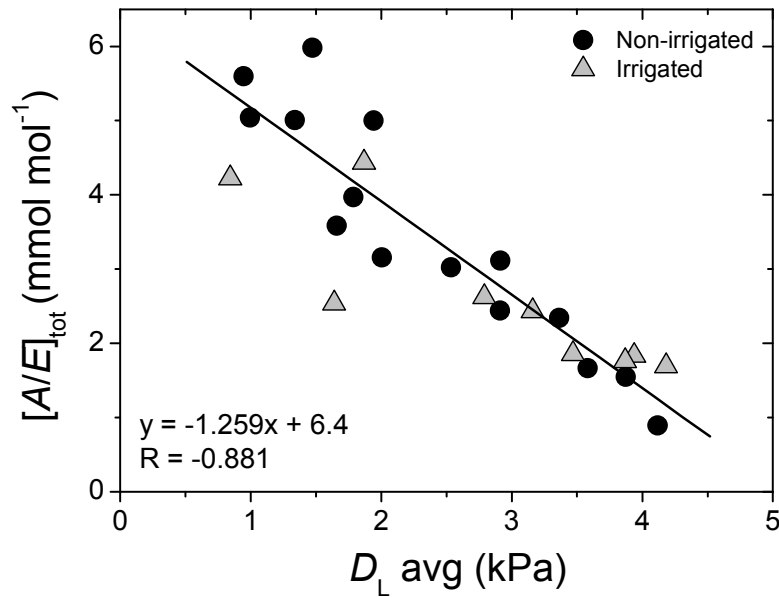


Figure 3.13. The dependence of the total daily carbon gain to water loss ratio ($[A/E]_{tot}$) on daily average D_L from diurnal scale gas exchange measurement on both non-irrigated and irrigated trees.

The importance of D as a driving factor in carbon-water relations

There is a differential response to stomatal closure between CO_2 and H_2O fluxes due to differences in diffusion parameters affecting CO_2 and water during photosynthetic gas exchange, and it is often assumed that WUE_{Ph} increases as soil water content declines as a consequence (Larcher 2003, see Inclán *et al.* 2005 for an example in *P. halepensis*). However, this appears to be only applicable when other conditions (environment, plant state) remain relatively stable, and field observations in other Mediterranean species at both leaf (Moriana *et al.* 2002, Llorens *et al.* 2003) and canopy (Reichstein *et al.* 2002) scale have shown that A/E is lowest in drought periods. While various explanations have been proposed for the low WUE_{Ph} under drought (Reichstein *et al.* 2002), it is clear from the results presented here and in other studies (Verhoef *et al.* 1996, Moriana *et al.* 2002, Ponton *et al.* 2006) that soil water content can not be considered in isolation as a driving factor on water use efficiencies. Indeed, the (often concurrent) changes in D exert a potentially much stronger influence, and the effect of soil water content may be quite minor other than at very low water contents.

The values of A/E observed in this study are high compared to those of other pine species (2 – 4.4 mol mol⁻¹; Teskey *et al.* 1994) and in other Mediterranean species (Moriana *et al.* 2002, Llorens *et al.* 2003). A high WUE_{Ph} can be attributed to the high photosynthetic capacity in this species and the inherently low rates of water loss typical of pines (Teskey *et al.* 1994). Furthermore, having a large period of activity in the cool months, when D (and

relative transpiration) is low leads to very high A/E at times during the most productive period.

The lower values of intrinsic water use efficiency (at high REW), are similar to the lower values reported for other Mediterranean trees and shrubs, but the dry season maximums are higher (Flexas *et al.* 2001, Gulías *et al.* 2002), showing that *P. halepensis* maintained relatively higher net CO_2 assimilation rates under drought conditions. These results are consistent with measurements of organic matter $\Delta^{13}\text{C}$, which provides an integrated measure of WUE_{int} , which indicated a high WUE in *P. halepensis* (Ferrio *et al.* 2003, Grünzweig *et al.* 2003). The asymptotic nature of the A - g_s relationship (Fig. 3.3) results in an A/g_s ratio that is low when conductance is high (and A_{sat} is at or close to saturation relative to g_s), and increases as conductance decreases. Therefore the general increase in WUE_{int} with decreasing soil water content includes other factors affecting the seasonal change in stomatal conductance, including D .

The lowest WUE_{in} values were measured at times during the dry months, when WUE_{in} is also typically high, and indicate that periodic reductions in net assimilation occur with relatively little change in stomatal conductance. This may indicate an increase in non-stomatal limitations to photosynthesis due to, for example, changes in turgor in the cells, and the values do tend to occur when the combined stress factors are highest, i.e. low soil water content, together with high D and high temperatures. However, due to the very low absolute fluxes measured under these conditions, errors associated with calculating g_s become significant, due in particular to the proportionally larger contribution that cuticular water loss makes to the transpiration flux.

Water conservation and productivity

Minimizing water loss and operating at high water use efficiencies, even in periods of high water availability, has the effect of reducing the rate at which the soil dries out through canopy transpiration in spring, thus extending the period of potential activity during the dry period. At the annual scale, water lost through transpiration in Yatir Forest has been shown to be close to balancing that received by precipitation (Schiller and Cohen 1998, E. Rotenberg unpubl. data). We have also shown that even when growing in the conditions experienced in Yatir Forest, with no access to ground water, very little annual precipitation and a long rain free period, that this close balance between water use and water availability also involves ongoing water use throughout the whole year. By identifying the factors controlling water loss

and associated productivity, it may be possible to identify potential productivity under various hydrological regimes hydrological limits to forest survival.

Overall, these results show that *P. halepensis* employs a ‘drought avoidance’ strategy in which water stress is avoided by minimizing water loss. Water use efficiencies are maintained high and water conservation assumes greater importance than maintaining higher absolute rates of carbon gain at any point in time. This strategy contrasts with a prodigal or ‘drought tolerant’ strategy, often seen in *Quercus* species for example (Epron and Dryer 1993), in which stomatal resistances are maintained relatively low and high rates of soil water uptake are maintained to balance water loss (Passioura 1982, Llorens *et al.* 2003). While a water spending strategy enables higher CO₂ fixation rates, higher fixation rates in the short term or under mild drought do not necessarily mean greater survival under long term drought (Filella *et al.* 1998). A conservative strategy is considered to be more successful for optimising carbon gain and avoiding mortality as aridity increases and is an important factor of overall drought tolerance influencing both current and changing plant distributions in regions that experience seasonal water deficit (Zhang *et al.* 1997, Filella *et al.* 1998, Gulías *et al.* 2002, Zavala 2000). In considering potential ecosystem response to water deficits, we have also shown that an atmospheric-driven sensitivity to water loss is critical for water conservation, maintaining relatively high levels of productivity and for long term survival, and will possibly be a key feature in determining the relative success of a species experiencing warmer and drier conditions than at present (Zavala 2004).

3.3 Summary

The physiological responses of *P. halepensis* are characteristic of arid and dry Mediterranean adapted species, with very sensitive stomatal control over water loss. The main period of photosynthetic activity is in the winter-spring period, when relatively high rates of photosynthesis are observed. Assimilation rates are predominantly a function of stomatal conductance, and stomata respond rapidly to both soil and atmospheric water deficits. Low soil water content and high *D* limit photosynthetic carbon gain, but the effect of *D* on conductance is dependant on the soil water content. Low atmospheric vapour pressure deficits during the period of maximal photosynthesis result in high water use efficiencies and overall *D* was an important factor controlling carbon gain and water use efficiency. Although photosynthetic rates were low over summer months, carbon uptake was maintained through exploiting the early and late hours of the day.

Chapter 4

Environmental and physiological aspects of dry summer leaf phenology in *P. halepensis*

4.1 Introduction

Phenology is the study of the periodic occurrence of life cycle events, or phenophases, such as flowering, leaf growth, or fruiting. Phenological processes typically have evolved in order to maximise fitness in a particular environment, in response to both biotic and abiotic interactions (Fenner 1998). A constrained time frame for the particular phenophase acts to buffer individuals against short term climate uncertainty and fluctuations (e.g. in precipitation, temperature) and optimise behaviour in response to the long term average conditions. As phenological events often depend on an environmental trigger, including accumulated temperature ('degree-days'), soil moisture or photoperiod (Dougherty *et al.* 1994, Rathcke and Lacey 1985), there has been renewed interest in recent years looking at phenological events as indicators of climate change. There is now a wealth of evidence from various plant types from many regions of the world showing that the timing of a number of phenological events are shifting as a result of climate change (temperature increase) over the last century (Bradley *et al.* 1999, Menzel 2000, Peñuelas and Filella 2001, Ahas *et al.* 2002, Parmesan and Yohe 2003, Chen *et al.* 2005). For example, in the Mediterranean region, leaf unfolding occurs on average 16 days earlier (Peñuelas *et al.* 2002). However, detailed understanding of the relationship of phenological traits with meteorological variables and underlying physiological processes remains poor (Chuine and Cour 1999).

Mediterranean pines have their origins in a pre-Mediterranean climate (Klaus 1989) and while some adaptation to Mediterranean climate conditions has occurred in a few species, including *P. halepensis* (Liphschitz and Lev-Yadun 1986), they also share many characteristics similar to temperate pine species. Needle phenology (emergence and growth) in pines involves spring flushing and summer growth (Dougherty *et al.* 1994) and is similarly seen in *P. halepensis* (Weinstein 1989a, 1989b). As a result, needle growth in *P. halepensis*, and other Mediterranean pines, occurs through the potentially most stressful period of the year.

Leaf phenology generally occurs when conditions are optimal for carbon gain i.e. when temperatures are increasing, the risk of frost is low and soil water availability is high. Often the key for the initiation of leaf development is temperature, but may be water availability in Mediterranean and desert plants and tropical dry forests, where critically low temperatures are not common (Kemp 1983, Borchert 1994, Penuelas *et al.* 2004). Needle elongation in *Pinus* species involves the continuous deposition of cellular material and cellular division at the basal meristems and therefore requires a continuous supply of carbon (Kozlowski 1971, Cannell *et al.* 1976, Wright and Leavitt 2006). As needle growth occurs through the most stressful period, when carbon uptake declines to minimum values, it is pertinent to consider the carbon source maintaining this growth. Initial bud formation may rely on current photosynthate from the older needle cohorts and / or stored photosynthate from earlier periods, which in temperate species is generally the end of the previous years growing season. The ongoing elongation may involve current photosynthate from both the older and the developing needles themselves (once photosynthetically active) or remobilised starch from storage (Wright and Leavitt 2006). Therefore the observation that needle phenology occurs over summer in *P. halepensis*, when carbon gain at the leaf and ecosystem level is at the lowest (Chapter 3, Grünzweig *et al.* 2003) and drought stress is the highest presents an interesting situation.

Vegetative growth in Mediterranean environments commonly occurs in the spring to early summer period, with few species showing onset of growth after about May. Little growth is seen through the entire summer, including in those species that are winter deciduous (Orshan *et al.* 1989, Castro-Díez and Montserrat-Martí 1998, Milla *et al.* 2005a). However, leaf and shoot growth during summer has been observed in some species growing in Mediterranean-type climates under conditions in which carbon gain is low (Poljakoff 1945, Mooney *et al.* 1974, Orshan *et al.* 1989). The persistence of summer growth suggests that leaf phenology is an evolutionarily constrained feature with incomplete adaptation to the Mediterranean-type climate (Orshan *et al.* 1989). Analogous phenological responses are observed in species from old tropical evergreen flora surviving in Mediterranean refugia (Herrera 1984). However, through affecting the amount of photosynthesizing tissue, the timing and extent of annual leaf growth are critical features controlling ecosystem productivity (Menzel and Fabian 1999, Vose *et al.* 1994). This would suggest that the retention of dry summer foliage growth may represent an advantage for ecosystem function that has reduced the likelihood of major evolutionary adjustments. Furthermore, with the possibility that future climate change within evolutionary time frames may disrupt current

climate-physiology-phenology relationships, more ecosystems may experience growth phases over a dry summer. Therefore, understanding the underlying physiological processes related to dry summer leaf phenology is important for not only understanding controls on current ecosystem productivity, but potentially also future ecosystem responses.

In addition to carbon, growth of new tissue is dependent on a supply of nitrogen (and other nutrients). The seasonality of precipitation in Mediterranean environments can result in low soil N availability during the summer drought period, adding additional constraints to the relationship between phenology and nutrient demands (Milla *et al.* 2005b). Wellburn *et al.* (1996) showed that drought impairs root nitrate reductase activity in *P. halepensis* and measurements in Yatir Forest show that soil nitrogen availability is very low over summer (I. Gelfand, unpubl. data). Growth may also rely on the remobilisation and internal translocation of nutrients (Nambiar and Fife 1987, Milla *et al.* 2004). Often this is assumed to involve the retention of nutrients of senescing leaves prior to leaf fall, but mature leaves and wood can also be important sources (Pugnaire and Chapin 1993, Nambiar and Fife 1991, Cherbury 2001).

This chapter addresses mechanisms and implications of dry summer leaf phenology. The variability and control of leaf phenology is investigated through comparing both the timing and extent of needle growth inter-annually, between sites and from the defoliation and irrigation experiments. Leaf phenology is compared with stem growth phenology, representing above-ground wood growth and another major carbon sink. The hypothesis that the foliage growth continues to rely predominantly on the available photosynthate is tested through analysis of ecosystem carbon fluxes, the manipulation experiments and needle C content and $\delta^{13}\text{C}$ composition. The influence of foliage growth on needle N allocation is investigated and the hypothesis that the N demands of the new needles are met by a large amount of N remobilisation and resorption is tested through analysis of leaf nitrogen content.

4.2 Results and Discussion

4.2.1 Needle and stem growth dynamics

Measurements of needle length through the elongation phase were made on trees at the tower (Tow) site in three consecutive years (see Chapter 2). In the 2003 season the measurements were made on the trees in the defoliation experiment and included both the control and defoliated (Def) branches, in which all previous years needles on the branch ends were

removed just after bud-burst. This data was combined with elongation measurements made on the trees at the irrigation site from the two years of irrigation, including both non-irrigated (Con) and irrigated (Irr) trees. The results from the total of eight data sets are shown in Fig. 4.1. The final length of the needles in the non-treatment trees (i.e. Tow and Con samples) ranged between 57 ± 2.3 mm (Tow, 2004) to 82 ± 2.8 mm (Con 2001). Irrigation increased final needle lengths relative to the non-irrigated trees by 34% in 2001 ($P < 0.0005$, to 110 ± 3.1) and 33% in 2002 ($P < 0.0001$, from 72 ± 2.3 mm to 96 ± 3.2 mm). Defoliation of the previous year's needles significantly reduced the final needle length in the current season's needles by 22% relative to the new needles on non-defoliated branches on the same tree ($P < 0.001$), from 59 ± 1.6 mm to 46 ± 1.2 mm.

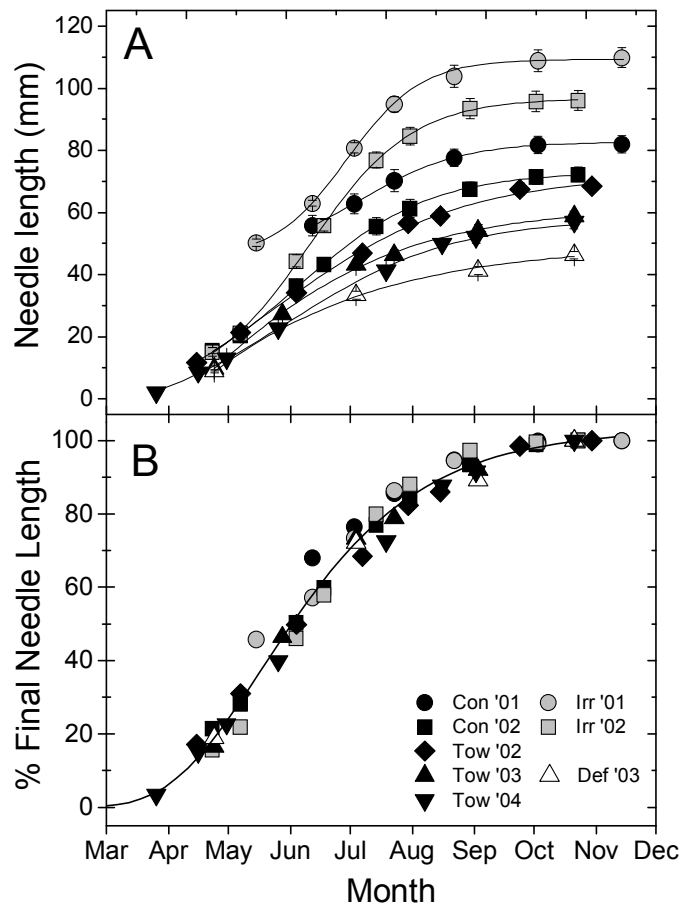


Fig. 4.1 Needle lengths measured during the elongation period in a number of samples, from different years and different treatments. (A) Absolute needle length and (B) needle length relative to final length. The legend is the same for both panels.

Despite the differences in absolute length, the period of growth was similar between all samples. Bud-burst (emergence of the needle tips from the shoot) in all samples was in mid-late March (~day 80) and while the needles had reached 90% of their final length by mid August, elongation continued for a total of about 200 days, into October. Furthermore,

relative needle length at any time (i.e. length relative to final length) was very similar, irrespective of site, year or treatment (Fig. 4.1B) and indicated a control on growth that is independent of the variable factors that determined absolute growth rate.

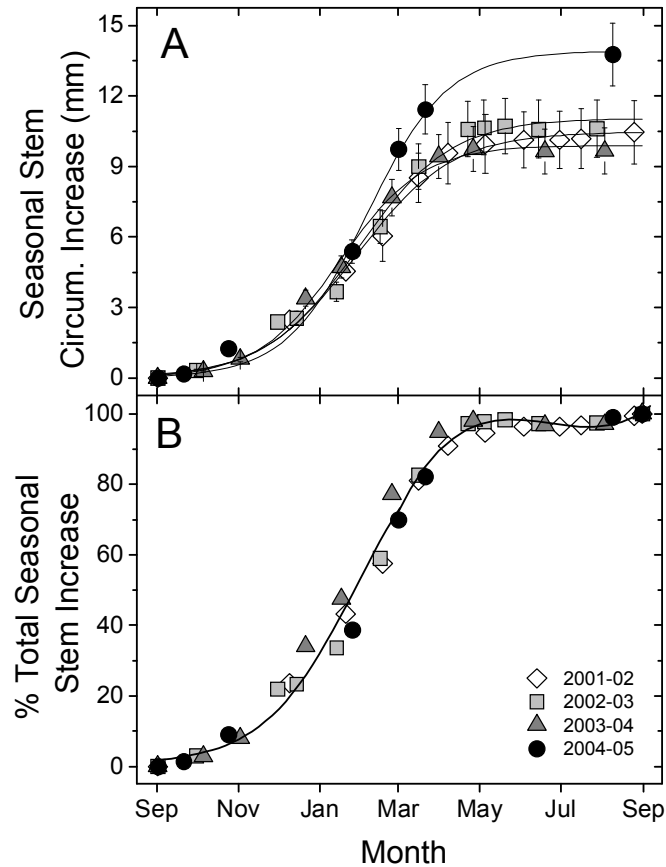


Fig. 4.2 Stem circumference measurements from four years of measurement. (A) Absolute circumference increase from Sept. 1st of each season and (B) the change in circumference relative to the circumference at the end of the season.

Stem growth predominantly occurred between November and May (Fig. 4.2A). There was little or no stem growth over the summer months; even some shrinkage in stem circumference was observed, probably due to cellular dehydration (Linder *et al.* 1987). Annual stem circumference increase was between 10.0 ± 1 mm and 10.9 ± 1.2 mm for the three seasons of 2001-02 to 2003-04, and 13.9 ± 1.3 mm in the 2004-05 season; a ~30% increase in circumference growth relative to the previous three years. The timing of the relative increase in stem circumference through the season was also very similar between years (Fig. 4.2B). Therefore there was a clear separation between the phenophases of stem and foliage growth in these trees.

A representative phenogram (phenophase diagram) of stem growth and needle elongation, depicting also the relative growth rates, is shown in Fig. 4.3. Relative growth rates

were determined from the relative increment data in Figs. 4.1B and 4.2B, and were used to categorise the phenograms in Fig. 4.3. Also shown are net ecosystem carbon uptake (NEE) normalised to the yearly maximum and soil water content (REW) for the 2002-03 year. Stem growth predominantly occurred during the period when soil water content and carbon uptake were high. There was a slight delay between the period when stem growth and carbon gain were maximal, with NEE peaking later in the season, but overall the seasonal pattern demonstrates that stem growth occurred when environmental conditions were favourable and physiological activity high. Needle elongation, on the other hand, is initiated when conditions are near optimal and carbon uptake is maximal, but peaks when both soil water content and NEE are low and continues as water stress increases and carbon uptake declines.

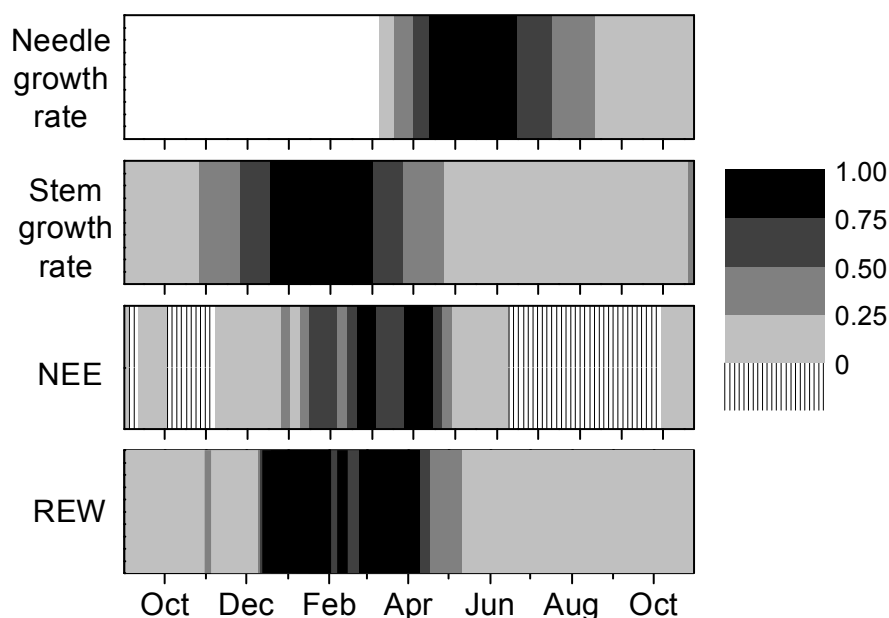


Fig. 4.3 Representative quantitative phenograms of stem and needle growth, together with ecosystem carbon flux (NEE) and soil relative extractable water (REW). Growth data are growth rates normalised to the seasonal maximum. NEE is represented with positive values indicating carbon uptake. Shading levels represent ranges of the variable relative to the seasonal maximum, with darker levels indicating higher growth rates, greater CO₂ uptake and higher soil water content. Absence of shading (in needle growth) indicate no activity, and the striped shading in the NEE indicates negative NEE (carbon loss). The growth rate phenograms (both needle and stem) were determined from the derivatives of the fit to the normalised increment data in Figs. 4.1B and 4.2B.

Control of needle phenology

There was some inter-annual and inter-site variability in final needle length, but the results are consistent with lengths characteristic for *P. halepensis* (50-100mm, Quézel *et al.* 2000). The timing of needle emergence was similar to that found by Weinstein (1989a,

1989b) for *P. halepensis* in Israel, but earlier than the date (day 122, or May 2) given by Mediavilla and Escudero (2003) for a cold Mediterranean site in Spain and mid-April to early May estimated from the data of Borghetti *et al.* (1998) at a site in southern Italy. Bud-burst in temperate trees, including conifers, is a phenological trait considered to be predominantly under the control of temperature and possibly photoperiod (Vegis 1964, Dougherty *et al.* 1994, Chuine and Cour 1999, Menzel and Fabian 1999, Badeck *et al.* 2004). There is a certain degree of inherent phenotypic plasticity within a species that enables phenological acclimation to local conditions to accommodate variations in climate over time at one location (Kramer 1995). Weinstein (1989b) also observed variability in the timing of vegetative growth between different ecotypes of *P. halepensis* growing at the same location in Israel of up to three or four weeks but there was no record of needle emergence later than mid April. Therefore the earlier appearance of the needles in Israel (i.e. this study and in Weinstein 1989a, 1989b) compared to northern Mediterranean sites (Mediavilla and Escudero 2003, Borghetti *et al.* 1998) is consistent with both ecotype adaptation and intra-species phenological variation across geographical gradients (Kramer 1995, Ahas *et al.* 2002).

Irrigation resulted in higher growth rates but did not extend the period of needle growth in both this and other studies (Garrett and Zahner 1973, Borghetti *et al.* 1998, Irvine *et al.* 1998, Raison *et al.* 1992, Sheffield *et al.* 2003). Unless a strong stress effect stops meristematic activity, the cessation of needle elongation in pines, and other growth and phenology parameters in woody plants in general, is considered to be under the control of a photoperiodic stimulus (Wareing 1956, Nitsch and Nitsch 1959, Cannell *et al.* 1976). Notably, across all samples measured in our study, including the inter-annual and inter-site variation and the irrigation and defoliation treatments, growth not only lasted for the same length of time, but the relative growth rate at any point in time was also similar (Fig. 4.1B). Therefore it appears that exogenous control from a highly consistent environmental parameter, or combination of parameters, such as daylength, light spectrum or temperature has primary control on the rate of cell division and expansion during the entire growth period in these trees. The factors affecting carbon supply appear superimposed on the factors controlling relative growth rate and therefore their influence on absolute growth will depend on the time during the growth period.

Leaf production during the dry season has also been seen observed in some other seasonally dry environments. Species in some seasonally dry tropical forests in India leaf out in the hottest and driest months; a strategy that ensures productivity over the ensuing short wet season is maximised (Singh and Singh 1992). Likewise, in the case of the evergreen *P.*

halepensis, productivity is not compromised by a lag associated with the production of new foliage while environmental conditions are favourable. Whether off season (i.e. summer) foliage growth would represent an advantage compared to the (hypothetical) situation of foliage production in the wet season depends on how closely needle flushing and senescence would remain linked (due to, for example, nutrient remobilisation requirements). If the oldest cohort of needles were maintained on the tree (and retained high photosynthetic capacity) during the growth of the new needles, there would not be a lag in the development of maximal leaf area, and in fact LAI would be temporally higher during the growth of the new needles.

Control of stem growth

The pattern of stem growth agrees with investigations into the cambial activity in *P. halepensis*, which has been found to have particularly plastic behaviour depending on location and climate, but is considered to be indicative of adaptation to the Mediterranean climate (Oppenheimer 1945, Liphshitz *et al.* 1984, Liphshitz and Lev-Yadun 1986). Low rates or absence of cambial activity was seen over summer in trees in Israel, where summer rains are absent, and winter inactivity may occur if temperatures are low. However, in Italy and France, where summer rains do occur, the cambium was active from spring through to autumn but was inactive in winter (in Liphshitz and Lev-Yadun 1986). Irrigated *P. halepensis* showed cambial activity and stem growth all year round in Israel, indicating summer inactivity is more of a rest phase than dormancy, and the trees are able to make opportunistic use of suitable conditions for growth if they occur at any time of the year (Liphshitz and Lev-Yadun 1986, Klein *et al.* 2005). The relationship between stem growth and REW and NEE observed in this study indicates that stem growth is closely linked to photosynthesis and carbon gain, and the plasticity of cambial activity is linked to the plasticity of physiological activity (see Chapter 3). Furthermore, there is good agreement between annual estimates of above ground productivity made on the basis of the stem growth measurements and site specific allometric equations and estimates of total ecosystem productivity from the flux measurements (data not shown).

Stem growth measurements can include any and all aspects of cambial activity, i.e. xylem, phloem or phellem cell production; or volume changes in the cambial cells. Liphshitz *et al.* (1984) found that xylem production exceeded phloem production in both duration and number of cells and phloem production was confined to a month in May in non-irrigated trees, but occurred for five months in spring and late summer in irrigated trees. It is assumed that the early stem growth in autumn and winter in the trees in Yatir is therefore due to xylem

production in response to the change to more favourable hydrological conditions, with phloem production later in the season, when rates of carbon gain were high (see Chapter 3).

Needle and stem phenophase separation

The flexibility of stem growth and the more constrained needle growth resulted in a temporal separation of the two important carbon sinks. The growth phases of stems, needles and leaders in pines growing in temperate regions typically overlap to a large extent, occurring in the summer period (Dougherty *et al.* 1994, Irvine *et al.* 1998). Similarly for conifers, including *Pinus* species, in the montane Mediterranean climate of Sierra Nevada the growth phenophases predominantly overlapped, occurring between June and August (Royce and Barbour 2001). Within Mediterranean plants, species with both concentrated overlapping phenophases (in spring) and sequential phenophases have been identified (Castro-Díez and Monsterrat-Martí 1998, Milla *et al.* 2004). Phenophase separation represents a trade off between lower resource availability during certain phenophases and reduced intra-plant competition. Therefore the shift in phenological behaviour observed in *P. halepensis* in this study relative to other conifers and separation of the stem and foliage growth periods might also be part of the adaptation process to the dry Mediterranean climate. By spreading growth demands over two periods in which growth activity is possible, but potentially limited to some extent (i.e. due to either cold or drought), intra-plant competition for resources are reduced.

The separation of phenophases and carbon sinks assumes greater significance if root production is also considered. Root growth in pines can occur through mild winters, but decreases during leaf unfolding and shoot growth due to the strong sink demand of the aboveground biomass (Konôpka *et al.* 2005). However, fine roots in conifers live on starch reserves that are deposited when the roots are formed (Waring and Schlesinger 1985), and therefore do not require a continuous supply of new photosynthate. Annual fine root biomass production is also greater than aboveground biomass (Waring and Schlesinger 1985), therefore the seasonal separation between foliage and root growth would enable the larger absolute sink of carbon (roots) to be formed when the supply of carbon is high.

4.2.2 Carbon supply and needle growth

Ecosystem carbon balance

If needle growth is sustained by current photosynthesis then the amount of carbon fixed by the canopy over the growth period needs to at least equal the amount of carbon added to the canopy in the new foliage. The total amount of carbon in the new (y_0) flush of needles in the canopy (C_{Toty_0} , in g C m⁻² ground area) can be estimated from:

$$C_{Toty_0} = LAI \times f_{y_0} \times LMA_{y_0} \times C_{y_0} \quad (4.1)$$

where LAI is canopy leaf area index (m² m⁻²), or area of needles per area of ground, f_{y_0} is the fraction of the canopy that is in the y_0 age class, LMA_{y_0} is the specific leaf mass area of the y_0 needles (in g DW m⁻², being the inverse of SLA, or the specific leaf area) and C_{y_0} is the carbon content (in % DW) of the y_0 needles. At the end of the growing period (October) LMA_{y_0} was determined as 197 g DW m⁻² and C_{y_0} was 57% (see Fig. 4.7). Recent measurements of canopy LAI are 1.5 ± 0.2 (E. Rotenberg, unpubl. data) and as on average about 3 cohorts of needles are retained on the trees at this site f_{y_0} was estimated at 0.3. These values provide an estimate of C_{Toty_0} of 50 g C m⁻².

The daytime and night-time NEE (net daytime or night-time ecosystem C exchange) rates for the April to October period from 2002-04 is shown in Fig. 4.4. Negative values mean net C gain by the ecosystem. The patterns were similar between years, with daytime NEE high in April and declining to close to zero by August, and then starting to recover in late September. Although it reached low values, daytime NEE was usually, but not always, negative meaning that the level photosynthetic activity that is sustained through the summer (Chapter 3) is able to sustain a net uptake of carbon for most days through the year. Night-time NEE (ecosystem respiration) was correlated with daytime NEE, being relatively high in April and declining to minimal values in mid-late summer, despite the seasonal increase in temperature (see Chapter 4).

As no year had a complete data set for whole period, total ecosystem carbon gain (sum of daytime and night-time NEE) was averaged across each day for the three years. The summed NEE of the 3 year daily averages for the April to October period (inclusive) was 47 g C m⁻², very similar to that estimated for the amount of carbon added in the new foliage. Therefore there is good agreement between the estimates of ecosystem productivity as determined from the flux data and the biomass gain in the new foliage. As needles are the strongest sink for photoassimilated carbon (Mooney 1972, Webb 1975) and have priority in terms of allocation for growth during drought (Raison *et al.* 1992) it is therefore reasonable to

assume that the physiological activity over the summer, despite being highly suppressed relative to the winter and spring periods, continues to sustain the foliage development.

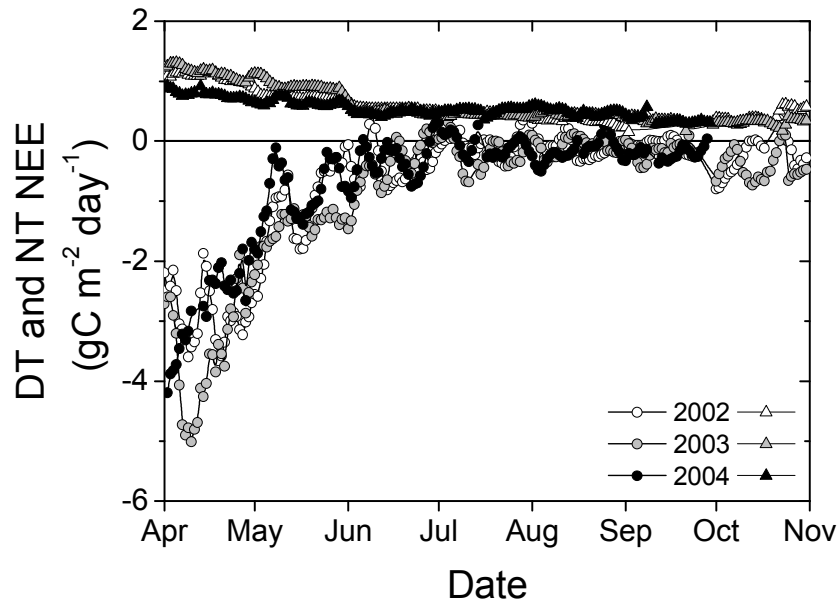


Fig. 4.4 Daytime (DT, circles) and night-time (NT, triangles) net ecosystem carbon flux (NEE) over the needle elongation period (April – October, inclusive) for the three years 2002 – 04. Values are day and night sums of half hour data, with the day / night separation of data on the basis of PAR > 0.

Effects of irrigation and defoliation treatments

In the defoliation experiment, the defoliation took place shortly after the buds had emerged, so the reduced growth could not be attributed to any limitation in the initial supply of carbon that may be used for bud formation. A sustained branch level wounding effect on growth relating to the defoliation can also not be ruled out, but gas exchange measurements in mid-summer (June) showed no adverse effects on the physiological performance of needles on the defoliated branches relative to the controls (see Fig. 3.10). In fact, the new needles on the defoliated branches tended to have higher A_{sat} rates than those of the control branches on the same tree (Fig. 3.10 inset). Other defoliation studies have found that photosynthesis is stimulated in remaining leaves following defoliation (Reich *et al.* 1993), but this does not compensate for the loss of the photosynthetic tissue, and growth (including height and needle length) is significantly reduced (Li *et al.* 2002). Therefore it appears the lower growth rate in the defoliated needles was due to the loss of a C source during their development, being either photosynthate and/or translocated C stores that would normally be supplied from the adjacent mature needles.

The irrigated trees showed a clear stimulation of needle length, and higher gas exchange rates that were maintained over the summer (TB Lin, unpubl. data) could provide the additional carbon for this growth. As there was no irrigation prior to the growth period in the first year of irrigation, it is unlikely the enhanced growth was due to stored carbon. The effects of water availability during growth on final needle length have been widely studied in pines through site and inter-annual rainfall variation, irrigation and drought experiments. Reduced water availability (induced or natural drought) tended to reduce needle length (Garrett and Zahner 1973, Raison *et al.* 1992, Irvine *et al.* 1998, Sheffield *et al.* 2003), whereas a (positive) response to irrigation was generally only observed when water supply was limiting (Garrett and Zahner 1973, Linder *et al.* 1987, Raison *et al.* 1992, Murthy and Dougherty 1997). Rain exclusion at a site in Italy reduced final needle length in *P. halepensis* by about 45% (Borghetti *et al.* 1998). Therefore it can be concluded that the positive growth response to irrigation indicates a response to conditions affecting photosynthetic carbon supply during the growth phase, and is consistent with evidence from the stable carbon isotope composition of the needles and tree rings from the irrigated and control trees (Klein *et al.* 2005).

Needle carbon content and $\delta^{13}\text{C}$ composition

Needle carbon concentration (on a dry weight basis) and $\delta^{13}\text{C}$ composition was measured on samples from the two mature needle cohorts from spring through to winter in 2002 (labelled for their year of development, i.e. *b00* grew in 2000 and *b01* in 2001), and on the new needles of 2002 (*b02*) through to the spring of 2003 (Fig. 4.5). Needle C concentration was found to increase through the needle growth period in all age classes. In April, soon after the emergence of the new needles, needle C concentration was $46.0 \pm 0.5\%$ in the new (*b02*) needles and $50.1 \pm 0.6\%$ in the *y1* (*b01*) needles, and had increased to $57.3 \pm 0.6\%$ and $59.0 \pm 0.8\%$ by mid-November in the *y0* and *y1* needles respectively (Fig. 4.5). There was a strong correlation between leaf C concentration and the $\delta^{13}\text{C}$ composition of the leaf carbon across all age classes, with the carbon becoming depleted in ^{13}C as concentration increased (Fig. 4.6).

An increase in needle carbon content, despite a decrease in photosynthetic rates and possible sink demands of the new foliage, may be due to a change in the ratio of compounds with a high to those with a low carbon weight percentage, such as the lipid to carbohydrate ratio. Lipids tend to be relatively depleted in $\delta^{13}\text{C}$ relative to carbohydrates (Melzer and

Schmidt 1987, Schmidt and Gleixner 1998), so the trend of decreasing ^{13}C content and increasing C as a percent of dry weight is consistent with a relative increase in the lipid fraction of the non structural carbon content. A decreasing carbohydrate content in the mature needles would indicate that levels of soluble sugars and starch were being metabolised at a greater rate than they were replenished, and is consistent with low rates of photosynthesis over summer and a strong sink effect driving the export of available carbon (stores or current photosynthate) to the new foliage (Cherbury *et al.* 2001)

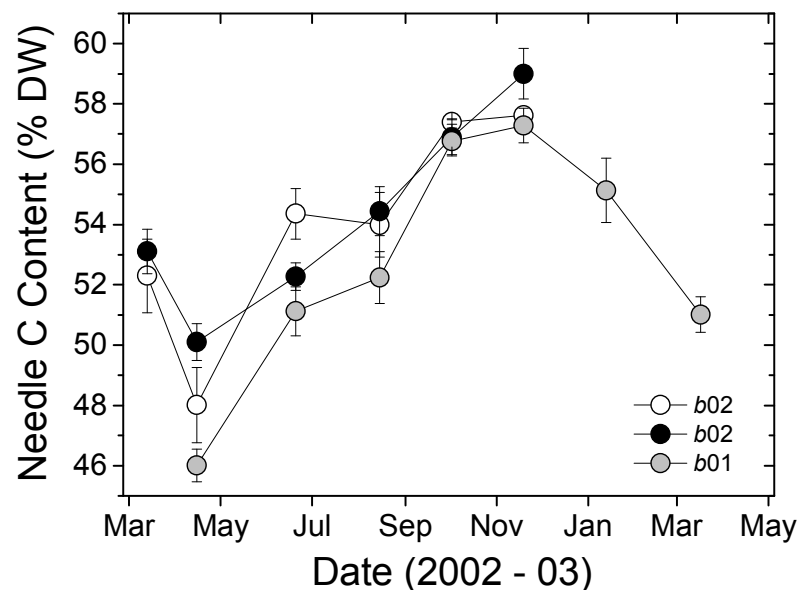


Fig. 4.5 Needle C concentration (as percent of dry weight) in mature (b00, b01) and the new (b02) needles between March 2002 and March 2003.

Carbon content was lower in the new needles, indicating a higher carbohydrate fraction, which is consistent with their strong sink effect. An overall increase in the lipid fraction in the new needles is possibly related to the construction of cellular components (e.g. membranes). There is one outlier in the $\text{C}\%-\delta^{13}\text{C}$ relationship, which is the y0 sample just after bud burst. Relative to the other samples, this sample was depleted in ^{13}C for the (low) carbon content. This is possibly due to having high soluble carbohydrate levels due to remobilisation of stored material from elsewhere in the trees to initiate growth, before significant anabolic and lignification processes have taken place.

The increase in carbon concentration may also be due to an accumulation of ‘compatible’ solutes (i.e. that do not interfere with cellular structure or function) in response to bulk cytoplasmic water loss to prevent protein denaturation and membrane fusion (Hoekstra *et al.* 2001, Jones and Corlett 1992). These solutes include, but are not restricted to, proline, glutamate, mannitol, sorbitol, trehalose, sucrose and oligosaccharides. Fructose and

glucose have been found to accumulate in pines in response to water stress (Koppenaar *et al.* 1991, Meier *et al.* 1992). However, Borghetti *et al.* (2004) did not observe osmotic adjustments in *P. halepensis* in response to drought in the field.

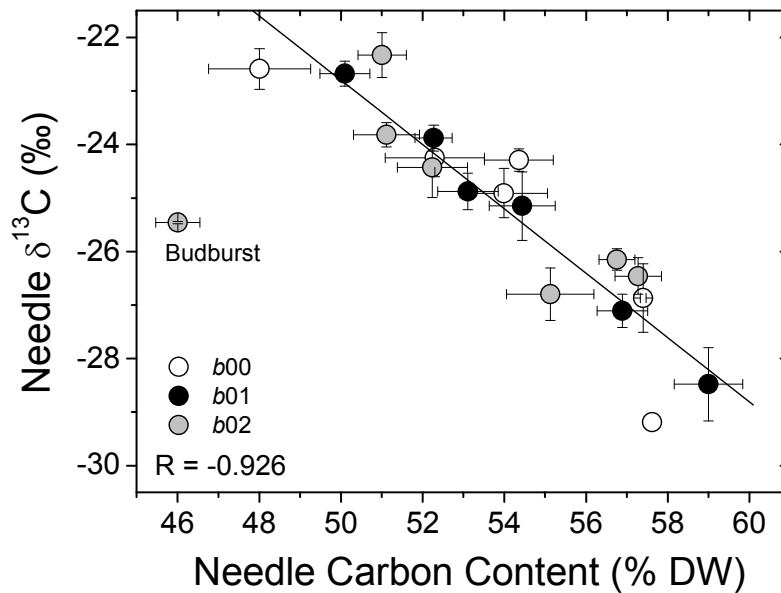


Fig. 4.6 The relationship between needle carbon content and the $\delta^{13}\text{C}$ composition of the needles, in the samples from Fig. 4.5. The liner regression is to all data except the b02 outlier at budburst.

A number of lines of evidence therefore indicate that needle growth was maintained predominantly by current photosynthesis, possibly supplemented by the remobilisation of stored carbon. Consequently, needle growth will be sensitive to the climatic conditions affecting photosynthesis during the growth period, in particular the rate at which soil water content declines at the end of spring when growth rates are maximal. The direct climate effect of variable needle growth on overall annual productivity may be small, but that fact that the amount of foliage produced in a season affects rates of productivity will amplify the impact. Reduced foliage growth and lower leaf area in one year will reduce the potential productivity from that age class for the duration of the needles on the tree (about 3 years) and successive years of low leaf area production will affect productivity more than each year independently (Kramer *et al.* 2000). Therefore forest productivity in this system is possibly sensitive to persistent influences of inter-annual climatic variations during the ‘off-season’ that extend beyond the effect on annual scale productivity.

If total foliage production is dependant on the prevailing conditions in summer, maximal leaf area index will be constrained by the hydraulic limits in summer i.e. high evaporative demand and low hydraulic conductivity. If, for example, leaf production occurred

under more mesic conditions, leaf area might be greater, but a larger leaf area would also be a larger evaporative surface in summer, which may lead to stress on the hydraulic system. Foliage development during summer therefore maintains leaf areas within manageable hydrological limits with respect to the most limiting period.

4.2.3 Needle total nitrogen and chlorophyll content

Needle nitrogen measurements were made on the same samples as the measurements for needle C content. Leaf nitrogen declined in the mature needles during summer from maximum levels in winter of about 1.1% to about 0.8% in mid-summer. Recovery started earlier in the 1 yr old (*b01*) than the 2 yr old (*b00*) needles (Fig. 4.7A). While it is difficult to determine the total level of remobilisation if uptake is occurring concurrently (Proe *et al.* 2000), we can assume that soil uptake is minimal over summer in Yatir Forest. Therefore the net seasonal change over summer in the mature needles likely represents remobilisation of about 25-30% of total leaf N in these needles. Litterfall occurs predominantly between April and August, overlapping the period of needle growth. The N content of litter collected just after leaf fall was about 0.5% (J. Grünzweig, pers. comm.), indicating that about 55% of total leaf N of the senescing age class is retained by the trees prior to their fall.

For a first approximation, if it is assumed that total N contents are similar between needle cohorts between years (i.e. similar biomass and N concentration), then the ~55% of N from the senescing needles and ~25% from each of the older age classes would be sufficient to meet the demands of the new foliage, on a mass balance basis. This, however, does not account for temporal dynamics. The dynamics of leaf N concentration during the growth phase are influenced by the initial N concentration in the buds, further rates of N incorporation, relative growth rate and concurrent growth in other organs (Mediavilla and Escudero 2003, Milla *et al.* 2004). A large proportion of the final N content may already be present at bud-burst (Millard 1994, Mediavilla and Escudero 2003). Initial N concentrations in the new needles were high, at over 1.6% (Fig. 4.5A), but rapidly dropped to less than 50% of the initial concentration due to dilution by the early increase in biomass. N levels remained at about 0.7% for the rest of the growth period and started to increase to the winter maximal levels once extension was complete. When the total N content of the needles (i.e. mass x concentration) is compared it is seen that the initial high N content represented more than 30% of the total N incorporated by the new needles over the growth period.

Concurrent with the reductions in total leaf N were seasonal reductions in chlorophyll content in the mature (*b01*) needles of nearly 50%, from a spring maximum (2.83 mg Chl g DW⁻¹) to summer minimum (1.49 mg Chl g DW⁻¹) values (Fig. 4.7B). Chlorophyll contents of the new (*b02*) needles were lower than the previous year's needles during their growth phase, at 0.9 – 1.0 mg Chl g DW⁻¹, but increased rapidly to maximal levels in autumn-early winter. There were also changes in the content of total carotenoids that were linearly related to total chlorophyll with a common relationship across all age classes (Fig. 4.5B inset).

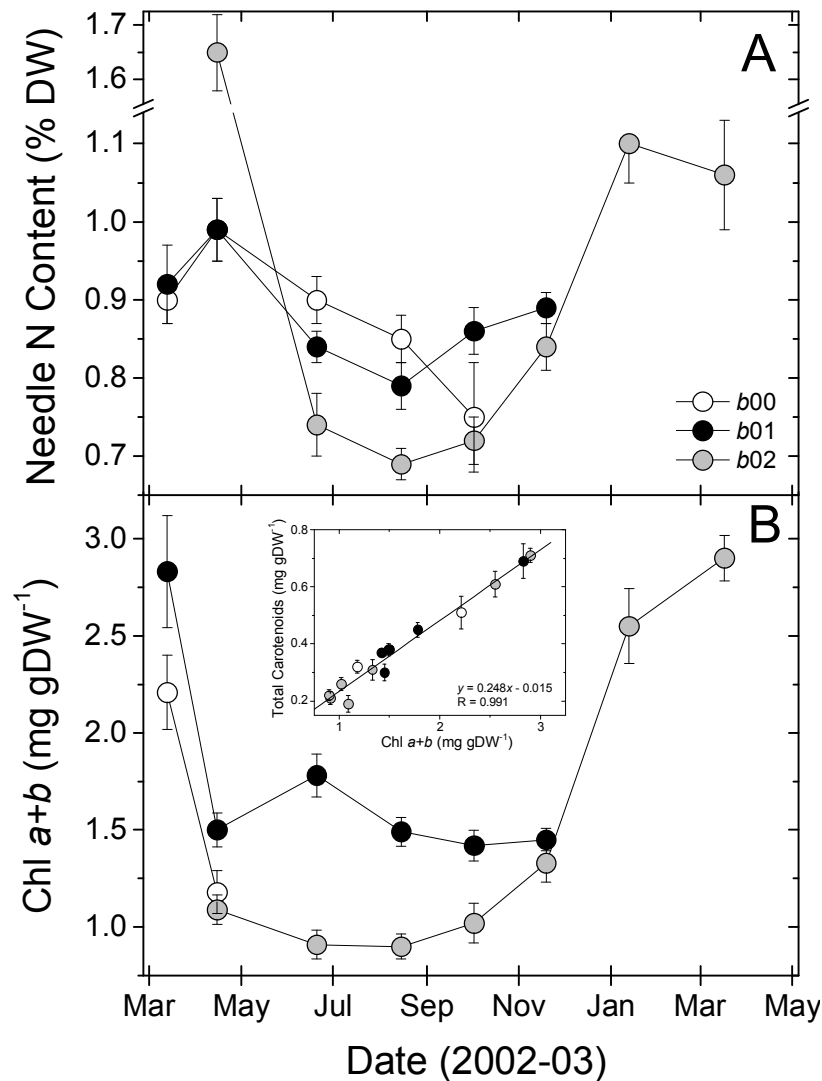


Fig. 4.7 Needle leaf N concentration (**A**), chlorophyll content (**B**) in the mature (*b00*, *b01*) and the new (*b02*) needles between March 2002 and March 2003. The inset in **B** shows the relationship between the chlorophyll content and total carotenoid content in the same samples. The liner regression is for all samples combined. Symbols for all panels are the same, as shown in **A**.

Internal reallocation of leaf N

The N concentrations and seasonal dynamics reported here are similar to those measured in 2001 in 1 year old needles (TB Lin unpub. data) and are similar to or slightly lower than those reported elsewhere for *P. halepensis* (Mediavilla and Escudero 2003, Inclán *et al.* 2005, Sardans *et al.* 2005). The N concentrations are only about a third of the values found in fertilized seedlings by Le Thiec *et al.* (2003) and are at the low end for a range of Mediterranean species, including other pines (Mediavilla and Escudero 2003).

Nitrogen contents in the needles at bud-burst were high, due to the common N loading of new leaves at the start of growth. However, after an initial decline, a relatively stable N concentration was maintained during the growth phase as opposed to the continual decline observed in other Mediterranean species (Mediavilla and Escudero 2003). The stable N contents indicate that N incorporation rates were well balanced with growth rates and that the slow growth rate of the needles over summer has the advantage that despite little, if any, uptake of N from the soil, rates of remobilisation from the older needles and resorption of N from senescing needles maintains stable levels of N in the new needles during their growth. Furthermore, the initial depletion in the 1 yr old needles (*b01*) started to be replenished before the end of the growth and drought period.

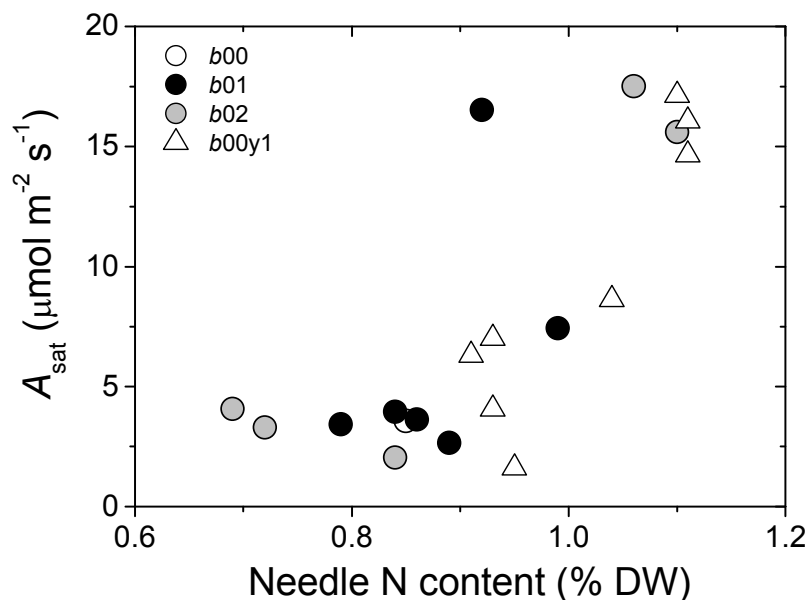


Fig. 4.8 The relationship between A_{sat} and leaf N content, in the samples collected between March 2002 and March 2003 (Fig. 4.5C) and samples collected in 2001 (*b00y1*).

The level of N remobilisation from the mature needles is comparable to that seen in other pine (20-40% in *P. radiata*; Nambiar and Fife 1987) but higher than another Mediterranean species (15% in *Q. ilex*; Cherbury *et al.* 2001). The level of N resorption from the senescing needles

observed in *P. halepensis* in this study was higher than the level of 20-30% observed in unfertilized young (5 yr) *P. halepensis* by Sardans *et al.* (2005), the range of ~35-50% given for selected Mediterranean conifers by Mediavilla and Escudero (2003) and the average value of 47% across 108 evergreen species in the survey of Aerts (1996). Site nutrient levels may influence resorption efficiency, with higher levels of resorption on low nutrient soils (Pugnaire and Chapin 1993, Enoki and Kawaguchi 1999, Sardans *et al.* 2005), although this is not an unequivocal response (Schlesinger *et al.* 1989, Aerts 1996). While total N levels in the soil in Yatir are typical of unfertilized systems, N availability, which is low over summer, is a more relevant parameter than total N for plant N uptake (I. Gelfand, pers. comm.). Therefore it appears that the drought induced decoupling between N availability and N demand for foliage growth drives high rates of N remobilisation and particularly N resorption. The ability of the trees to meet the growth demands through internal reallocation is therefore an important trait ensuring success under these conditions. The retranslocation of N to growing leaves has been found to be relatively independent of soil N availability in other species (Nambiar and Fife 1987, Proe *et al.* 2000) and may therefore represent a general trait important for success in possible climate change induced perturbations to phenology – resource availability relationships.

Physiological implications of N dynamics

The phenologically driven changes in leaf N have the potential to reduce photosynthetic capacity. Lower chlorophyll contents can reduce light absorption and a large proportion of leaf nitrogen is used in the various photosynthetic components, particularly the enzyme Rubisco. The chlorophyll contents observed in the needles in Yatir are comparable to those reported elsewhere for *P. halepensis* (Alonso *et al.* 2001, Martínez-Ferri *et al.* 2000), although Le Thiec *et al.* (2003) and Manes *et al.* (2001) report higher values. The levels are also similar to those of other Mediterranean species, in which reductions were also observed in summer (Ain-Lhout *et al.* 2004). A seasonal reduction in chlorophyll content of 40-50% over summer was also seen in *P. halepensis* by Elvira *et al.* (1998), and reductions were also observed in response to short term drought treatments by Alonso *et al.* (2001) and Manes *et al.* (2001). A seasonal chlorophyll reduction in Mediterranean species is possibly a protection response to reduce the energy load on the leaves in summer (Kyparissis *et al.* 1995) and is discussed in Chapter 5.

The relationship between needle N content and the assimilation rate (A_{sat}) measured on the needles prior to collection (see Chapter 3) is shown in Fig. 4.8. Included in this figure is

data from previous measurements on the *b00* needle cohort, when the needles were 1 year old (TB Lin unpubl. data.). There was a general decline in A_{sat} with leaf N in the leaf N range of about 0.9 – 1.1%, but high A_{sat} rates were also seen when leaf N was ~0.9%. In the lower range of leaf N (less than 0.9%, associated with the remobilisation and growth phase), A_{sat} remained quite constant as leaf N decreased. The lower range of N (i.e. $N < 0.9\%$) is associated with the low, but relatively stable, summer stomatal conductance values when the level of N in the new needles was lower than the older needles, but overall photosynthetic rates did not differ between age classes. The change in A_{sat} over the upper range of leaf N (i.e. $N > 0.9\%$) is associated with the period when stomatal conductance (g_s) is rapidly decreasing due the increase in soil and atmospheric water deficits driving the changes in A_{sat} (see Chapter 2). Overall, the relationships between leaf N, N allocation and assimilation rate indicate that, relative to the limitations imposed by the stomatal conductance, N remobilisation is not further limiting photosynthesis to a great extent, and an important level of coordination exists between phenological and physiological behaviour under drought.

4.3 Summary

Dry summer needle growth in *P. halepensis* is a highly constrained feature with an apparent strong photo-period control over timing and growth rate. By comparison, stem growth is plastic and linked to physiological activity. To maintain growth over summer despite the soil water deficits and low rates of carbon uptake, priority is given in allocating available photosynthate to the growing needles, but some remobilisation of carbon stores may also be used. The high sink demand of leaf phenology reduces carbon allocation to maintenance respiration elsewhere in the tree, which results in metabolism of stored carbon and, for a period in mid-summer, a net loss of carbon by the ecosystem. Leaf growth nitrogen demands are met by remobilisation from mature and resorption from senescing needles, and are largely independent of soil N availability. Reallocation of N does not appear to reduce photosynthetic capacity over summer relative to the stomatal limitations and soil uptake rapidly restores N levels to maximal levels in the wet season. Dry summer leaf phenology has advantages in that there is no lag period to reach maximal canopy photosynthetic potential in the wet season and separation from other phenophases reduces intra-plant competition for carbon. The extent of foliage growth each year is however dependent on conditions during the drought period, and may represent an important sensitivity to future climate change, particularly early spring soil drying.

Chapter 5

Photoprotection strategies and energy allocation in *P. halepensis* during extensive annual drought

5.1 Introduction

The prevailing climatic conditions during the Mediterranean summer are potentially harmful for plants due to the combination of high irradiance with high temperatures and vapour pressure deficit and low soil water availability. In addition to the limitations on productivity due to low rates of carbon assimilation (see Chapter 3), the reduced capacity of CO₂ fixation to provide an adequate sink for the absorbed energy may lead to over-reduction of the light harvesting and electron transport components of the photosynthetic apparatus. Formation of singlet oxygen or reactive oxygen species by transfer of excitation energy from triplet state chlorophyll or the photoreduction of oxygen by both photosystem I and II can result in protein damage and membrane peroxidation, and subsequent impairment of photosynthetic activity (Smirnoff 1993, Long *et al.* 1994, Horton *et al.* 1996).

A number of mechanisms exist that minimize the chances that excess light energy (which is common even under non-stress conditions) is transferred to potentially damaging pathways. However, when the capacity of these processes is exceeded such as during high or prolonged stress, damage, or photoinhibition, may result (Long *et al.* 1994, Werner *et al.* 2002). Protection strategies include mechanisms that both reduce energy transfer to the electron transport chain, and alternative sinks for photochemical energy other than CO₂ fixation. Summer-time reductions in chlorophyll pigments in Mediterranean species, including *P. halepensis* (Elvira *et al.* 1998, see Chapter 4) may be a response to reduce light absorption under an excess radiation load (Kyparissis *et al.* 1995). An important process dissipating excess energy within the chlorophyll pigment bed and reducing excitation pressure on the reaction centres is one mediated by increases in xanthophyll cycle pigments. In the xanthophyll cycle, violaxanthin (V) is de-epoxidated to zeaxanthin (Z) and antherxanthin (A), which are able to dissipate excitation energy from the excited state chlorophyll as heat (Demmig-Adams 1990, Horton *et al.* 1996). This process of thermal energy dissipation requires conformational changes in the photosynthetic membrane that are facilitated by

formation of a thylakoid pH gradient and the product of the PsbS gene (Li *et al.* 2000, Horton and Ruban 2005).

Antioxidants, enzymes and carotenoids capable of scavenging and quenching reactive oxygen species prevent oxidation damage and maintain oxygen reduction as an alternative oxygen sink and energy dissipation pathway (Smirnoff 1993, Hernandez *et al.* 2004). Due to the properties of Rubisco, photochemical energy may also be used for the fixation of O₂ in addition to CO₂ (Ogren 1984). As this process results in the loss of CO₂ in the regeneration of the Calvin Cycle intermediates, it is termed photorespiration and is considered a wasteful process. However, it does provide an additional sink for electrons when CO₂ fixation is restricted, maintaining continual electron flow through the electron transport system and preventing over reduction of the photosynthetic apparatus. Therefore the photorespiratory process is considered to be an important process for maintaining photosystem functionality under drought and other stressful conditions (Kozaki and Takeba 1996, Wingler *et al.* 2000).

In Chapter 3 it was shown that photosynthetic activity is low during the summer drought period but this limitation is primarily due to stomatal closure and periods of activity are maintained each day throughout the drought period. The sustained activity, which is a critical feature for the ongoing survival of the trees, indicates that a degree of protection exists to maintain the photosynthetic apparatus in a functional state and this protection underlies the successful persistence through the long annual drought period. Observations on *P. halepensis* seedlings have shown summertime increases in antioxidants and xanthophyll and other carotenoid pigments under drought and ozone exposure (Elvira *et al.* 1998, Alonso *et al.* 2001), but little information exists on responses under field conditions (Martínez-Ferri *et al.* 2000). Structural and functional adjustments within PSII, such as a reduction in reaction centre density but an increase in electron transport rate per reaction centre, also indicate alternative electron sinks may be important in the response to high light under drought in *P. halepensis* (Manes *et al.* 2001).

From measurements of chlorophyll fluorescence, it is possible to determine the efficiency at which absorbed photosynthetically active radiation (PAR) is used in photochemistry or is dissipated via thermal dissipation mechanism prior to transfer to the electron transport chain. As most fluorescence at ambient temperature originates in photosystem II (Kraus and Weis 1991), the efficiency with which PAR is used in photochemistry is known as the quantum efficiency of PSII, or Φ_{PSII} (Genty *et al.* 1989). Reductions in Φ_{PSII} with increasing light indicate that PAR exceeds the level with which it

can be used in photosynthesis, and may be related to damage to the photosynthetic apparatus or a non-photochemical quenching (NPQ) of excited state chlorophyll by the thermal dissipation mechanisms. The NPQ processes are important for redox control in the chloroplast and maintaining the balance between ATP and NADPH production and demand, particularly under conditions of limited CO₂ assimilation, and measurements of NPQ have often been found to be correlated with changes in the xanthophyll cycle pigments (Björkman and Demmig-Adams 1994).

The conditions during summer that lead to stomatal closure and reductions in CO₂ fixation are the low soil water contents and the high vapour pressure deficits (D) (see Chapter 3). Increasing soil water deficits reduce g_s and assimilation in a progressive manner resulting in low, but relatively stable, photosynthetic capacity over a prolonged period of time. On the hand, atmospheric vapour pressure deficits, while showing an average increase with time through summer, are more variable and may result in more transient and short term effects on photosynthetic capacity. Therefore, the response to the two types of stress, if considered separately, may differ. In order to isolate the atmospheric effects from those of soil water deficits, summertime irrigation was applied to set of trees over two consecutive summers. The irrigation relieved the stress of the soil water deficit, as seen by stable and high pre-dawn water potentials in the irrigated trees (data not shown), therefore it can be assumed that the stress responses observed in the irrigated trees were largely independent of soil water content effects.

This chapter presents the results from a study aimed to investigate the photochemical protection strategies in mature *P. halepensis* trees under the extreme drought and high irradiance experienced in Yatir Forest, through measurements of chlorophyll fluorescence made in conjunction with leaf gas exchange. It was hypothesized that the continuous photosynthetic activity and summer leaf phenology relies on effective photo-protection mechanisms, including investment in pigment mediated dissipation and the reliance on photorespiratory photochemical energy use. It also aimed to determine to what extent, if any, photoinhibitory damage did occur in spite of protection mechanisms due to the extent of the stress period, and the relative importance of the various protection strategies was estimated. Measurements on both irrigated and non-irrigated trees provided an opportunity to determine how the trees respond to the effects of temperature and atmospheric water deficit effects, independent of soil water effects.

5.2 Results and Discussion

5.2.1 Diurnal and seasonal patterns of Φ_{PSII} and energy allocation

Representative examples of the diurnal pattern of photosystem II efficiency (Φ_{PSII}) of mature needles from two points in the season (May and August) are shown in Fig. 5.1. The initial points of each curve are from pre-dawn measurements, and are therefore the dark adapted F_v/F_m values, or maximal efficiencies. The results from all of the measurements made between December 2001 (low stress period) and October 2002 (end of the drought period) are summarised in Fig. 5.2. This figure shows the pre-dawn (PD), midday minimum (MD) and evening (EV, at the end of the photoperiod) values of Φ_{PSII} for each measurement day for current (y0) and 1 yr old (y1) needles of both the control and irrigated trees.

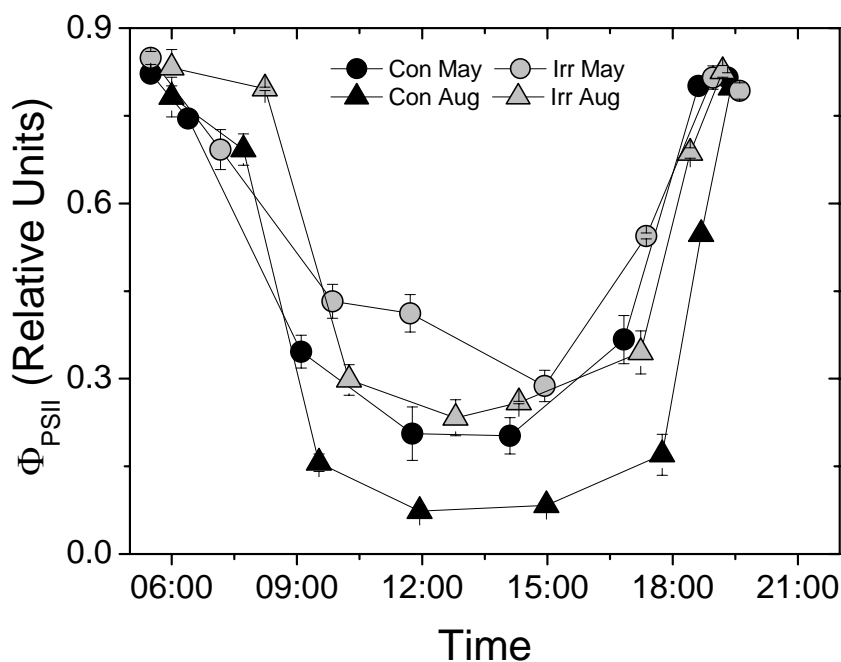


Figure 5.1 Examples the diurnal time courses of photochemical efficiency of photosystem II (Φ_{PSII}) from May and August in the control and irrigated trees.

In both the control and irrigated trees and both y0 and y1 needles the PD values remained high, at about 0.8, at all times of the year, indicating no long term damage to the photosynthetic apparatus due to the high energy loads during the drought period. The first measurements on the new needles for 2002 were made in July (i.e. the y0 age class in Dec-May became y1 for Jul-Oct, and the y0 class from then are the developing needles for Jul-Oct.). There were large reductions in Φ_{PSII} in all samples during the day, consistent with the increase in radiation load.

In the case of the MD minimum Φ_{PSII} values there were significant differences between the irrigated and control samples at each measurement date (Table 4.1, Fig. 5.2). The highest MD Φ_{PSII} values in the non-irrigated samples of ~ 0.2 were observed in December and May and the lowest values of 0.06 were observed in October. The irrigated samples, on the other hand, had MD values between 0.2-0.3 for most of the year (except for y0 needles in December, at 0.46). In most instances, differences in the MD Φ_{PSII} between age classes of the same treatment were not significant (Table 4.1). When significant differences were observed, the y0 needles had the higher Φ_{PSII} .

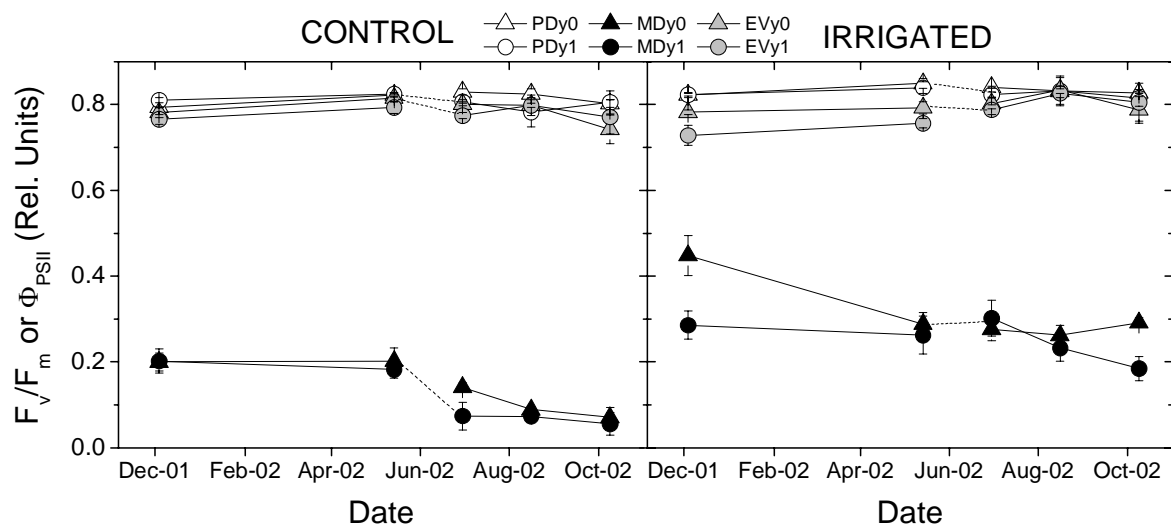


Figure 5.2 Seasonal changes in pre-dawn (PD, or F_v/F_m), midday (MD) and end of day (EV) Φ_{PSII} values determined from diurnal time courses depicted in Fig. 4.1, for both age classes and both treatments. The gap in the lines indicate the change in age class (first measurement of new, or y0, needles), with the dotted line connecting the needles of the same cohort (i.e. y0 in 2001 and y1 in 2002).

However, despite the reductions in Φ_{PSII} during the day, PSII efficiencies always returned to near pre-dawn values by the end of the photoperiod (Fig. 5.2, EV data). This indicated that there were no more than transient or short-term dynamic adjustments or very mild damage to PSII under the high radiation loads during the peak radiation hours. This supports the view that PSII is relatively stable under drought (Havaux 1992, Yordanov *et al.* 2000). Furthermore, it is also evident that the functionality of the photosynthetic apparatus in these trees is not impaired by development under high light and drought conditions, as was clearly demonstrated in the non-irrigated new needles that developed during the stressful summer period.

Light that is absorbed by the photosynthetic apparatus can be partitioned into that used in photochemistry (electron transport), that which is dissipated by non-radiative (thermal)

mechanisms and any remainder, termed an ‘excess’, that is not effectively used in photochemistry or dissipated through the non-photochemical dissipation mechanisms, and therefore has the potential for oxidative damage (Demmig-Adams *et al.* 1996, see Chapter 2). An example of the daily time course of the allocation of absorbed light energy between Photochemistry, thermal Dissipation processes and the remaining Excess is shown in Fig. 5.3. From this data, the relative allocation of absorbed PAR to each process for the whole day was determined by integration for and is shown in Fig. 5.4. The results presented are from four dates between May and October for each age class and treatment.

Table 4.1 The ratio of mean MD Φ_{PSII} between treatments (Irr/Con) and age classes (y0/y1) at different periods in the year. Asterisks indicate the level of significance in the difference between the means (* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$).

Month	Treatment				Age			
	y 0		y 1		Control		Irrigated	
	Irr/Con	sig.	Irr/Con	sig.	y 0/y 1	sig.	y 0/y 1	sig.
Dec	2.31	***	1.41	*	0.99		1.61	**
May	1.42	*	1.45	*	1.11		1.08	
Jul	1.95	***	4.08	***	1.91	*	0.91	
Aug	2.94	***	3.20	***	1.23		1.13	
Oct	4.10	***	3.29	*	1.27		1.58	*

The overall seasonal decrease in MD Φ_{PSII} (Fig. 5.2) equates to a decrease in the photochemical allocation of absorbed light energy from 42% in May to 25% in August in the non-irrigated trees. Over the same time, the allocation to photochemistry declined from 51% to 44% in the irrigated trees, while the allocation to dissipation increased from 45% to 61% and 35% to 43% in the control and irrigated trees, respectively (see Table 5.2). These allocation values represent the averages of the y0 and y1 age classes and the difference between the age classes was, on average, less than 5%. The decrease in photochemical allocation in the irrigated trees in August represents a decrease of 13.5% from the May value, and is an indication of the effect that the summer conditions (hot, dry atmosphere and high radiation) have on reducing photosynthetic light use efficiency, regardless of soil water content. The relative decrease in the non-irrigated trees over the same period was 40%, or three times that in the irrigated trees. However, all reductions in photochemical allocation (between treatments, age classes and dates) were effectively balanced by proportional increases in thermal dissipation, and the Excess parameter remained a relatively constant at 12 -14% of total absorbed energy across all samples for all dates.

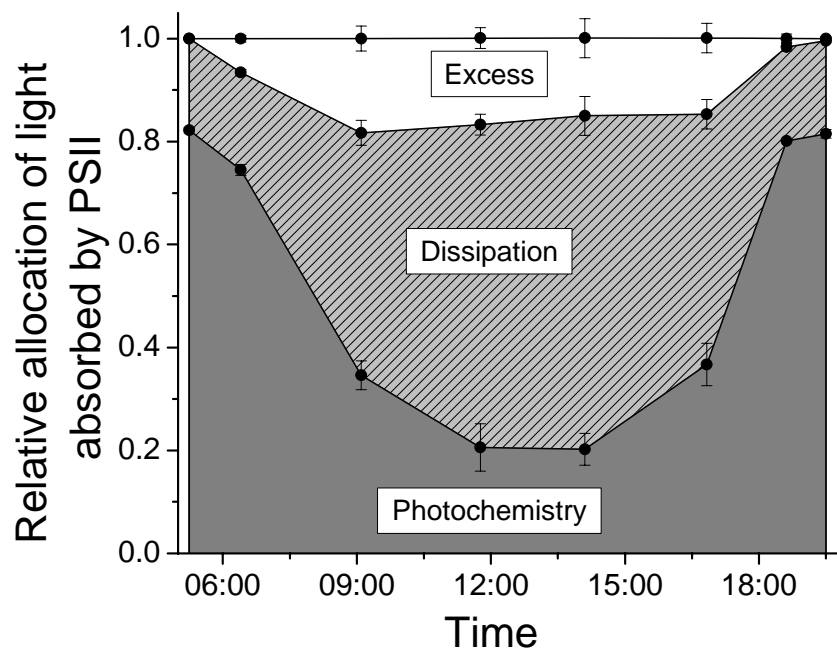


Figure 5.3 An example of the diurnal time course of the relative allocation of light energy absorbed by PSII to the various pathways as determined from the chlorophyll fluorescence measurements.

Seasonal and diurnal Φ_{PSII}

Similar long term stability in the F_v/F_m has been seen in the pre-dawn measurements of other tree species growing in the Mediterranean (Faria *et al.* 1998), and dynamic diurnal regulation of Φ_{PSII} , with complete recovery by the end of the day, has also been observed in a number of tree and shrub species (Damesin and Rambal 1995, Valentini *et al.* 1995, Castillo *et al.* 2002, Levizou *et al.* 2004). However, seasonal reductions in photosystem II performance have been observed in a number of Mediterranean tree, shrub and grass species (Faria *et al.* 1998, Fernández-Baco *et al.* 1998, Zunzunegui *et al.* 1999, Castillo *et al.* 2002, Llorens *et al.* 2003).

Differences in drought duration and extent of rooting depth (and therefore access to water) may underlie many of the different responses between species (Faria *et al.* 1998, Castillo *et al.* 2002). While access to water (in the irrigation samples) improved PSII performance in summer, it is clear from the data presented here that *P. halepensis*, growing on shallow soil and experiencing a long period without rain, was able to maintain PSII functionality over the entire summer season. Although daytime Φ_{PSII} values were higher in the irrigated trees than the non-irrigated trees in summer, irrigated summer time values were lower than winter time values. Therefore, even when soil water content was not limiting, the trees were unable to completely utilize the increased energy load in summer for photochemistry.

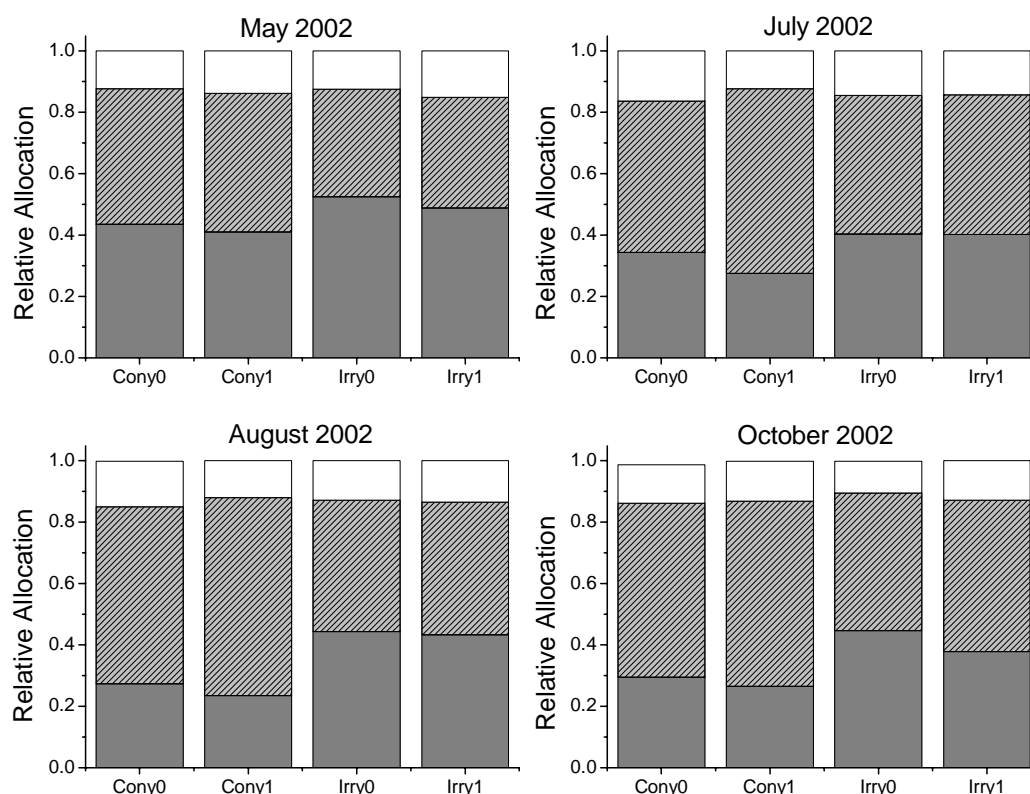


Figure 5.4 Daily integrated allocation of absorbed light between photochemistry, thermal dissipation and excess processes at four points in the drought season, determined from diurnal time courses represented in Fig. 5.3. For each date, individual bars are for a treatment (control: Con; irrigated: Irr) and needle age class (y0 or y1).

Partitioning and regulation of absorbed light energy

The maximum levels of allocation to photochemistry observed in this study are similar to the range (40 and 60%) typical for species growing under non-stressed conditions in the sun (Demmig-Adams *et al.* 1996, Faria 1998) or for Mediterranean species in spring (Oliveira and Peñuelas 2001). The maximum values of allocation to thermal dissipation in summer photochemical allocation were lower (and allocation to photochemistry higher) than values reported for Mediterranean species after a period of drought (67-77%; Faria *et al.* 1998) or dryland species under high temperatures with a favourable water supply (Shirke and Pathre 2003). Therefore it is evident that *P. halepensis* maintained a relatively high level of energy allocation to photochemistry considering the extent of the summer drought.

The reductions in photochemical allocation were effectively balanced by increases in thermal dissipation in all samples, and the proportion of excess remained constant. The Excess parameter observed is similar to that seen in other Mediterranean and arid region species (Faria *et al.* 1998, Oliveira and Peñuelas 2001, Martínez-Carrasco 2002, Shirke and Pathre 2003). A relatively stable Excess value is commonly seen at both seasonal and diurnal

cycles in plants and indicates the coordination between photochemical and thermal dissipation mechanisms. A regulation between photochemical and thermal dissipation pathways is of selective advantage for plants, particularly under stressful conditions, as it reduces excitation energy reaching closed reaction centres, minimizing the lifetime of chlorophyll excitation and therefore the production of potentially harmful triplet state chlorophyll (Laisk *et al.* 1997, Kornyejev *et al.* 2004).

While there has been some criticism of what the Excess parameter in the formulation of Demmig-Adams *et al.* (1996) as used in this study represents (Hendrickson *et al.* 2004, Kramer 2004), it has been shown to correlate with the rate of photoinactivation under photoinhibitory conditions (Kato *et al.* 2003), and was used to provide an indication of any break down in the coordination between photochemical and thermal dissipation processes under the stressful summer conditions. However, it is clear that the lack of any chronic photoinhibition during summer was due in large part to the effective up-regulation of thermal dissipation balancing the down-regulation of photochemistry.

5.2.2 Rates of the dissipation processes

As Φ_{PSII} and the Dissipation parameters are efficiencies of light use, the rates of light energy dissipation via either photochemistry (PDR) or thermal dissipation (TDR) can be calculated as (Demmig-Adams *et al.* 1996):

$$PDR = \Phi_{PSII} \times PAR \quad (5.1)$$

$$TDR = TD \times PAR \quad (5.2)$$

where TD is the allocation to thermal dissipation and PAR is photosynthetic active radiation, in $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$.

The photochemical and thermal dissipation rates in relation to incident PAR are shown in Fig. 5.5. There was in general similarity between dates within a treatment, but the response differed between treatments. In the control trees there was little increase in the rates of photochemistry as PAR increased above about $900 \mu\text{mol m}^{-2} \text{ s}^{-1}$ and rates of thermal dissipation exceeded photochemistry when PAR was above about $500 \mu\text{mol m}^{-2} \text{ s}^{-1}$. At maximal PAR rates of photochemistry were only ~20% those of thermal dissipation. Under irrigation, rates of photochemistry and thermal dissipation were similar up to a PAR of about $900 \mu\text{mol m}^{-2} \text{ s}^{-1}$, whereupon photochemistry started to saturate and thermal dissipation rates increased proportionally more. At the highest PAR, photochemistry rates were still about 50%

those of thermal dissipation, and nearly double those of the non-irrigated trees. A more quantitative analysis of photochemical energy utilisation is provided by analysis of electron transport rate (ETR):

$$\text{ETR} = \Phi_{\text{PSII}} \times \text{PAR} \times 0.5 \times \alpha \quad (5.3)$$

where α is the absorption coefficient for the leaves, which was determined from chlorophyll contents (see below). Higher Φ_{PSII} values in the irrigated trees resulted in electron transport rates exceeding those of the non-irrigated trees when PAR was above about $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Fig. 5.5). As can be inferred, there was a greater seasonal reduction in light saturated ETR in the control trees, from about $150 \mu\text{mol m}^{-2} \text{s}^{-1}$ in December to $50\text{--}75 \mu\text{mol m}^{-2} \text{s}^{-1}$ in July–October. Maximal ETR in the irrigated samples was about $250 \mu\text{mol m}^{-2} \text{s}^{-1}$ in December and about $150 \mu\text{mol m}^{-2} \text{s}^{-1}$ in the summer months.

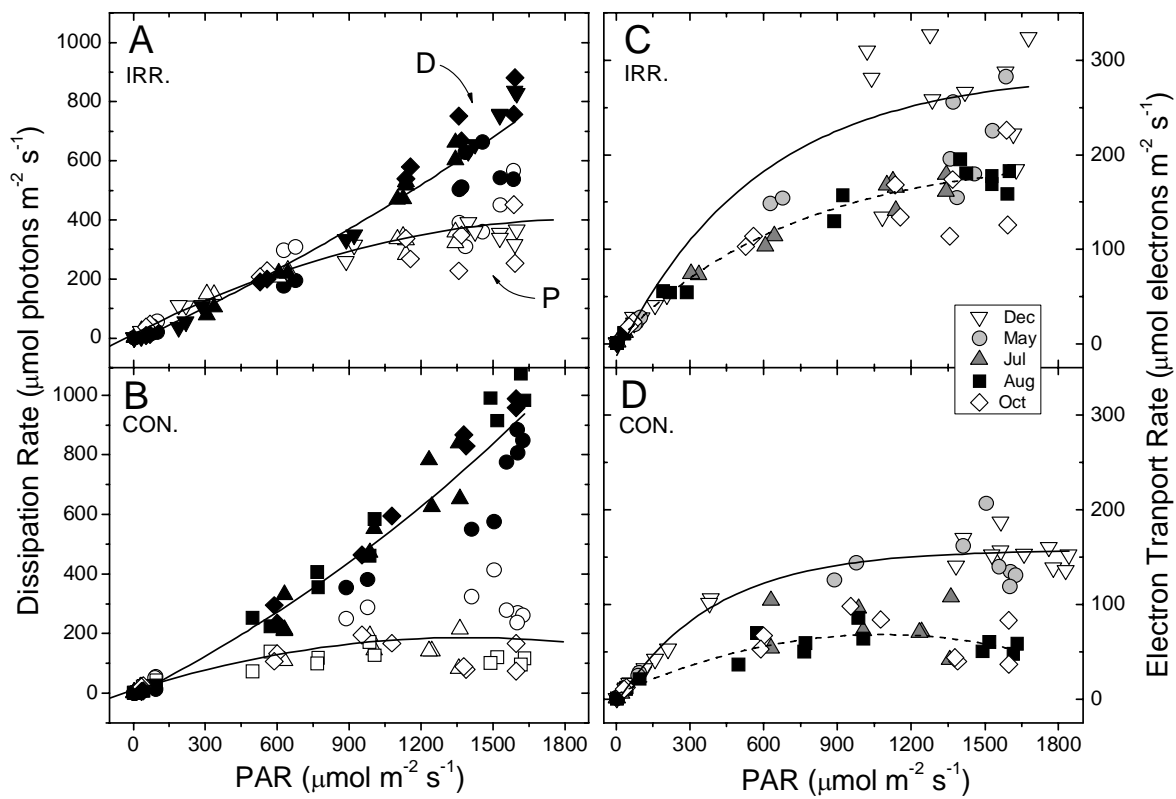


Figure 5.5 *Left panel:* PAR response of estimated rates of energy dissipation by photochemistry (P, open symbols) and thermal dissipation (D, solid symbols) in the irrigated (A) and control (B) samples. Different symbols represent data from different dates (May: circles; July: triangles; August: squares; October: diamonds) of both age classes. Curves are fits to all data combined. *Right panel:* PAR response of ETR in the irrigated (C) and control (D) samples of both age classes from different dates, as indicated in the legend. Curves are fits to the December (solid line) and August (dashed line) data of both age classes combined.

Gas exchange measurements were also made on the trees that received irrigation, and a suppression of photosynthesis was also seen in these trees over summer (TB Lin, unpubl. data). A_{sat} rates in the irrigated trees were higher than the non-irrigated trees for much of the year, but declined to minimums of $\sim 7 \mu\text{mol m}^{-2} \text{s}^{-1}$ in midsummer from maximum rates in winter-spring that were similar between the irrigated and control trees ($15 - 20 \mu\text{mol m}^{-2} \text{s}^{-1}$).

From the relationship between PSII electron transport rates and CO_2 fixation, rates of photorespiratory CO_2 release (R_i) can be calculated according to (Valentini *et al.* 1996):

$$R_i = J_o / 8 \quad (5.4)$$

where J_o is the rate of electron flow to photorespiration, and is calculated as:

$$J_o = 2/3(ETR - 4(A + R_F)) \quad (5.5)$$

where A is the net rate of CO_2 assimilation and R_F is the rate of foliage dark respiration, measured at the end of the day after sundown.

The relationship between R_i and incident PAR is shown in Fig 5.6. Overall, photorespiratory CO_2 release was greater in the irrigated trees as a result of the overall higher electron transport rates in the irrigated samples. However, the ratio of electrons used in photorespiration as a proportion of the total electron transport (J_o/ETR), determined under saturating light conditions, differed significantly between treatments (Fig. 5.7). When soil water content was not limiting (Dec), there was little difference between treatments, and J_o/ETR was estimated at $0.50 (\pm 0.017)$ and $0.48 (\pm 0.015)$ in the control and irrigated trees, respectively. This increased to $0.64 (\pm 0.009)$ in the control and $0.56 (\pm 0.008)$ in the irrigated trees in August, representing a 27% and 17% increase in J_o/ETR relative to the December values for the control and irrigated trees, respectively (see Table 5.2). The proportional allocation of electrons to photorespiration in midsummer (July and August) in the non-irrigated trees was 13% higher than the irrigated trees.

Rates of dissipation and electron transport

More energy was dissipated thermally than was used in photochemistry in the non-irrigated samples at most light levels and the rate of photochemical dissipation was saturated at relatively low light levels. Therefore, for much of the day, the rates of energy dissipation prior to transfer to electron transport exceeded the flux directed to photochemistry (Fig. 5.4). Under irrigation the rates of photochemical dissipation were higher than the control trees and were similar to, but did really not exceed, the rates of thermal dissipation up to the level close to saturating PAR ($\sim 900 \mu\text{mol m}^{-2} \text{s}^{-1}$). These responses suggest that when the potential for

high CO₂ fixation is maintained, as in the irrigated samples, energy is relatively equally distributed between photochemical and non-photochemical dissipation until the photochemical capacity is saturated. When the reduction in potential maximum CO₂ fixation rates, as occurs with soil water deficits, energy allocation is shifted away from photochemistry at all but the lowest light levels.

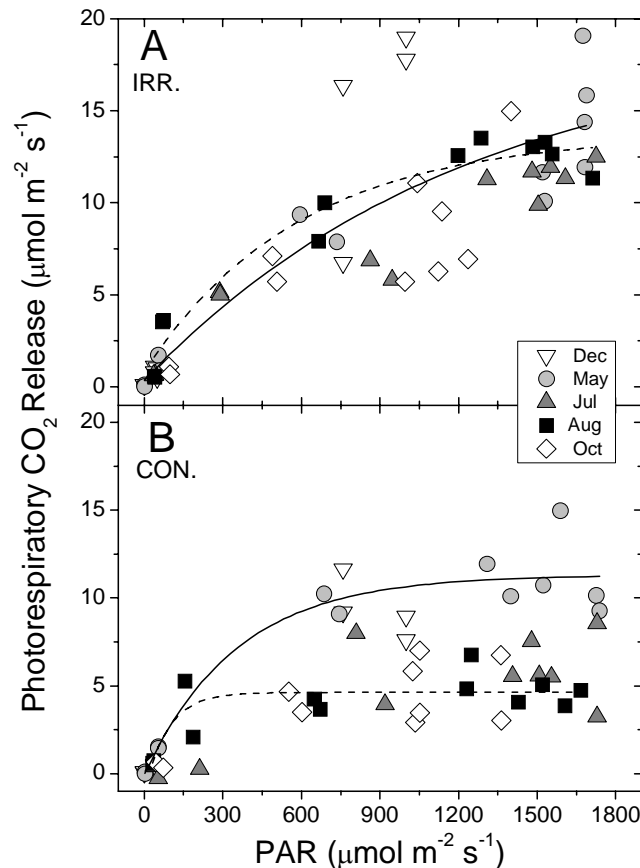


Figure 5.6 PAR response of the photorespiratory CO₂ release in the irrigated (top) and control (bottom) samples. Different symbols are data from different dates, as indicated in the legend, from both needle age classes. Curve fits are to the May (solid line) and August (dashed line) samples of the age classes combined.

In both irrigated and control trees, electron transport rates were lower in summer than winter months, consistent with the depression of CO₂ fixation rates that was observed in both treatments over summer. However, in the non-irrigated trees the light level at which maximal rates of electron transport were observed was also lower in summer, with even a decrease in ETR at high light levels. This may reflect an acclimation to the period of maximal photosynthetic activity, which was confined to morning and afternoon hours in the summer (see Chapter 3), and further illustrates the regulatory processes that exist between the thermal dissipation and photochemical processes. In summer months, as light levels increased during

the day, CO₂ fixation rates decreased. It appears that a proportional increase in thermal dissipation rates reduced the overall electron transport rates at the higher light levels, preventing back pressure on the electron transport chain due to reduced rates of regeneration of NADPH and ATP from the lower rates of CO₂ fixation.

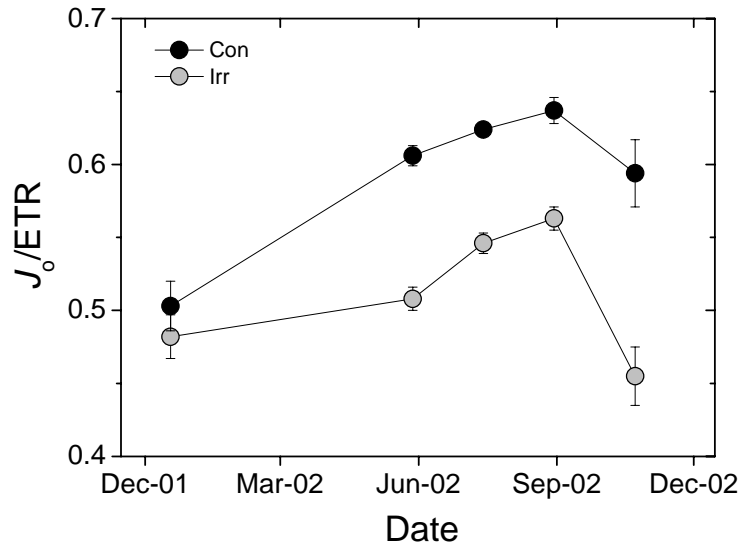


Figure 5.7 The proportion of total electron transport used in oxygenation in the control (Con) and irrigated samples (Irr) under light saturated conditions at different points in the season.

The seasonal range of electron transport rates observed in the control trees is similar to that observed in other Mediterranean species (Valentinin *et al.* 1995, Llorens *et al.* 2003, Ogaya and Peñuelas 2003) growing in locations where annual precipitation is about double, and the rain free period about half (or less), of that in Yatir. The summer time rates in the control trees were about 50% lower than those of *P. halepensis* estimated from summer time measurements made in the field under a lower degree of drought stress by Martínez-Ferri *et al.* (2000) but much higher than the rates ($\sim 10\text{--}35 \mu\text{mol m}^{-2} \text{s}^{-1}$) in saplings of other Mediterranean species under conditions of severe drought stress (Filella *et al.* 1998). The relative reductions in CO₂ assimilation rates in summer were much greater, however, than the reductions in ETR in the control trees (data not shown). Therefore, while the CO₂ fixation was highly depressed at midday in summer, the capacity for electron transport through PSII was maintained relatively high.

The role of photorespiration in energy dissipation

A relatively greater reduction in CO₂ assimilation compared to ETR implies that, in order to sustain continued linear electron transport through the photosystems, other processes

regenerating NADPH and ATP or alternative electron sinks are required. An important process that is involved in the energy dissipation is photorespiration. The analysis of photorespiration rates made here assumes that processes such as the Mehler reaction (involving oxygen reduction) play a minor role under both irrigation and drought conditions (Cornic and Briantais 1991, Flexas and Medrano 2002). However, as accurate measurements of this process are difficult to make, and some estimates have been as high as 30% of total electron flux (see Ort and Baker 2002 for review), it is unknown to what extent this, and other alternative electron sink processes, contribute to the energy dissipation.

Increased photorespiration comes at a cost in terms of carbon, however, due to the release of CO₂ during glycine decarboxylation, reducing net CO₂ assimilation rates and productivity. The estimates of CO₂ release from photorespiration in summer were equivalent to the rates of CO₂ uptake in the less stressful periods, and up to an order of magnitude greater than the concurrent net assimilation rate. Rates of photorespiratory CO₂ release have also found to be similar to or exceed net CO₂ uptake other Mediterranean and savannah species, and were considered as a potentially important limitation for leaf carbon balance under high irradiances (Valentini *et al.* 1995, Franco and Lüttge 2002). Therefore it is interesting to note that the rates of photorespiratory release were also high in the irrigated samples. In absolute terms, they exceeded the rates from the control trees, and were up to 1.5-2 times the rates of net CO₂ assimilation in summer. These high estimates may indicate that rates of photorespiration were over estimated, and that other reduction processes have some role in the overall energy dissipation (Ort and Baker 2002). A proportion of the summer increase in photorespiration rates may be related to the differential temperature sensitivity of oxygenation and carboxylation. Due to both a decrease in the solubility of CO₂ relative to O₂ in water and a decrease in the CO₂/O₂ specificity of Rubisco with increasing temperature, oxygenation rates increase proportionally more than carboxylation with temperature (Brooks and Farquhar 1985). This would affect both irrigated and control trees as the seasonal changes in leaf temperature were relatively similar for both treatments.

Despite high absolute photorespiration rates in the irrigated trees, the estimated allocation of electrons to photorespiration, as a proportion of the total electron transport, was higher in the non-irrigated trees, reaching 0.64 and 0.56 in late August, respectively. These values are similar to, or slightly higher than the estimates of 0.4-0.5 for a Mediterranean *Quercus* species (Valentini *et al.* 1995) and 0.4-0.6 for an exposed stressed herbaceous species (Muraoka *et al.* 2000). These results support the notion that photorespiration has an important photoprotection role under stressful conditions, including drought, serving as an

sink for excess energy and possibly providing metabolites (glycine, serine) for antioxidant systems (Osmond 1981, Cornic and Briantais 1991, Wingler *et al.* 2000). Importantly, the high rates of photorespiration were also evident when soil water content was not limiting but high temperatures and atmospheric water deficits were limiting photosynthesis.

5.2.3 Non-photochemical quenching and xanthophyll pigments

An important component of the thermal dissipation processes is the thylakoid pH mediated xanthophyll cycle. The non-photochemical quenching (NPQ) parameter derived from chlorophyll fluorescence is a measure of the dynamic light dependant components of the overall thermal dissipation mechanisms and as such is often correlated with the changes in xanthophyll cycle pigments (Demmig-Adams 1990, Björkman and Demmig-Adams 1994). Fig. 4.9 shows the daily time course of NPQ and the de-epoxidated state of xanthophyll pigments (DPS, where $DPS = [V+A]/[V+A+Z]$) for three dates in May August and October for y1 needles of both treatments (xanthophyll pigment data from TB Lin, unpubl. data). There was a high correlation between the NPQ and DPS parameters at each date (Fig. 4.9), particularly during the higher stress periods (August and October).

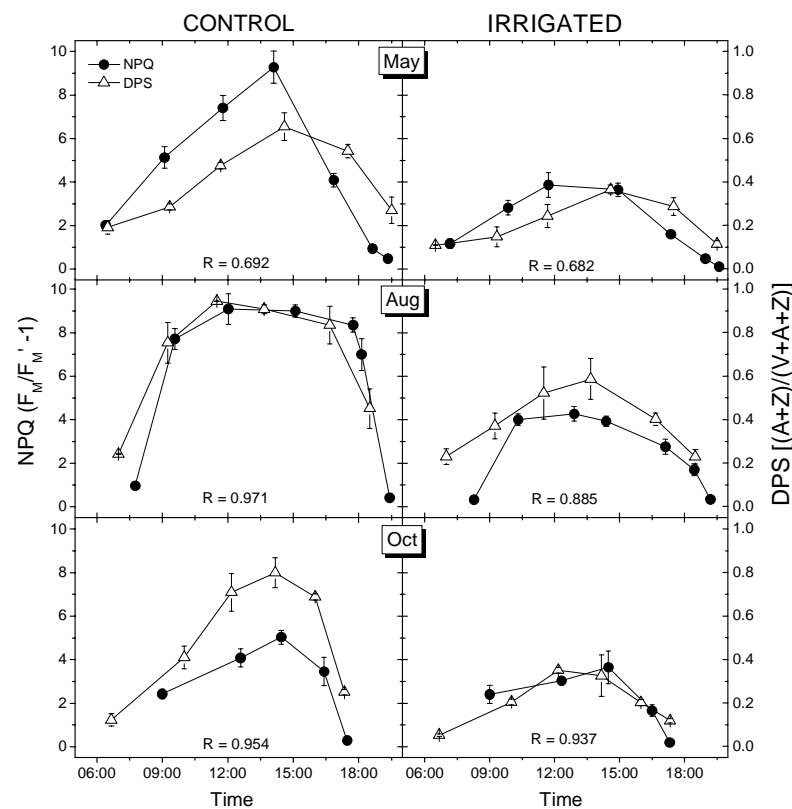


Figure 5.8 Diurnal time courses of non-photochemical quenching (NPQ) and the de-epoxidated state of xanthophyll cycle pigments (DPS) for three points in the dry season. The correlation coefficients (R) for the NPQ-DPS relationship are indicated in each panel.

In addition to xanthophyll pigment changes, there were seasonal reductions in total chlorophyll content in both irrigated and non-irrigated trees similar to the changes described already in Chapter 4 (data not shown, TB Lin, unpubl. data). Total chlorophyll content decreased by 53% in the control and 46% in the irrigated samples between March and August. Chlorophyll content can be converted to the fraction of PAR absorbed by the leaf (absorbance, α) according to the equation (Evans and Poorter 2001):

$$\alpha = \chi / (\chi + 76) \quad (5.6)$$

where χ is chlorophyll content per unit leaf area ($\mu\text{mol m}^{-2}$). The estimates of leaf absorbance decreased from 0.87 to 0.76 (control) and from 0.89 to 0.81 (irrigated) between March and October. The seasonal (March-October) reduction in chlorophyll content represented about 13% and 8% less of the incident PAR being absorbed by the needles, in the control and irrigated samples, respectively, and therefore represents an important initial step in reducing in the energy load on the leaves in the summer.

5.2.4 The relative importance of the dissipation processes under contrasting conditions

A summary of the seasonal change in each of the processes investigated in this study, together with parameters from gas exchange measurements, is presented in Table 5.2. This table lists the spring (May) and summer (late August) values for each parameter of both irrigated and control samples, the spring – summer difference of each parameter, the relative difference this change represents (percent change from the spring values) and the ratio of Control/Irrigation values for each parameter in summer.

The data from Table 5.2 were used to estimate the relative importance of each process in dissipating or utilising incident light energy under three principal conditions. The three conditions considered were: low water stress (from spring data from the irrigated trees); with atmospheric but without soil water deficit (mid-summer in the irrigated trees); and with both atmospheric and soil water deficit (mid-summer in the non-irrigated trees). This analysis involved estimating the distribution of light energy incident at the leaf surface among the various processes under the three conditions. The results are shown in Fig. 5.9 in a schematic representation of the processes involved.

A number of interesting features emerge from this analysis. In all parameters there was a seasonal change in the irrigated samples that was of the same direction as the non-

irrigated samples, but the relative change was lower in magnitude and differed between parameters. Therefore the warmer conditions and higher atmospheric water deficits are an important factor that influence productivity and induce light protection responses even when soil water is not limiting, but the overall response under summer drought depended on the nature and extent of the drought stress.

Table 5.2 The spring and summer values of the various photosynthesis related processes, the absolute and relative change in these parameters, and the estimated effect that the low soil water content (SWC) has on the seasonal change in the non-irrigated trees, as a percentage of the total change.

	Parameter value				Seasonal Difference				
Process	Control		Irrigated		Summer - Spring		Rel. Change (%)		Summer
Units	Spring	Summer	Spring	Summer	Control	Irrigated	Control	Irrigated	Con/Irr
Photoprotection									
Absorbance (fraction of PAR)	0.874	0.799	0.887	0.821	-0.074	-0.066	-8.5	-7.4	0.974
Energy allocation (% absorbed PAR)									
Photochemistry	42.3	25.4	50.7	43.9	-16.91	-6.86	-40.0	-13.5	0.578
Thermal Dissipation	44.7	61.3	35.5	43.1	16.51	7.53	36.9	21.2	1.423
Excess	13.0	13.4	13.8	13.1	0.31	-0.68	2.4	-4.9	1.019
Photorespiration (J _o /ETR)	0.503	0.637	0.482	0.563	0.134	0.081	26.7	16.9	1.131
Gas Exchange									
Maximal g _s (mol m ⁻² s ⁻¹)	0.286	0.016	0.349	0.076	-0.270	-0.273	-94.3	-78.4	0.215
A _{sat} (μmol m ⁻² s ⁻¹)	15.23	0.849	18.31	7.44	-14.38	-10.88	-94.4	-59.4	0.114
Carbon gain (mg C m ⁻² day ⁻¹)	867.1	32.7	990.8	426.2	-834.4	-564.6	-96.2	-57.0	0.077

Chlorophyll content and light absorbance

Reductions in light absorbance through changes in chlorophyll content were similar between the treatments and relatively small compared to the changes in the other parameters. However, in summer, up to 20% of the incident light did not enter the leaf. As has been discussed, photochemistry decreased and thermal dissipation and photorespiration increased in summer in both treatments, but the proportional change in thermal dissipation was greater than the increase in photorespiration for both treatments. Under low stress, 32% of the incident light was thermally dissipated and 45% was used for photochemistry, which was equally partitioned between photorespiration and CO_2 fixation. In summer, the allocation of incident PAR to photochemistry decreased both with (to 20%) and without (to 36%) soil water deficit. In the summer irrigated trees, the reduction in the amount of incident light

absorbed was similar to the reduction in the amount of incident light used in photochemistry, hence the amount dissipated thermally showed only a small increase (Fig. 5.9). This suggests that the changes in chlorophyll content are coordinated with photosynthetic capacity and are an important feature of the photoprotection response under these conditions (Kyparissis *et al.* 1995).

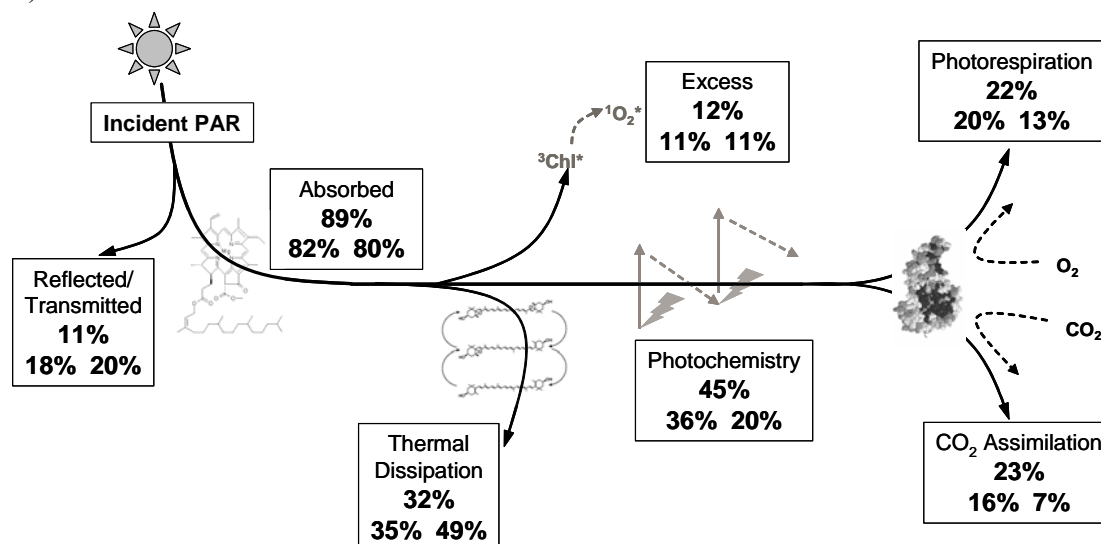


Figure 5.9 Schematic view of the processes involved in the utilisation and dissipation of light energy. The values in the boxes are estimates of the fraction of incident PAR directed to each process under three conditions. The upper value is spring (low stress) conditions, the lower left is with high summer soil water content (atmospheric deficit only) and the lower left value is low summer soil water content (soil and atmospheric water deficits).

Thermal Dissipation and Photorespiration

In the summer irrigated trees the proportion of incident light energy used in photorespiration remained similar between spring and summer (22-20%). Under both atmospheric and soil water deficit, the estimated proportion of light energy dissipated thermally increased to 49% (from 32%), and the proportion ultimately used in photorespiration decreased to 13% (from 22%). Therefore, with both soil and atmospheric water stress, greater emphasis was placed on the thermal dissipation mechanisms to dissipate energy than the utilisation of photorespiration. Under soil drought, CO₂ fixation capacity decreased in a more long term and stable manner compared with the more variable limitations due to only atmospheric water deficit. The greater reliance on thermal dissipation mechanisms with increased stress suggests that the costs involved with thermal dissipation are less than those associated with photorespiration when CO₂ fixation rates are consistently low. For example, only a few molecules of zeaxanthin per reaction centre may be sufficient for

effective thermal dissipation (Bukhov *et al.* 2001). It is also interesting to note that while there was a reduction in the proportion of incident light used in CO₂ fixation in the irrigated trees in summer (16%), the proportion was only slightly less than that used in photorespiration (20%, Fig. 5.9). The thermal dissipation mechanisms prevent light energy being used for both CO₂ fixation and being ‘wasted’ through photorespiration, indicating that the cost of the greater reduction in CO₂ fixation capacity relative to photorespiration under both soil and atmospheric drought may induce the up-regulation of the thermal dissipation mechanisms. This implies that the notion that photorespiration is an important pathway for energy dissipation under high light conditions (Osmond 1981, Winger *et al.* 2000) may depend on the extent of the concurrent limitations to CO₂ fixation.

Implications for productivity

In terms of potential productivity, we can see from the gas exchange data that A_{sat} , maximal g_s and daily total leaf level C gain was estimated to be about 95% lower in summer than spring in the non-irrigated trees, i.e. all got close to zero (Table 5.2). In the irrigated trees, on the other hand, while maximal g_s declined by nearly 80%, A_{sat} and total C gain declined by about 60%. The difference in the proportional change in g_s and C gain between treatments can be attributed to the fact that the assimilation response to g_s is non-linear (see Fig. 3.4), with a proportionally greater reduction in assimilation with reductions in g_s as g_s declines below the inflection point of the relationship. In summer it was estimated that 16% of the incident light on the leaf was used for CO₂ fixation when soil water content was not limiting, but only 7% was used under both soil and atmospheric water deficit (Fig. 5.9). Therefore, when the combination of stresses reduces stomatal conductance to increasingly lower levels, there is a proportionally greater reduction in the rates of CO₂ fixation. These results show that interactive effects of both soil and atmospheric water deficits are crucial features determining ecosystem productivity under drought conditions.

Despite the greatly reduced rates of carbon gain during drought, the fact that both no chronic or long term photoinhibition was observed, and a dynamic diurnal regulation of energy utilisation was apparent through the long period of water stress has positive implications for tree productivity and survival at this study site. Sustained photoinhibition effects have been seen to lead to decreased plant productivity and negatively affect plant distribution under semi-arid conditions (Werner *et al.* 2001, Valladares *et al.* 2005). In addition, the afternoon recovery of Φ_{PSII} and long term stability of the photosynthetic

apparatus enabled the periods of early morning and late afternoon to be exploited for carbon gain (see Chapter 3). Although low, the morning and afternoon photosynthetic rates were enough to maintain a net daytime CO₂ uptake at the ecosystem level in these periods, even in the middle of summer. Furthermore, having a completely functional photosynthetic apparatus minimized the time required to up-regulate the photosynthetic system to take advantage of improved conditions when the first rains arrived, maximising carbon gain over the entire wet season.

5.3 Summary

Overall, it is clear that a critical feature of the ability of *P. halepensis* to survive even the extreme drought conditions experienced in Yatir, at close to the limit of its natural distribution, is the efficient protection of the photosynthetic system against light stress for a prolonged period of time. This was conferred by adjustments and regulation at various levels in the photosynthetic pathway, the net result of which was an apparent lack of any photoinhibitory damage to the photosynthetic apparatus over the extensive drought period. The lack of photoinhibition was apparent in both the mature and the new needles that developed during the drought period, and underlies the ability for photosynthetic activity in more favourable periods that is observed through the drought period.

There were reductions in the amount of light initially absorbed by the needles as irradiance levels increased in summer. The increase in the xanthophyll cycle mediated thermal dissipation process was a critical feature that dissipated the majority of excess energy absorbed by the trees under that experienced both soil and atmospheric water deficits. As a proportion of total electron transport, photorespiration was a greater energy sink in summer than the less stressful periods, but absolute rates of photorespiratory CO₂ release were reduced due to the pigment bed energy dissipation. In the trees that only experienced atmospheric water deficits, less emphasis was placed on thermal dissipation mechanisms, therefore maintaining the potential for higher CO₂ fixation. Consequently, photorespiration accounted for a greater proportion of the energy use than in the non-irrigated trees, but it was still less than the non-photochemical processes. It is concluded that energy dissipation prior to photochemical electron transport is a more cost effective protection strategy than reliance on photorespiration, but the relative distribution of energy between dissipation and photochemistry may depend on the potential CO₂ fixation capacity under the environmental conditions.

Chapter 6

Foliage and stem respiratory CO₂ fluxes and $\delta^{13}\text{C}$ composition in *P. halepensis*

6.1 Introduction

Respiration is a key factor in plant and ecosystem productivity (Amthor 1989, Valentini *et al.* 2000). The process of respiration involves oxidising photo-assimilated carbon substrates to provide the energy and intermediates for biosynthesis and function. Hence flux rates through the respiratory pathways are by necessity the most significant after photosynthesis. During the conversion of photosynthate to structure and metabolites, anywhere between 35 and 80% of assimilated CO₂ is released back to the atmosphere (Amthor 2000). The rate at which CO₂ is released (respired) therefore provides an integrated estimate of metabolic demands at any time. The proportion of the assimilated carbon that is retained by the plant is the measure of plant productivity. Respiratory CO₂ release integrated over a period of time can provide information on the efficiency at which assimilated carbon is retained and used by the plant. Therefore understanding how respiration responds to various plant demands and environmental conditions is critical for a mechanistic understanding of and any predictions regarding plant and ecosystem productivity (Cannell and Thornley 2000).

Respiration is a temperature sensitive process (Amthor 1989) and until quite recently a relatively simple treatment of respiration was used in most models of ecosystem productivity (Gifford 2003). A common parameter used to predict the temperature response of a reaction is the Q_{10} , or proportional increase in rate with a 10°C increase in temperature. Many carbon balance models treat respiration by using fixed values for the ratio between photosynthesis and respiration to set the base respiration rate and a fixed Q_{10} , commonly around 2, to estimate the actual rate dependent on temperature (Gifford 2003). This approach treats respiration as a static response function that makes no account for changing plant demands and ability to acclimate to changing environmental conditions. However, with the increasing attention that has been applied to studies of carbon cycling and ecosystem and crop productivity, more detailed studies of respiration have shown that the Q_{10} is not necessarily constant between species, environments or over time (Amthor 1989, Tjoelker *et al.* 2001, Zha

et al. 2004). Acclimation processes have been identified as important aspects of the seasonal and long term responses of plant respiration and can involve both the change in rate at a common basal temperature and a change in the sensitivity to temperature (Atkin and Tjoelker 2003). Furthermore, when considering whole plant or ecosystem behaviour, the total respiratory release is a composite of individual components, including foliage (needles), stems (trunks), branches, roots and soil organisms. Each component may respond independently from the other to either common or different drivers, hence a complete understanding of ecosystem respiration and productivity requires knowledge of the behaviour of the different components (Ryan *et al.* 1996, Lavigne *et al.* 1997).

Component level field measurements of respiration have increased in recent years, but there still remains a lack of information obtained over seasonal or annual cycles, particularly in semi-arid or drought affected regions (Carey *et al.* 1997). Much work has focussed on foliage or soil respiration, as they are considered the dominant components of the ecosystem respiratory flux. However, woody (stem and branch) respiration, which may account for anywhere from between 5 and more than 50% of total autotrophic respiration, remains less well understood (Lavigne *et al.* 1997, Damesin *et al.* 2002). It has been common in the treatment of respiration to separate growth and maintenance components i.e. the portion of the total respiratory CO₂ release that is due to either growth or maintenance (protein turnover, maintaining ion gradients, etc.) respectively. However, ambiguity in separating energetic demands between maintenance and growth processes has led to attempts to create more mechanistic based models that estimate costs associated with individual processes (Amthor 2000, Cannell and Thornley 2000).

Mostly, respiration involves the sequential oxidation and decarboxylation of glucose, pyruvate and citric acid. However, if the supplies of glucose (from either recently assimilated carbon or stored carbohydrate) cannot meet respiratory demands, alternative sources such as lipids or proteins may be employed (ap Rees 1980). The switch to other substrates may be of potential importance under conditions of extensive summer drought, when photosynthetic carbon uptake is at the minimum, temperatures are high and stress responses may add to the metabolic demands. With the additional significant C demand of concurrent foliage growth as is the situation in Yatir, the potential for the plants to operate near or beyond their short term carbon balance increases. Identifying strategies such as shifts in respiratory substrate can provide further information on how plants respond to different conditions and stresses and improve the mechanistic understanding of respiration.

Identifying the respiratory substrate may be possible through the analysis of the $^{12}\text{C}/^{13}\text{C}$ ratio of the respired CO_2 (Jacobson *et al.* 1970, Tcherkez *et al.* 2003). Measurements of stable isotopes at natural abundance are often used in ecophysiological and environmental research due to their useful role as tracers of processes and material fluxes (Yakir and Sternberg 2000), and combining isotope and flux or concentration measurements can be used to partition ecosystem respiration and photosynthetic fluxes (Yakir and Wang 1996, Bowling *et al.* 2001). Recently, increased attention has been directed at the measurement of the ^{13}C composition respiratory CO_2 due to revision of the assumption that the signature of respiratory CO_2 closely resembles that of the bulk tissue from which it was derived (Ghashghaie *et al.* 2003). The isotopic composition of respired CO_2 may differ from that of the bulk tissue due to compound specific differences in substrates and possible fractionation effects associated with respiration metabolism. Differences in substrate composition can arise over time due to different levels of fractionation during the original photosynthetic assimilation of carbon under different environmental conditions (Farquhar *et al.* 1982), and differences occur between compounds due to post-assimilation fractionation effects and during compound synthesis (Hobbie and Werner 2004). For example, photosynthetic fractionation is higher (the carbon more depleted) in times of low stress and high activity, and lipids are more depleted than carbohydrates formed from the same original photosynthate pool (Park and Epstein 1961, Schmidt and Gleixner 1998).

In this chapter the results of field scale investigations of above-ground (foliage and stem) respiration are presented. This work is apparently the first on the respiratory responses of *P. halepensis* under natural environmental conditions and annual growth cycles, and one of the few in general to address these responses in semi-arid or drought prone regions. Specifically, the study is aimed at testing the hypothesis that respiration rates, and temperature sensitivity, are seasonally variable but not related to the seasonal change in temperature. High photosynthetic rates (substrate supply) and growth demands (for the stem) are expected to increase respiration rates in winter and spring. However, possible stress related demands such as the synthesis and maintenance of osmoregulatory compounds (Amthor 1989), growth demands for the new needles and a lack of temperature acclimation may increase respiratory rates in summer. Rates of CO_2 efflux from needles (foliage) and stem were measured periodically over 3 – 4 years, and short term temperature responses were used to determine temperature sensitivity. Novel measurements were also made of the $\delta^{13}\text{C}$ composition of the respired CO_2 , primarily to provide an indication of possible substrate switching in response to

respiratory demands exceeding photosynthetic supply. The measurements of the respiratory $\delta^{13}\text{C}$ were also some of the first seasonal scale field measurements of respiratory CO_2 , particularly under drought conditions, and some of the first in general of stem respired CO_2 , therefore providing valuable information relevant for understanding ecosystem respiration responses.

6.2 Results and Discussion

6.2.1 Foliage respiration fluxes

The rates of foliage respiration (R_F) measured over the course of three years in both the mature (y1) and developing (y0) needle age classes is shown in Fig 6.1. Rates in the y0 needles, first measured in about May, were initially much higher than the mature needles, but had declined to similar rates by August or September. Foliage respiration in the y1 needles was generally highest in March, up to $\sim 0.8 \mu\text{mol m}^{-2} \text{s}^{-1}$, and lowest between June and September (usually less than $0.4 \mu\text{mol m}^{-2} \text{s}^{-1}$). When the measured R_F rates are shown in relation to measurement temperature (Fig. 6.2) it is clear that the seasonal variation in R_F was not a simple function of temperature. Across the entire of temperatures range at which measurements were made, R_F rates were generally within the range of $0.2 - 0.6 \mu\text{mol m}^{-2} \text{s}^{-1}$ and showed no clear dependence on temperature. Therefore no singular temperature response function was applicable over the annual cycle for describing the respiration response of foliage in this system.

Short-term temperature responses of the foliage were measured at different times in the year by adjusting the temperature in the measuring chamber, and representative examples of some responses are shown in Fig 6.3. The R_F temperature response curves showed that R_F at a common temperature differed between seasons, e.g. in the examples presented, R_F was highest in January, intermediate in March, and lowest in November. From the exponential fits to the temperature response data using equation 2.3 (see Materials and Methods), the temperature sensitivity of R_F can be calculated in terms of the Q_{10} parameter. The Q_{10} values derived from the response data at different times in the year are shown in Fig. 6.4. Data from different years and from both y0 and y1 needle age classes are placed on the same relative year axis to determine the seasonal trend. Overall, the R_F Q_{10} values ranged between 1.49 and 2.76, with lowest values in June and a broad period of relatively high Q_{10} values between

October and March. To provide a general function applicable to all years describing the seasonal dependence of Q_{10} a 2nd order polynomial was fitted to the R_F Q_{10} data ($R^2 = 0.582$):

$$Q_{10} = -2.23 \times 10^{-5} x^2 + 0.0098x + 1.550 \quad (6.2)$$

where x is the day of a year from day 1 at May 1st, or starting about 1 month after new needle emergence.

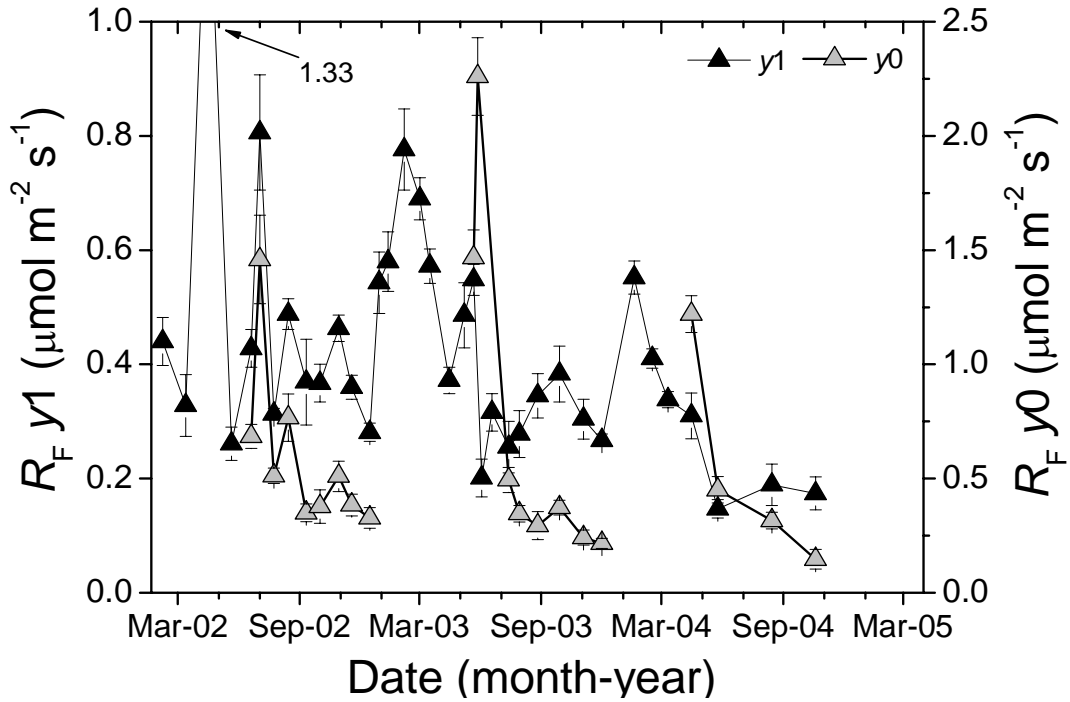


Figure 6.1 Seasonal trends in foliage respiration of mature ($y1$, black symbols) and new ($y0$, grey symbols) foliage measured after dark at ambient temperatures. Note the different scales for the different age classes. The $y1$ measurement made in April 2002 (of 1.33) was made during a heat wave.

Using the seasonal Q_{10} function, all the R_F values measured across the seasons were normalised to a respiration rate at a common temperature of 20°C (R_{F20}). Fig. 6.5 shows R_{F20} of the $y1$ needles from each year overlaid on a common axis together with the light saturated photosynthetic rates (A_{sat}) made over the same time period (see Chapter 3). There was a more than four-fold seasonal variation in R_{F20} from the lowest rates of about $0.25 \mu\text{mol m}^{-2} \text{s}^{-1}$ in mid-late summer (July –October), to a to above $1.0 \mu\text{mol m}^{-2} \text{s}^{-1}$ in the wet period, between January and March. The early season increase in R_{F20} was more rapid than the end of season decrease, and overall the response is qualitatively very similar that seen for A_{sat} . The regression coefficient for the relationship between R_{F20} and A_{sat} from measurements on the same day is 0.851, indicating a strong relationship between photosynthetic capacity and foliage respiration capacity.

The normalised respiration (R_{F20}) of the y0 needles during their development period from each of the years is shown in Fig. 6.6, together with the average relative needle growth rate (see Chapter 4). The high rates of respiration measured in the young growing needles (Fig. 6.1) represent a respiration capacity that was initially up to 7 times greater than the capacity at the end of the growing phase. The normalised and measured respiration rates declined in the new needles as growth progressed and growth rate declined, but remained above those of the y1 needles until about September, when the new needles were over 80% of influence on respiratory demand, but this was confined to the growing tissue only and resulted in the growing foliage to respond quite differently than the mature foliage.

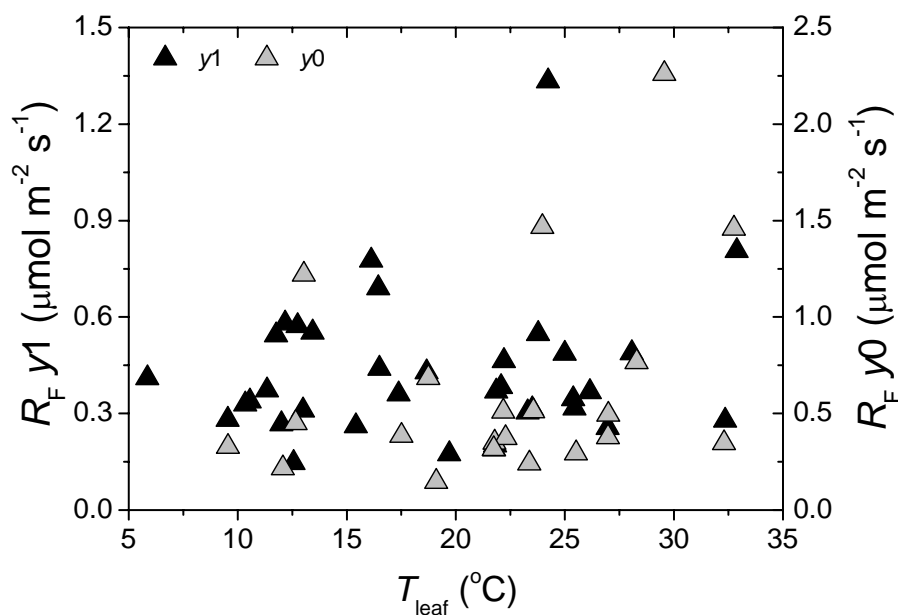


Figure 6.2 The relationship between leaf temperature (T_{leaf}) and respiration rate (R_F) from the data in Figure 6.1 for both y1 and y0 age classes. Note different scales for different age classes.

Seasonal patterns of R_F

This data is the first report of field based and seasonal scale measurements of foliage respiration in *P. halepensis*. The R_F rates measured here in the mature needles are at the lower range of rates found in other *Pinus* or conifer species in temperate or boreal localities and are more similar to those of dormant season rates (Ryan *et al.* 1997, Shibistova *et al.* 2002). Of particular interest was the finding that rates in the summer tended to be similar to or even lower than those in winter or spring, showing a strong down-regulation of R_F in summer. This contrasts with species in temperate regions in which the highest rates of R_F tend to be found in summer, when both temperature and photosynthetic activity are high (Law *et al.* 1999, Vose and Ryan 2002, Curtis *et al.* 2005).

A reduction in respiration in response to drought has been reported from short term and controlled studies in a number of crop species (see Amthor 1989), and (Melzack *et al.* 1985) observed that R_F declined in *P. halepensis* seedlings as water stress was imposed. The results of this study showed an important response of foliage respiration under natural conditions of summer drought combined with high temperatures. Under field conditions, the (non-growing) foliage maintained similar rates of respiration for much of the year and showed little response to the large seasonal scale temperature variations. This indicates that respiration rates were responding to other drivers and estimates of respiration across annual time scales will either over or under estimate rates if temperature is considered the main driver. The relative homeostasis, or constancy of respiration rate, also indicates that the trees had similar energetic requirements throughout the year, albeit due to possibly different metabolic demands. These results also indicate that caution may need to be applied when interpreting an apparent temperature response derived from seasonal scale temperature variations when both temperature and metabolic demands may be changing in relative unison. While such a response may be empirically interpreted as a mechanistic response based on temperature, it may in fact be the result of concurrent changes in temperature and metabolic demands.

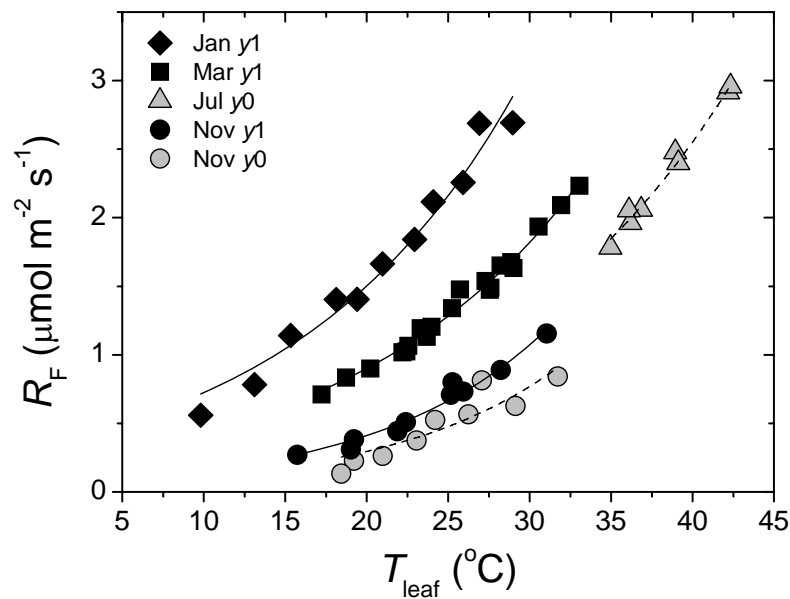


Figure 6.3 Representative examples of the relationship between foliage respiration rate and short term temperature changes from measurements made at different times of the year on y1 (black symbols) and y0 (grey symbols) needle age classes. The range of temperatures for each sample was obtained by modifying the cuvette temperature. Each data set is a composite of 2-3 samples.

Short term temperature response and temperature sensitivity of R_F

The relatively low R_F rates observed in summer (Fig. 6.1) and seasonal homeostasis in the y1 foliage were the result of a seasonal acclimation of foliage respiration that resulted in both the rate at a common temperature and the sensitivity to temperature (Q_{10}) decreasing in summer. High respiration rates were observed in summer in the new needles during their growth period however. As the Q_{10} of the y0 and y1 needles show a similar trend through the season, the higher rates seen in the new needles was due to the high respiratory demands associated with growth increasing the base respiration rate, and not an enhanced temperature response.

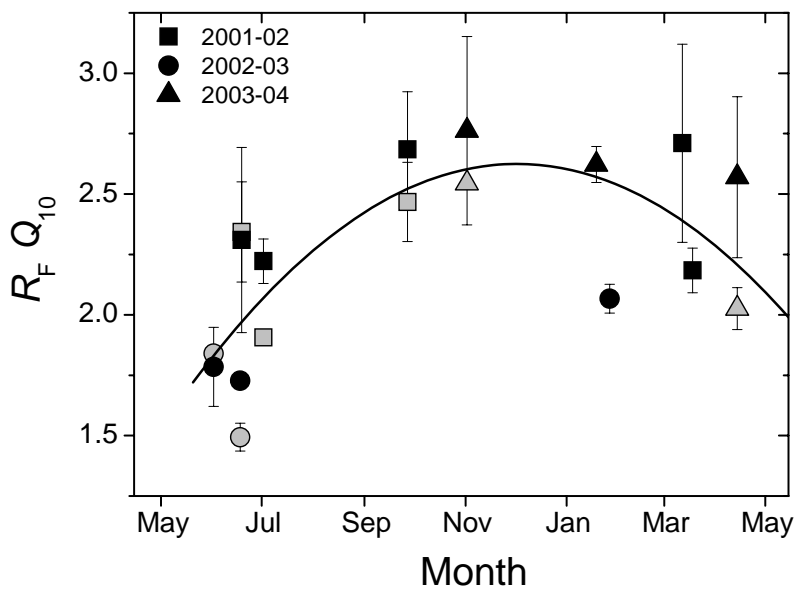


Figure 6.4 Seasonal variation in the Q_{10} values of the R_F temperature response derived from the temperature response functions as depicted in Fig. 6.5. The different symbols refer to measurements made in different years, as shown in the legend (where the year is the hydrological year i.e. from Oct-Sept). Black symbols are y1 foliage and grey symbols y0, with the same symbol type for the same year in each age class. The fit ($R^2 = 0.582$) is to all data combined.

The range of Q_{10} values we observed is typical for plant respiration (Atkin and Tjoelker 2003). Seasonal variations in temperature responses and Q_{10} values are now recognised as important features of plant respiration responses, and it is evident that short term temperature responses do not always predict long term responses and *vice versa* (Gifford 2003, Zha *et al.* 2004, Atkin *et al.* 2005). While some authors have found a relatively invariable Q_{10} across the year (Damesin *et al.* 2002), other studies have shown that the Q_{10} tends to be inversely related to growth temperature across growth environments (Fukai and Silsbery 1977, Atkin *et al.* 2000, Tjoelker *et al.* 2001). We also observed that the Q_{10} was higher in the cooler conditions,

due to a dynamic continuous adjustment of the Q_{10} through the season, as opposed to a fixed relationship with the environment. The results presented here indicate a potential contrast that may exist with more temperate regions. The seasonal adjustment leads to the Q_{10} being down regulated in summer, when photosynthetic activity and respiration rates are also low. Hence there is a seasonal (positive) correlation with activity as well as an inverse relationship with temperature. A low Q_{10} in warmer conditions in temperate regions would coincide with higher plant activity, raising the possibility that both an inverse relationship with temperature and positive relationship with activity lay lead to a relatively invariant Q_{10} across the season in temperate regions (Damesin *et al.* 2002).

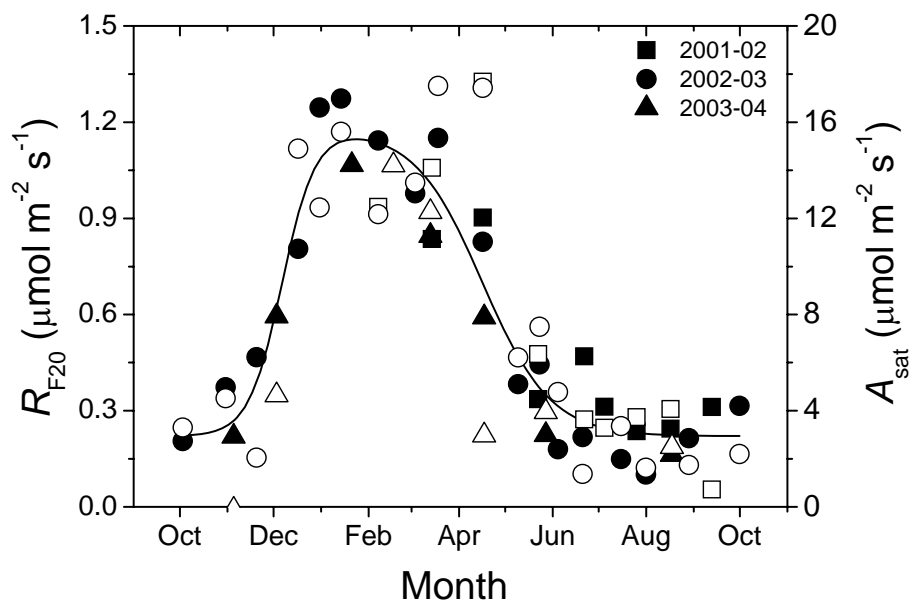


Figure 6.5 The seasonal response of R_{F20} (R_F normalised to 20°C) of y1 needles for different years (solid symbols) and light saturated photosynthetic rate (A_{sat}) over the same period (open symbols). The different symbols refer to data from different years, as indicated in the legend, and apply to both R_{F20} and A_{sat} data. The fit is to all R_{F20} data combined ($R^2 = 0.911$).

Normalised R_F and photosynthesis

The large seasonal variation in normalised R_F shows that the physiological or biochemical factors underlying needle respiration rate have a strong influence on respiration. The mechanisms underlying the acclimation of respiration in plant tissues are complex, but respiration capacity and temperature sensitivity have been found to be associated with changes in leaf nitrogen and carbohydrate levels (Ryan *et al.* 1996, Tjoelker *et al.* 1999, Lee *et al.* 2005), including in response to the retranslocation of N to developing needles (Vose and Ryan 2002). As was shown in Chapter 4, there was a seasonal decrease in leaf N in the summer that may be related to the changes in R_F . However, we found that needle respiratory capacity (R_{F20}) was more strongly correlated with photosynthetic capacity. While Vose and

Ryan (2002) found little relationship between the seasonal variation in A and normalised respiration rate in *Pinus strobus*, a relationship between photosynthetic carbon gain in the previous light period and respiration rate has been found (Azcón-Bieto 1992, Whitehead *et al.* 2004), suggesting a role of substrate supply in the link between photosynthesis and respiration.

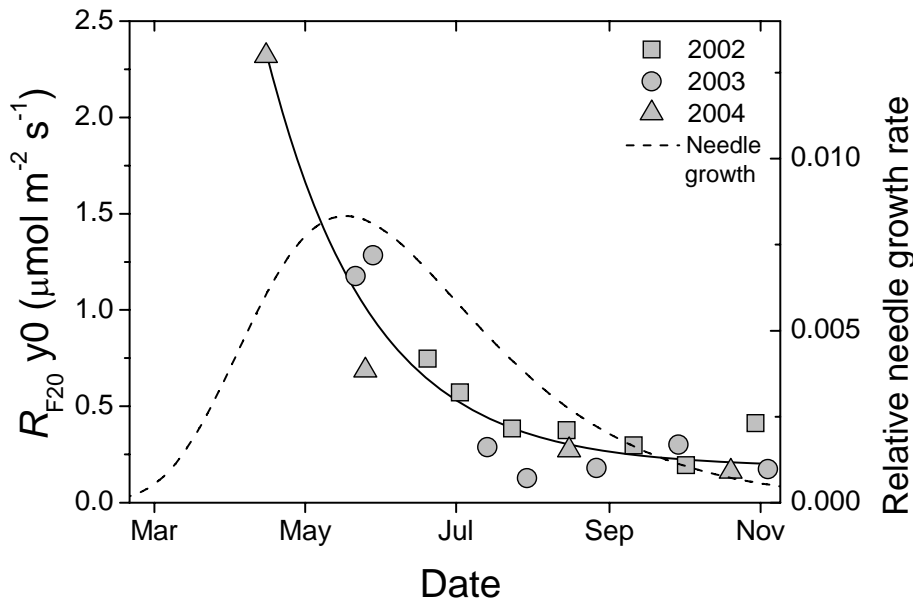


Figure 6.6 R_F of new (y_0) needles normalised to 20°C during the needle growth period in different years, as indicated in the legend. The fit (solid line, $R^2 = 0.926$) is to all data combined. Also shown is the needle growth rate, being the derivative of the fit to the relative needle length data in Fig. 4.2A.

Therefore it is concluded that the seasonal changes in foliage respiratory capacity were a substrate induced a response. With the higher photosynthetic activity in the cool and mild months, substrate availability and requirements for photosynthate metabolism would presumably have been high at a time when temperatures were potentially more limiting for respiration. Hence it may be inferred that both respiratory capacity and temperature sensitivity was increased in the winter and spring period to reduce possible temperature related limitations to respiration, and reduced in summer to minimise temperature induced wastage. Overall it appears that respiratory regulation is an important feature of the carbon relations in this system and operate at such a level that respiratory metabolism becomes relatively independent of seasonal temperature changes.

6.2.2 Stem respiration fluxes

The measurements of stem respiration (R_T) made periodically for more than three years are shown in Fig. 6.7. In each year there was a peak in R_T in April-May, in which R_T reached

rates above $3 \mu\text{mol m}^{-2} \text{s}^{-1}$. For the period from July to January R_T was low, less than $1 \mu\text{mol m}^{-2} \text{s}^{-1}$, and relatively stable. Therefore, as with foliage respiration, there was no common relationship between the measured rates of R_T and the measurement stem temperature across the annual cycle (Fig. 6.8). There was an apparent increase in respiration rate with temperature from the point where both seasonal temperatures and R_T are at their minimum (in January) to the maximum rates (in April) as temperatures increase from about 10°C to $25 - 30^\circ\text{C}$. This was followed by a sharp decrease in R_T of about 50% with little change in temperature, and then a further decrease in R_T to their minimum values as temperatures increased to their maximum ($30 - 35^\circ\text{C}$, in June - July). R_T then remained stable at these low rates despite a seasonal temperature decrease of up to 25°C .

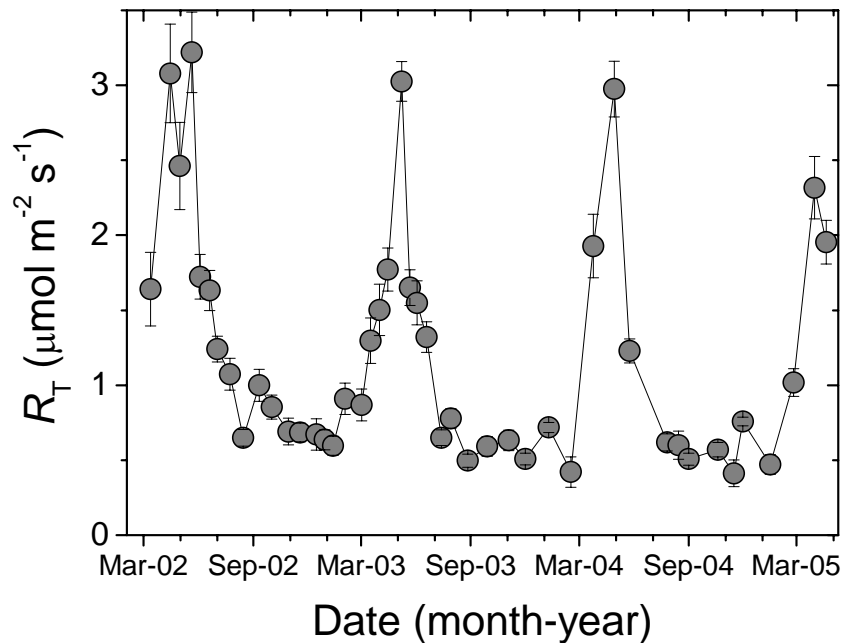


Figure 6.7 Seasonal trends in stem respiration rate measured mid-afternoon at ambient temperatures.

The short-term temperature response of R_T was determined from repeated measurements made over the diel (day-night) period. Representative diel time courses of R_T and the associated temperature response are shown in Fig. 6.9. Temperature responses of R_T were also determined from combining measurements made on different days in the season within a short time period ('Seas.'), or by combining data made at a similar time of the year in different years ('MultiSeas.'). Changes in R_T over the course of the day (Fig. 6.9A) did appear strongly dependent on temperature (Fig. 6.9B) although a certain degree of hysteresis was sometimes apparent, with rates slightly higher in the afternoon than the morning at a similar temperature.

As with foliage respiration, there were seasonal differences in the temperature response of R_T in terms of the rate at a common temperature, but the seasonal trend is not clear. For example, high rates were seen in May (mild-warm temperatures) and low rates in January (cool temperatures), with June (warm-hot temperatures) intermediate between the two.

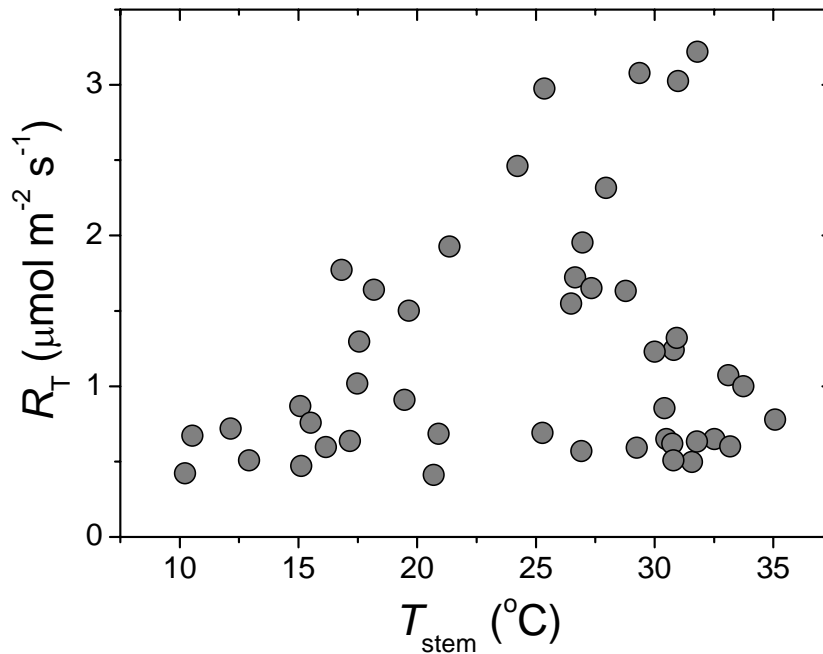


Figure 6.8 The relationship between stem temperature (T_{stem}) and measured stem respiration rate from the data in Figure 6.3.

The Q_{10} values for the R_T temperature responses are shown in Fig. 6.10. Overall the Q_{10} values were lower in the stem compared to the foliage, ranging between 1.38 and 1.83. Highest R_T Q_{10} values were seen in March and the lowest in summer. A Gaussian function was fitted to the R_T Q_{10} data ($R^2 = 0.880$):

$$Q_{10} = 1.378 + 0.435e^{(-0.5(x-158)/57.4)^2} \quad (6.2)$$

where x is the day of the hydrological season used in at this site, with day 1 being October 1st.

The R_T Q_{10} function is similar in nature to that of stem growth rate, a process that may have an important influence on respiratory activity. The average seasonal stem growth rate was derived from the relative stem increment data in Fig. 4.2B (see also Fig. 4.3) and is shown in Fig. 6.10. Both the fit to the stem respiration Q_{10} data and the stem growth rate were maximal in March, and are similarly distributed about this peak.

Using the seasonally dependent Q_{10} function, the R_T data were normalised to 20°C and are shown in Fig. 6.11. There is a consistent seasonal pattern of R_{T20} that was observed over the 3 – 4 years of observations. R_{T20} rates were low in October, at the start of the season,

increasing gradually through February and then rising to a peak in May, before declining sharply to the minimal rates by late July. As with foliage respiration, respiration at a constant temperature is lowest in mid-summer, when overall activity of the trees is low but the seasonal R_{T20} pattern differs from that of R_{F20} , and also differs from that of stem growth rate.

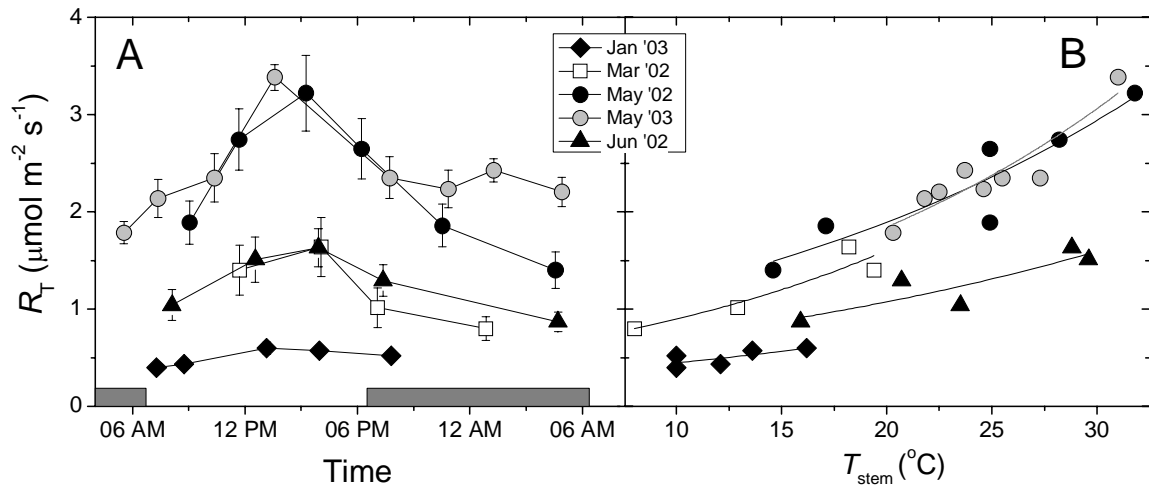


Figure 6.9 Representative examples of diel responses of R_T (A) and the relationship between R_T and measurement temperature from the diel measurements (B) from different dates. Values are averages of 6 – 12 trees. The legend applies to both panels.

Seasonal patterns of R_T

This data is the first report of stem or wood respiration in *P. halepensis*, either under field or controlled conditions, and the first study known on seasonal or inter-annual scale responses of stem respiration in conditions of seasonal annual drought. The range of R_T rates measured for *P. halepensis* are in the range reported for other conifers (Sprugel and Benecke 1991, Shibistova *et al.* 2002, Bowman *et al.* 2005). As with R_F , the lowest values of R_T occurred during the warmest period of the year and the summer R_T values are typical of dormant season or lower values of stem respiration reported elsewhere (Ryan *et al.* 1997, Maier *et al.* 1998). However, similarly low values were also observed in the coolest part of the year, showing a large range of temperatures over which R_T homeostasis is maintained. The maximal rates (of up to 3 $\mu\text{mol m}^{-2} \text{s}^{-1}$, or 2 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at 20 $^{\circ}\text{C}$), however, are higher than those reported for growing season rates in a other trees (Ryan *et al.* 1997, Shibistova *et al.* 2002, Griffis *et al.* 2004, Zha *et al.* 2004).

Short term temperature response and temperature sensitivity of R_T

Overall, the stem respiration Q_{10} values measured here are at the lower range relative to other studies of stem respiration (Carey *et al.* 1997, Damesin *et al.* 2002). The R_T Q_{10} was

seasonally variable and, as in the $R_F Q_{10}$, was lowest in the warmest months. However, the highest $R_T Q_{10}$ values were observed in spring, and overall the $R_T Q_{10}$ showed a similar seasonal pattern to that of stem growth rate. Zha *et al.* (2004) found the Q_{10} of stem respiration was also highest in the growing season in a boreal pine forest, but therefore meaning also highest in the warmer months. A seasonal reduction in Q_{10} (between July and September) was also observed in *P. ponderosa* stem respiration in desert growing trees (Carey *et al.* 1997). The comparison of these studies, and in particular the seasonal shift in activity away from temperature dependence in our system, reveals that stem growth may be controlling the Q_{10} of stem respiration, as opposed to the temperature control apparent for leaf respiration (Atkin and Tjoelker 2003)

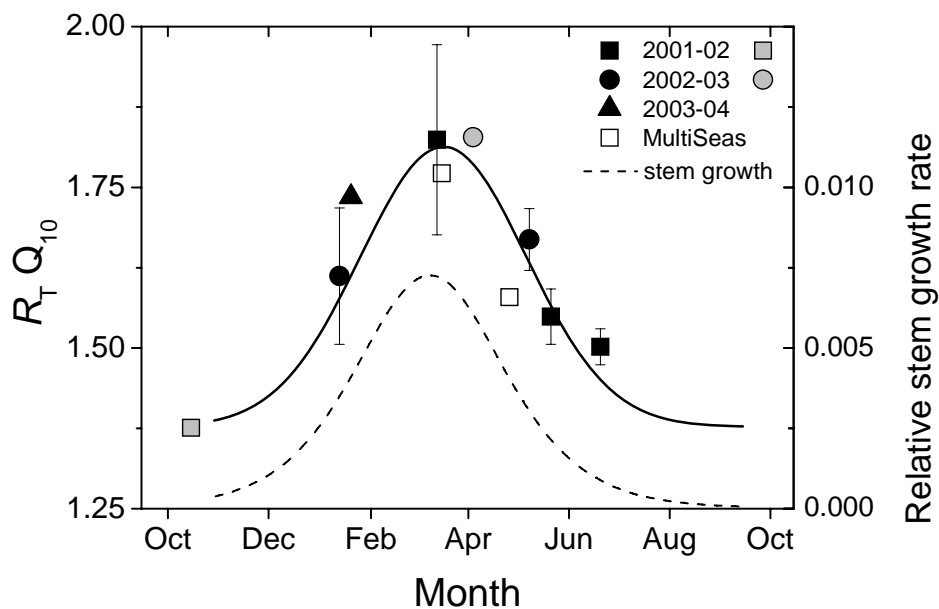


Figure 6.10 Seasonal variation in the Q_{10} of R_T derived from diel temperature responses, as in Fig. 6.7 (black symbols), or from temperature responses obtained by combining measurements from different, but proximate, days in the same year (grey symbols) or days from different years at a similar time of year (MultiSeas). The dates refer to the year from Oct – Sept and apply to the diel and seasonally proximate data. The fit (solid line, $R^2 = 0.880$) is to all data combined. Also shown is the seasonal stem growth rate (dashed line), being the derivative of the fit to the relative growth data in Fig. 4.2B.

R_T and stem growth

The growth component of respiration is considered to be an important component of the seasonal variation in stem respiration (Vose and Ryan 2002, Damesin *et al.* 2002). The acclimation responses observed in this study served to maintain stable stem respiration rates outside the growth period. During the much of the early growth period, R_T was similar to the rates outside the growth period, but this is also a period of low temperatures, which was serve

to offset the increase due to growth. However, we observed that although stem growth started in November, peaked in March and continued through until May, normalised stem respiration was slower to respond over the initial stem growth phase and peaked in May when seasonal stem growth had nearly finished. The peak in stem respiration also occurred after the peak in photosynthetic activity and as soil water contents were starting to decline (see Fig. 4.3). This indicates that instantaneous stem growth rates may not necessarily be a good indicator of total current stem respiratory activity (Edwards and Hanson 1996). The lag between stem growth rate and respiratory capacity may be related to the delay between wood synthesis and cell expansion, but the difference exceeded the expected delay of up to about a month proposed by Sprugel and Benecke (1991).

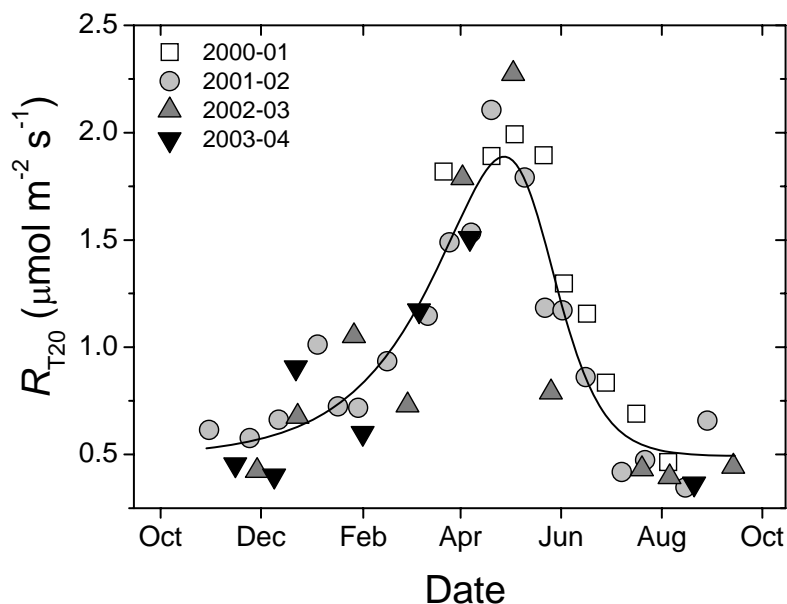


Figure 6.11 The seasonal response of R_T normalised to 20°C for different years, as indicated in the legend. The fit ($R^2 = 0.832$) is to all data combined.

The discrepancy between stem growth and stem respiration may be due to the difference in the relative numbers and metabolic requirements for producing different cambial cell types (e.g. phloem or xylem) or changes in the underlying ‘maintenance’ respiration component (Sprugel and Benecke 1991). Lavigne *et al.* (2004) found that the highest stem respiration rates occurred during xylem production, when phloem cell number did not increase. However, phloem cells have the highest construction costs of the cambial cells (Waring and Schlesinger 1985), and Liphshitz *et al.* (1984) observed that phloem production occurs in May in *P. halepensis* trees in Israel. In addition, the peak in stem respiratory activity occurred at the time when carbohydrate mobilisation was likely to be occurring for the initiation and early growth of the new foliage (see Chapter 4). If the stems are a source of carbohydrate remobilisation

then this process may form a significant component of the respiratory activity (Sprugel 1990). These results show that stem respiration appears to have had an important underlying phenological influence independent of environmental drivers, such as temperature and water availability, or physiological influences, such as carbon supply. Such a response may be prevalent in other systems, but the general overlap in phenology, physiology and temperature that exists in more temperate regions makes it difficult to separate the factors (Vose and Ryan 2002, Griffis *et al.* 2004, but see Lavigne *et al.* 2004). This result has important implications for predictions of ecosystem response and carbon balance to future climate scenarios and needs to be considered in addition to the more common environmental responses.

It has been discussed and recently demonstrated (Sprugel and Benecke 1991, Teskey and McGuire 2002, Bowman *et al.* 2005) that CO₂ released from the transpiration stream may form a significant component (up to 40% at times) of the total stem CO₂ efflux. Xylem transported CO₂ represents a relocation of respired CO₂ that is dissolved into the xylem at one location and released in another, and is therefore dependent on xylem cell and sap characteristics, the local rate of CO₂ production and sap flow rates (Bowman *et al.* 2005). While xylem transported CO₂ is not considered to be responsible for the seasonal pattern of R_T observed in this system, it may account for some the inter-annual variability of R_{T20} at a particular time of year and the temperature hysteresis behaviour sometimes observed on the diel scale.

6.2.3 Up-scaling respiratory fluxes and implications of respiratory acclimation

Using the seasonally dependent functions for R_{F20} and R_{T20} (see Figs. 6.5 and 6.11) and the relevant functions for the Q_{10} values (see Figs. 6.4 and 6.10), the foliage and stem respiration rates were estimated for each 30min period in the year using the canopy air temperature data collected at the tower for the four years of 2000-01 to 2003-04 (October to September). The yearly totals of the respiratory efflux were expressed on a ground area basis using an area weighting index (the component surface area per ground area) of 1.3 for the foliage (based on measurements of LAI) and 0.2 for the stems (based on average tree height of 10m, basal diameter of 19cm and tree density of 300 stems ha⁻¹, or basal area of 8.5 m² ha⁻¹). The average of the 4 years of estimates of the annual respiratory CO₂ release was 212 g C m⁻² y⁻¹ by the foliage and 65 g C m⁻² y⁻¹ by the stems.

Estimates of total annual foliage respiration in other ecosystems include 157 g C m⁻² y⁻¹ for a cool temperate / semi-arid *P. ponderosa* forest (LAI of 1.5, Law *et al.* 1999), 256 g C m⁻² y⁻¹ for a cool temperate / semi-arid *P. ponderosa* forest (LAI of 1.5, Law *et al.* 1999), 256 g C m⁻² y⁻¹ for a cool temperate / semi-arid *P. ponderosa* forest (LAI of 1.5, Law *et al.* 1999), 256 g C m⁻² y⁻¹ for a cool temperate / semi-arid *P. ponderosa* forest (LAI of 1.5, Law *et al.* 1999).

$^2 \text{ y}^{-1}$ for a deciduous hardwood forest (maximum LAI of about 3.6, Curtis *et al.* 2005) and $180 \text{ g C m}^{-2} \text{ y}^{-1}$ in a boreal jack pine forest (LAI of 1.8, Ryan *et al.* 1997). Comparisons across sites for stem respiration are more difficult due to the variability in tree characteristics (density, height and basal area). Annual stem respiration has been estimated at $198 \text{ g C m}^{-2} \text{ y}^{-1}$ in a boreal aspen forest (980 stems ha^{-1} , basal area $\sim 30 \text{ m}^2 \text{ ha}^{-1}$, height $\sim 20\text{m}$; Grifffis *et al.* 2004), $74 - 122 \text{ g C m}^{-2} \text{ y}^{-1}$ in a boreal pine forest (450 stems ha^{-1} , basal area $\sim 30 \text{ m}^2 \text{ ha}^{-1}$, height $\sim 20\text{m}$; Shibistova *et al.* 2002), $225 \text{ g C m}^{-2} \text{ y}^{-1}$ in a temperate beech forest (3480 stems ha^{-1} , height $\sim 13\text{m}$, basal area $\sim 27 \text{ m}^2 \text{ ha}^{-1}$; Damesin *et al.* 2002) and $257 \text{ g C m}^{-2} \text{ y}^{-1}$ in a warm temperate pine forest (275 stems ha^{-1} , height 21m , basal area $23 \text{ m}^2 \text{ ha}^{-1}$; Ryan *et al.* 1996).

The similarity between the annual foliage respiration rates in Yatir with other cooler climate forests of similar LAI serves to indicate that the acclimation processes have an important role in maintaining respiration rates at levels that are determined more by demand than response to environmental parameters, in particular temperature. After adjusting for the differences in tree characteristics, we see that the annual rates of stem respiration in Yatir were higher than those in the boreal sites, but similar to those of the temperate sites. Again the acclimation responses are shown to be an important feature in minimising carbon losses under hot summer climate, but the effect appears not as strong in the stems as in the foliage. However, this is complicated by the fact that at any time it is impossible to separate the growing tissue from the mature tissue in the stems, as can be done in the foliage. This is evident in the greater degree of homeostasis maintained through the year in the mature foliage respiration rates, compared with the phenology driven peak in stem respiration that was observed in the spring / early summer.

Minimizing respiratory carbon losses under high temperatures is important for the overall productivity for the forest, as it leaves more carbon available for structural growth. This is particularly important in the situation of growth during drought stress, as in this forest, when photosynthesis is reduced. Recent ecosystem productivity simulations that used a temperature dependant acclimation of foliar respiration parameters resulted in estimates of above ground net primary productivity being up to 38% higher across a range of ecosystem types than if a constant Q_{10} value was used (Wythers *et al.* 2005). Furthermore, using the acclimation algorithm resulted in productivity estimates closer to field measurements than with the static representation.

While temperature is considered a major driving factor in respiration responses, through the decoupling of temperature and activity at the annual scale we have found that respiration

of both foliage and stems appears driven more by metabolic demand than temperature, and effective acclimation operates to meet these demands, independent of the prevailing temperature. Furthermore, the different demands of different components result in contrasting temporal respiration responses between the components. Furthermore, we have shown that long term acclimation and adaptation to stress, such as drought, that occur on a regular (annual) basis can differ from responses to episodic events. Edwards and Hanson (1996), for example, found that respiration in temperate oaks was highest at the same time of year in both a normal and a drought year. Identifying and incorporating such acclimation processes is essential for understanding how ecosystem behaviour might respond to changes in the overall average state of the climate, particularly in response to warming conditions. For example, positive feedbacks associated with higher temperatures causing greater respiratory CO₂ release may not be realistic.

6.2.4 The $\delta^{13}\text{C}$ composition of respired CO₂

The results from the measurements of the $\delta^{13}\text{C}$ composition of the foliage ($\delta^{13}\text{C}_{\text{Rf}}$) and stem ($\delta^{13}\text{C}_{\text{Ri}}$) respired CO₂ made over nearly three years are shown in Fig. 6.12. In the first season, the age classes of the foliage were not separated, but in the subsequent two years the new (current year, y0) and mature (previous year, y1) needle cohorts were separated. There was a large inter-seasonal variation in the respired $\delta^{13}\text{C}$ composition of all components, which was similar in nature between the components across the seasonal scale. The most depleted values (-24.0 to -26.3‰ in the foliage, -27.2 to -27.5‰ in the stem) were observed in March. A progressive enrichment occurred through the summer and on until November - December, with values of -19.3‰ observed in the y1 foliage and stem and up to -18.6‰ in the y0 foliage. The CO₂ efflux from the stem tended to be depleted relative to that from the foliage, usually by about 1-2‰ but at times up to 3-4‰, relative to the y1 needles. The $\delta^{13}\text{C}_{\text{Rf}}$ from the new needles tended to be enriched relative to the mature needles, but was similar to or slightly depleted relative to the y1 needles in the early part of the growth season. There was also some significant inter-annual variability. In particular the seasonal minimum values measured in 2004 (in March) were about 2‰ enriched compared with the recorded minimum in 2003. The respiratory CO₂ became enriched more rapidly in the early summer in 2004 but also showed a shift to depleted values in mid-summer (August), before becoming enriched again by the end of the drought period in October.

There was quite a good agreement seen between the seasonal trends of respiratory $\delta^{13}\text{C}$ and those of assimilation rate and stomatal conductance (Fig. 6.13A. 6.13b)). Respiratory $\delta^{13}\text{C}$ was enriched in summer, when A_{sat} and g_s were low, and depleted when the gas exchange parameters were low. The timing of the peaks in A_{sat} and g_s coincided with the most depleted values of the $\delta^{13}\text{C}$ values. Estimates of the $\delta^{13}\text{C}$ composition of assimilated carbon (δ_p), accounting for the fractionation effects associated with photosynthesis, can be calculated from (Farquhar *et al.* 1982):

$$\delta_p = \delta_a - a - (b - a) \frac{C_i}{C_a} \quad (6.3)$$

where δ_a is the $\delta^{13}\text{C}$ composition of the atmospheric CO_2 ($\sim 8.5\text{‰}$), a is the fractionation occurring due to diffusion in air (4.4‰), b is the net fractionation associated with carboxylation (27‰) and C_i and C_a are the leaf internal and external CO_2 concentrations, respectively. The assimilate $\delta^{13}\text{C}$ estimated from the instantaneous gas exchange data is shown in Fig. 6.13C, with values of $\delta^{13}\text{C}_{\text{Rf}}$. In contrast to A_{sat} and g_s , there was little agreement between estimates of assimilate $\delta^{13}\text{C}$ composition and the respiratory $\delta^{13}\text{C}$ measurements. The best agreement occurred in the winter and spring periods, when values of both were more depleted.

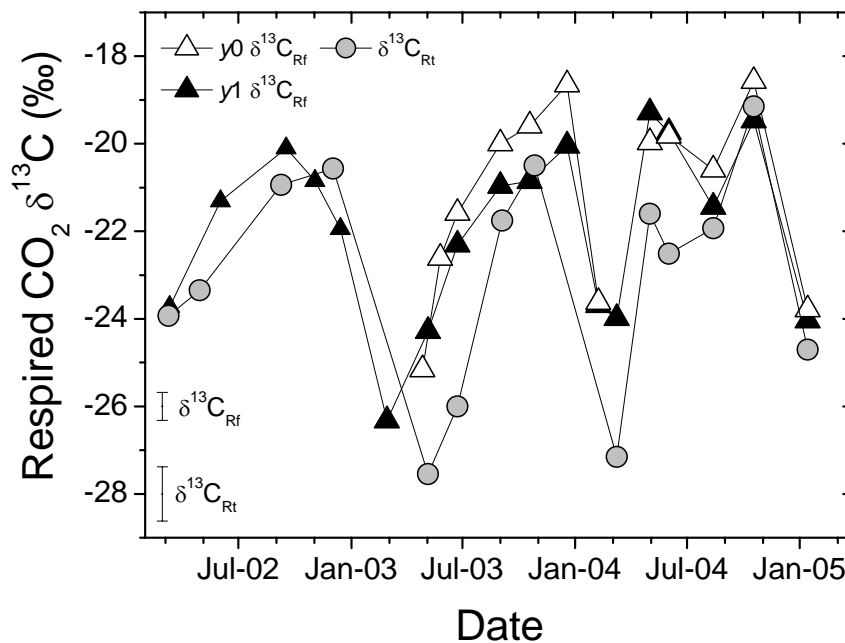


Figure 6.12 Seasonal patterns of the $\delta^{13}\text{C}$ composition of foliage ($\delta^{13}\text{C}_{\text{Rf}}$, triangles) and stem ($\delta^{13}\text{C}_{\text{Rt}}$, circles) respired CO_2 . In the first season, needle age classes were not separated, but in the subsequent collections needle age classes were separated into new (y0, open symbols) and 1 year old (y1, closed symbols) needle cohorts. For clarity, average error bar sizes are shown.

Seasonal variation of $\delta^{13}C_{Rf}$

There are few reports of seasonal scale measurements of foliage respired $\delta^{13}C$ composition made in the field, and none have addressed the response to seasonal drought stress, but the seasonal variation in $\delta^{13}C_{Rf}$ observed in this study is greater than what has been observed elsewhere. Seasonal variation in the $\delta^{13}C$ of respired CO_2 was between -22.1 and -26.3‰ in leafy twigs in beech (Damesin and Lelarge 2003) and between -27.3 and -31.0‰ in *Pinus taeda* foliage (Mortazavi *et al.* 2005). The large seasonal variation observed in our study, with enrichment through the drought period, indicates that extensive drought conditions are influencing not only the respiration rates but also have a strong effect on the $\delta^{13}C$ composition of the respired CO_2 .

Laboratory experiments on herbaceous species have revealed a tendency for both $\delta^{13}C_{Rf}$ and leaf sucrose to become enriched under short term drought as stomatal conductance and leaf internal CO_2 concentration (C_i) decreases, indicating a close link between recent discrimination during photosynthesis and the respired substrate (Duranceau *et al.* 1999, Ghashghaie 2001). Likewise, studies of the $\delta^{13}C$ of total ecosystem respiration indicate a close association with the factors driving changes in assimilate composition (soil and atmosphere moisture deficits), suggesting a fast turnover of assimilate (order of days) at the ecosystem level (Bowling *et al.* 2002, Fessenden and Ehleringer 2003, Knohl *et al.* 2005). Therefore the general enrichment trend measured through the summer in our study and the agreement with the seasonal variation in assimilation rate and stomatal conductance indicates that the seasonal enrichment is related to a seasonal decrease in photosynthetic discrimination affecting the composition in carbon coming into the system (Farquhar *et al.* 1982).

Another factor that may increase the extent of assimilate enrichment, and consequently that of the respired CO_2 , is the effect of photorespiration. The glycine decarboxylation during photorespiration may involve discrimination against ^{13}C of anywhere between 0.5 – 8‰ (Ivlev 1996, Gillon and Griffiths 1997). Therefore the remaining pool of assimilate will be enriched before it is incorporated into the leaf and ultimately used for construction or dark respiration. As was shown in Chapter 4, photorespiratory rates were similar to or exceeded those of net CO_2 assimilation during summer; hence the drought related increases in photorespiration may also have contributed to the enriched signal observed over summer.

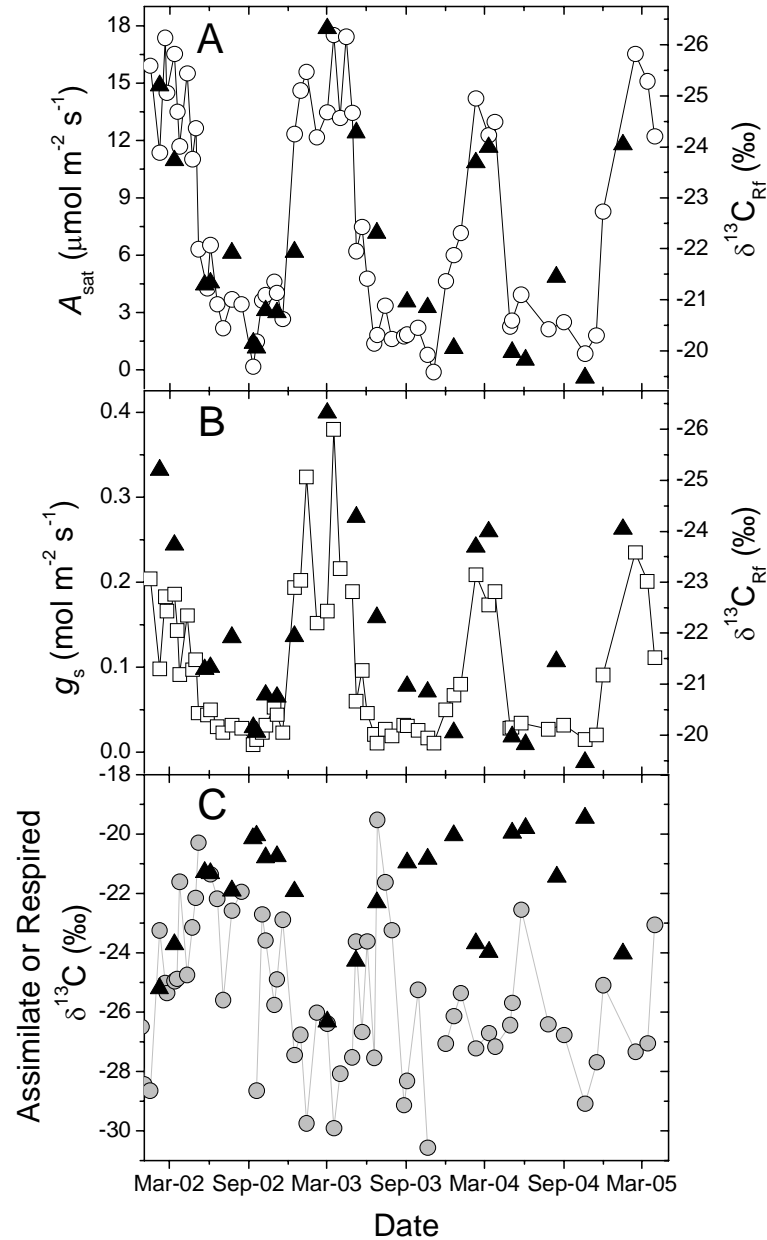


Figure 6.13 The seasonal pattern of respiratory $\delta^{13}\text{CO}_2$ and gas exchange parameters. For clarity, only the y1 $\delta^{13}\text{C}_{\text{RF}}$ values are shown, and are the triangles in each panel. **A:** light saturated photosynthetic rate (open circles); **B:** stomatal conductance (squares); **C:** the calculated $\delta^{13}\text{C}$ composition of assimilate based on the gas exchange data (grey circles). Note the reverse axis for the $\delta^{13}\text{C}_{\text{Rf}}$ data in **A** and **B**.

Respiratory substrate

From the relationship between the physiological parameters determining discrimination and respiratory $\delta^{13}\text{C}$, it may be inferred that respiration continues to use recently fixed photosynthate through the long drought period. However, estimates of the assimilate $\delta^{13}\text{C}$ composition based on the gas exchange data do not agree with those of the respired CO_2 for much of the year, particularly when stomatal conductance is low. This may therefore indicate

that the respiratory substrate is not (only) recently fixed photosynthate. However, the discrepancy between the respired and estimated assimilate $\delta^{13}\text{C}$ values is also quite possibly related to errors in the estimate of C_i/C_a , and consequently estimates of the assimilate $\delta^{13}\text{C}$, that can occur when stomatal conductance is very low and of similar magnitude to cuticular conductance (Boyer *et al.* 1997). This is supported by the observation that the better agreement between assimilate and respiratory $\delta^{13}\text{C}$ values was in the winter and spring periods, when g_s was above about $0.1 \mu\text{mol m}^{-2} \text{s}^{-1}$.

The differences between assimilate and respired $\delta^{13}\text{C}$ may also be related to fractionation effects during respiration. An enrichment of foliage respired CO_2 , relative to both bulk leaf material and carbon fractions (sucrose, starch, lipids) is now recognized as a common feature in plants, and has been observed in number of species in laboratory (Duranceau *et al.* 1999, Ghashghaie 2001, Tcherkez *et al.* 2003), large enclosure (Biosphere 2, Xu *et al.* 2004) and field studies (Hymus *et al.* 2005). However, the enrichment is not constant, varying between species and environmental conditions (Ghashghaie *et al.* 2003) and even diurnally by up to 7‰ (Hymus *et al.* 2005). Less diurnal variation has been observed in Yatir (K. Maseyk and G. Hymus, unpubl. data), but the apparent fractionation effects between respired CO_2 and the respiratory substrate therefore make it difficult to make direct inferences about the respiratory substrate from the $\delta^{13}\text{C}$ composition of the CO_2 alone.

Possible alternative respiratory substrates include carbohydrates stored earlier in the season or the degradation and use of lipids if carbon stores are exhausted and current photosynthetic supply is not enough to meet demands (Duranceau *et al.* 1999, Tcherkez *et al.* 2003). However, the $\delta^{13}\text{C}$ composition of stored carbon would be more depleted, due to the higher fractionation under the more favourable photosynthetic conditions, and lipids tend also to be depleted in ^{13}C relative to other carbon compounds in the leaf due to fractionation effects during compound synthesis (Melzer and Schmidt 1987). Therefore it appears more likely that overall respiration continues to rely on recent photosynthate through the drought period.

However, a depletion shift was noticed in the summer of 2004, after a wet season of lower than average rainfall and in which soil water content, and consequently stomatal conductance and assimilation rate, declined earlier in the season. The earlier decline in photosynthetic activity is consistent with the higher enrichment early in the season, and the depletion shift in mid-summer, when the stress is highest, may be indicative of a respiratory substrate shift associated with a long period of low assimilation rates. The return to the more

enriched values occurred in autumn, when conditions for photosynthesis had improved, indicating that the dependence on alternative substrates was temporary and associated with the extreme drought conditions of that year.

$\delta^{13}C_{Rf}$ of the new foliage

There was a tendency for the new foliage to be further enriched relative to the mature foliage. This trait was observed beyond the growth period and until the $\delta^{13}C$ values had returned to the low wet season values. This feature may be related to anabolic processes during the development of the foliage. For example, due to kinetic isotope effects during their synthesis, many secondary compounds, including lipids, proteins and lignin, are depleted in ^{13}C relative to the original pool of carbon from which they were formed (Schmidt and Gleixner 1998, Hobbie and Werner 2004). Thus the discrimination during their synthesis leads to enrichment of the primary carbohydrate pool, from which respiration is drawing its substrate. The fact that the relative enrichment of the new foliage persisted beyond the period of needle extension may indicate that growth or development related process were still occurring in the new foliage despite the completion of their structural development. Should the difference between the new and mature foliage respiratory $\delta^{13}C$ be related to growth processes, it represents an important distinction, and possible application, of respiratory $\delta^{13}C$ measurements under growth conditions. While respiratory $\delta^{13}C$ may be dependent on the respiratory substrate being utilised i.e. on catabolic reactions, it may also be related to anabolic processes when the formation of secondary products is high.

Seasonal variation of $\delta^{13}C_{Rt}$

Few studies exist on the $\delta^{13}C$ composition of stem respired CO_2 , and no work in the literature exists on seasonal scale measurements. Damesin *et al.* (2005) presented data from a preliminary study into measurement techniques on a range of temperate and Mediterranean species, made in early winter. Stem respired CO_2 was enriched by up to 3‰ in most species relative to the stem organic matter, but measurements were not made of other components. We found that the stem respired CO_2 tended to be depleted relative to the foliage respired CO_2 , which is consistent with a downstream effect resulting from the depletion of the soluble carbon pool prior to export from leaf as result of the release of enriched respired CO_2 (Ghashghaie *et al.* 2003). However, the magnitude of the difference between $\delta^{13}C_{Rf}$ and $\delta^{13}C_{Rt}$ was variable and sometimes quite large (up to ~3‰) and it is unknown whether there

are additional effects associated with the diffusion resistance of the bark. Another factor that may influence the $\delta^{13}\text{C}_{\text{Rt}}$ is a contribution of CO_2 transported in the xylem which may bring CO_2 from another region such as the soil or roots (Teskey and McGuire 2002) and may have a ^{13}C composition that differs from that respired in the stem itself.

Overall, the results of the investigations into the $\delta^{13}\text{C}$ composition of the above ground respired CO_2 were consistent with the use of photosynthate from the ongoing photosynthesis in maintaining metabolic activity (as well as providing structural material for growth, see Chapter 2). This indicates the ability of these trees to maintain a close balance of their carbon supply and demand despite the long dry summer, as is evidenced also by the down regulation of respiration rates in summer. However, it is not known whether the linkage between photosynthesis and respiration may also indicate a lack of ability to rely on stored carbon for an extended period of time. This could be of consequence, for example, if the system experiences a number of extremely dry years in a row. In addition, the respiratory $\delta^{13}\text{C}$ data presented here have broader implications relating to the use of ^{13}C data in ecosystem research. We have shown that there can be a large seasonal variation in the $\delta^{13}\text{C}$ composition of respiratory CO_2 and that individual components of total ecosystem respiration can carry different isotopic signatures, including phenologically related (foliage development) differences. Furthermore the differences between components were not constant over time. Therefore caution needs to be exercised when interpreting total ecosystem respiratory $\delta^{13}\text{C}$ measurements and making inferences based on these measurements, such as in the estimation of canopy discrimination (Buchmann and Kaplan 2001).

6.3 Summary

When considering the factors responsible for determining ecosystem productivity, it is essential to understand how respiration is responding to environmental and physiological drivers. In this study, measurements of both foliage and stem respiration over a series of annual cycles that includes a seasonal drought period have been made. A number of interesting results emerge that are of relevance to understanding ecosystem responses to temperature and drought and for the mechanistic understanding of respiration in general.

There were similarities between the response of foliage and stem respiration to the warm dry summer conditions, but these were contrasted by a number of important phenologically related differences that result in temporal differences in relative CO_2 efflux rates between the

components. Both foliage and stem respiration showed a strong down-regulation in summer, despite the high temperatures. In fact, when not influenced by growth (i.e. in the mature foliage and outside the stem growth period), measured respiration rates in the stem and foliage were relatively constant and independent of temperature through the coolest and warmest periods of the year. This was achieved by acclimation responses that reduced the respiration rate at a common temperature and the temperature sensitivity of respiration in summer. However, growth of the developing needles resulted in high respiration rates, but not Q_{10} , during the summer. Respiration capacity, as indicated by respiration rate normalised to 20°C, indicated that photosynthetic substrate supply was driving mature foliage respiration while the developing foliage was responding to growth demands. Stem respiration peaked in late spring (May) and seemed to be responding to more subtle phenological influences such as the timing of phloem cell development or remobilisation processes. The decoupling of temperature and activity that is present in this system revealed that respiration is driven more by metabolic demand than temperature. When a factor such as summer drought reduces photosynthetic carbon gain, acclimation processes reduce respiration rates despite increases in temperature, and are therefore key factors in the overall carbon balance and productivity of the system.

From measurements of the $\delta^{13}\text{C}$ composition of the respired CO_2 , it was concluded that respiration generally continues to use photosynthate from the ongoing photosynthesis in summer, but in particularly dry years may have to utilize reserves or other compounds. There was a large seasonal variation in the respiratory $\delta^{13}\text{C}$ composition that was not consistent between different components, which may have implications for the interpretation of total ecosystem respired $\delta^{13}\text{C}$ measurements. There was a general enrichment through the summer in the respired CO_2 from all components, reflecting the influence of leaf physiology processes. Foliage tended to be more enriched than that from the stem, and developing foliage more enriched than the mature. It is proposed that the enrichment in the developing foliage is a result of the high degree of biosynthesis occurring during their growth, enriching the primary carbon pool.

Chapter 7

Conclusions

The high tolerance to drought in *P. halepensis* has made it an attractive species for afforestation and land reclamation efforts in much of the Mediterranean region. Even in Yatir Forest, at close to the limit of its distribution, the Aleppo pine ecosystem maintains levels of productivity that are comparable to much more mesic sites. In this study, a number of important physiological features underlying the high level of forest productivity have been identified.

A key element of productivity is the rate of photosynthesis and the timing and duration of the active season. Due to the pivotal role of water availability in this system, photosynthetic rates at both diurnal and seasonal scales are regulated mainly by changes in stomatal conductance, and the main period of activity is in winter and spring. In addition to soil water content, stomata are highly responsive to atmospheric vapour pressure deficit (D). The maximal rates of photosynthesis reached in the active period are relatively high and, due to the seasonal shift in activity in response to soil water content, occur when D is low. Although water loss rates are highest at the same time as maximal photosynthetic rates, the low D and regulating influence of the stomata (and other water-loss minimizing traits) result in high water use efficiencies. A conservative use of water, even in the wet season, slows the rates of soil water depletion in the early summer and extends the period of photosynthetic activity. Importantly, photosynthesis is maintained through the entire dry summer, albeit at much lower rates, and is concentrated in the early morning and late afternoon hours. Stomatal restrictions mean that the lowest rates of water loss are in summer, despite the high temperatures and D , and water use efficiency at peak activity is not affected much by the decline in soil water content. However, water use efficiency was strongly affected by increases in D .

Maintaining a level of photosynthetic activity through summer is crucial for maintaining needle phenology. Needle growth occurs in a highly constrained time period over summer, and appears to rely predominantly on the carbon fixed over this period. The high degree of control over water loss maintains cell turgor essential for growth, but also limits rates of carbon gain. Therefore, to maximise photosynthesis in the available periods of activity, the photosynthetic apparatus needs to be maintained in a state as close to optimal as

possible. Photosystem functionality is maintained through the high-light stress period through a series of protection mechanisms, including reductions in chlorophyll content, xanthophyll pigment mediated thermal dissipation and photorespiratory activity. In particular, the role of thermal dissipation was seen to be a key process, particularly as the limitations to carbon fixation increased. An important point that emerged from this study was the extent and nature of the water stress can affect the photoprotection responses. When the capacity for photosynthesis remains high enough that appreciable CO₂ fixation can occur, proportionally lower rates of thermal dissipation are employed, and consequently higher rates of photorespiration are also realised. When overall photosynthetic capacity is very low, thermal dissipation mechanisms are of greater relative importance, minimizing the costs associated with photorespiration.

In addition to carbon requirements, dry summer leaf phenology decoupled nitrogen demands from soil N availability. High levels of N remobilisation from mature needle classes and resorption from senescing needles provided N to the new needles. The redistribution of N resulted in lower overall needle N during the summer, but levels were maintained relatively constant in the new and 1 yr old needles through the period of minimum N. However the minimal levels of N were not limiting for photosynthesis beyond the stomatal limitations, indicating coordination between physiological, growth demands and phenological responses.

Through the measurements of foliage and stem respiration, it was shown that respiratory metabolism of the two components was responding to different demands, resulting in qualitatively contrasting temporal dynamics. Both foliage and stem showed a down-regulation of respiration during the hottest and driest period of the year, minimising carbon loss when carbon uptake was low and sink demand the needle phenology was high. Changes in the response to temperature were continuous through the year, and led to both a lower rate of respiration at a common temperature and a lower sensitivity to temperature changes. As a result, the lowest respiration rates could be observed across the entire seasonal range of temperatures in both the foliage and stem. The large decoupling from temperature (at the seasonal scale) shows that (above-ground) respiration in this system is responding predominantly to current metabolic demands, reducing any temperature induced wastage carbon losses. The influence of various physiological and phenological drivers is seen in the response of normalised respiration. Growing foliage respiration capacity was related to the needle growth rate, and remained higher the mature foliage through much of summer. Mature foliage respiration capacity was highly correlated with that of photosynthetic capacity, and the

main driver for stem respiration appeared to be late season phloem development and possibly a source demand for needle bud-burst.

Analysis of the $\delta^{13}\text{C}$ composition of the respired CO_2 indicated that respiratory metabolism is sustained by current photosynthate in both the foliage and stems, and therefore implying that net ecosystem carbon losses in summer are driven by root and soil respiration. There is also evidence from changes in the carbon content and $\delta^{13}\text{C}$ composition of leaf organic matter that there is a depletion of carbohydrates in the needles over summer, which could be due to their use in respiration and / or remobilisation to the growing needles. There was no obvious indication of a reliance on lipid catabolism for most the stress periods, consistent with the role of respiration down-regulation being important for plant carbon balance. However, there was evidence for a shift in metabolism in a summer with a drier spring. The lack of true dormancy in the dry and stressful summer, while contributing to an overall relatively high level of productivity, also renders the system sensitive to more extreme conditions in the summer.

Appendix

The relationship between soil water content, plant water status and physiological activity

An important response that can contribute to the overall stress effect under drought is a change in leaf or branch water status, as seen in changes in branch water potential. It is proposed that plants regulate their water loss in order to prevent leaf water potentials dropping to a critically low level, at which both impairment of cellular function and the development of embolisms within the plant hydraulic pathway may occur (Tyree and Sperry 1989, Whitehead 1998). Therefore measurements of branch or leaf water potential (Ψ_L) can provide important information on plant responses under water limiting conditions. It is assumed that the trees are in equilibrium with the soil before sunrise and therefore the measure of predawn water potential (Ψ_{PD}) is equivalent to soil water potential (water availability). The measure of water potential at midday (Ψ_{MD}), when water potentials drop to the daily minimum, indicates plant water status at the peak of evaporative demand and will depend on the soil water potential, the soil-leaf hydraulic conductivity, the extent of water loss through transpiration and the ability of the plants to control this water loss.

In the context of a broader study, the results of which are not included in this thesis, leaf water potential has been measured on branch ends (i.e. containing all age class cohorts of needles) in *P. halepensis* trees in Yatir and at two other sites in Israel where *P. halepensis* forms the predominant vegetation; at a site on Mt. Carmel (MC) in the north and a forest in the Judean Hills (JH) in the centre of the country. The three sites experience a steep precipitation gradient with a mean annual precipitation of ~700 mm at MC, 400 mm at JH and 280 mm at Yatir. Measurements of both predawn and midday water potential and needle gas exchange were made at different times of the year representing different soil water contents and levels of photosynthetic activity. Results from these measurements are shown here together with additional data from the non-irrigated trees in the irrigation experiment (TB Lin, unpublished data).

The relationship between Ψ_{PD} and gravimetric soil water content (0 – 30 cm) at each site is shown in Fig. A1, and shows the decline in soil water potential with water content. Below a gravimetric soil water content of ~17% there is a sharp decline in soil water potential to values below -1.5 MPa. This water content is equivalent to a REW of 0.4, which is the point at which a strong negative effect on physiological function is observed (see Fig. 3.3 and Fig. 3.6), and therefore indicates the soil water

potential at which water extraction becomes increasingly difficult to maintain for the trees to meet evaporative losses.

During the day, as transpiration proceeds and water is lost from the leaves, branch water potential declines to a minimum value, usually around midday. The influence of the seasonal changes in available soil water and plant water status can be seen in the relationship between REW and Ψ_{MD} (Fig. A2). Minimum water potentials were relatively stable at between -2.2 to -2.4 MPa above the REW_c value of 0.4. As soil water availability declined below the REW_c value, the minimum branch water potential reached each day also declined. However, in each of the populations investigated, the daily minimum water potential remained above -3.0 MPa. Therefore it can be concluded that stomatal closure acts to maintain leaf water potentials above this minimum value of -3.0 MPa, and indicates a critical lower limit of Ψ in *P. halepensis* (Tyree and Sperry 1989, Borghetti *et al.* 1998, Whitehead 1998).

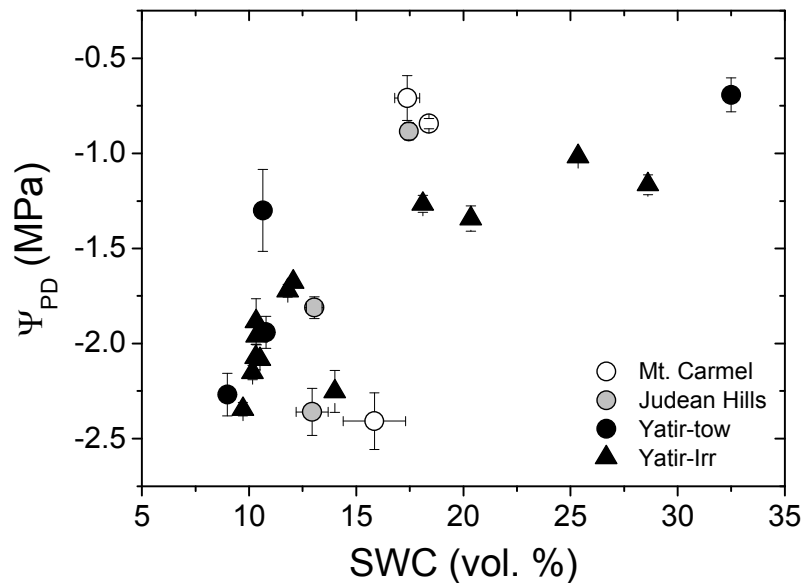


Figure A1. The relationship between gravimetric soil water content (SWC) and predawn branch water potential (Ψ_{PD}) in *P. halepensis* measured at three geographical locations in Israel at different times in the season. Measurements were made at two sites in Yatir Forest, at the location of the meteorological tower and at the site of the irrigation experiment. The results from the irrigation site are for the non-irrigated trees only.

The effect of the changes in soil water availability and on the whole plant hydrological system can be seen in estimates of the soil-to-leaf hydraulic conductance (K_L), which can be estimated from:

$$K_L = E / (\psi_s - \psi_L)$$

where E is leaf transpiration rate from the gas exchange measurements, $(\Psi_S - \Psi_L)$ is the water potential gradient between the soil and the leaves, and is determined by using the measurements of Ψ_{PD} and Ψ_{MD} for Ψ_S and Ψ_L , respectively. As soil water declined below the REW value of 0.4 there was strong decline

in K_L (Fig. A3), indicating that the lower branch water status observed under the low soil water conditions (Fig. A2) is related to a reduced supply of water through the soil-leaf hydrological pathway being unable to match the rate of evaporative loss from the leaves.

The decline in leaf water potential may negatively impact CO_2 assimilation rate if the leaf water content drops too low. In Fig. A4 the relationship between leaf water potential and needle assimilation rate measured on the same branches at different times during the day is shown. Maximal photosynthetic rates were observed when branch water potential was in the range of -2.0 to -2.4 MPa, but remained low as branch water potential declined below -2.4 MPa. However, low assimilation rates were also observed when Ψ was in the range at which high photosynthetic rates were also observed. The low assimilation rates in this range were the result of stomatal closure in response to increasing atmospheric vapour pressure deficit (D) during the day (data not shown, but see Fig. 3.6B). The response of stomatal closure in response to increases in D , despite leaf water potential still being in the range for high rates of assimilation, indicates a feed-forward response of stomata to evaporative water loss and highlights the conservative response of *P. halepensis* to controlling water balance, whereby water loss is minimized at the expense of carbon gain over the short term.

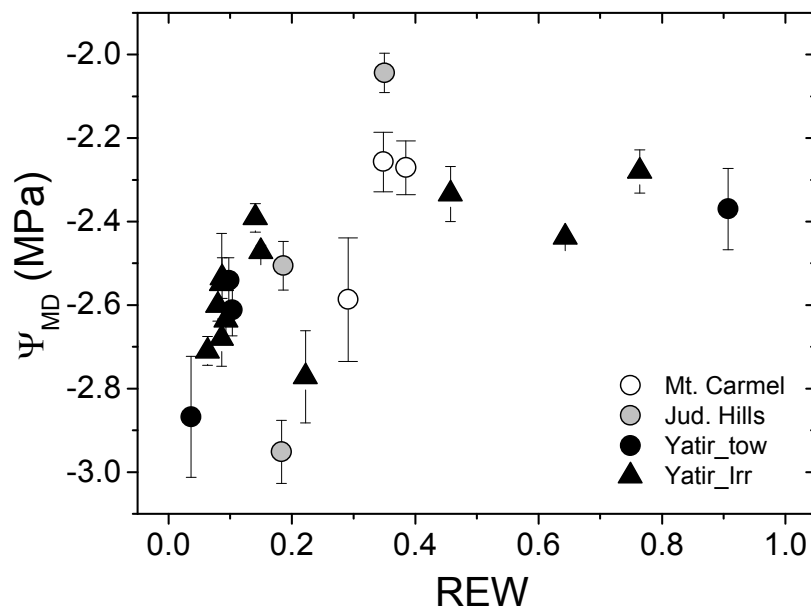


Figure A2. The relationship between the daily minimum branch water potential, measured at midday (Ψ_{MD}), and root Relative Extractable Water (REW) at different times in the season for the same samples as shown in Fig. A1.

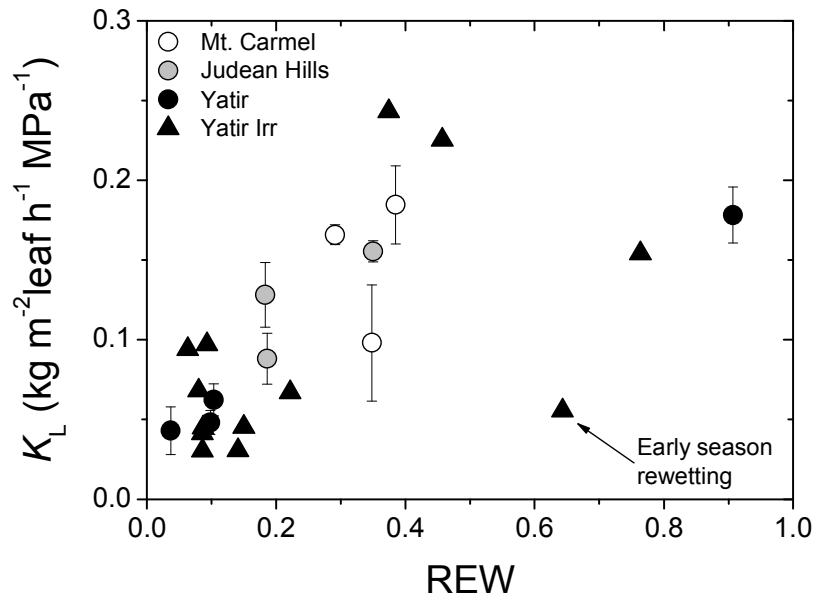


Fig. A3 Soil-to-leaf hydraulic conductance (K_L) in *P. halepensis* estimated from gas exchange and branch water potential measurements from the samples from three sites in Israel.

While the daily minimal Ψ_{MD} values and the Ψ_{MD} values measured under non-limiting soil water contents were similar between the different populations of *P. halepensis*, there were site differences observed in the relationship between branch water potential and soil water content. In the samples measured in the Judean Hills and Mt. Carmel sites, Ψ_{PD} was greater than -1.0 MPa when soil water content was about 16% (Fig. A1) and Ψ_{MD} was still at maximal values when REW was about 0.3 (Fig. A2). This may be related to using soil water content over a depth of 0 – 30 cm only as a measure of plant available soil water. Preliminary observations have indicated that the majority of fine root biomass in Yatir is located in this layer, but roots do extend to a depth of 1.2 m or more (J. Grünzweig, N. Raz Yaseef, unpublished data). Root mass density may differ with depth between sites of contrasting edaphic characteristics, and the relative access to water from deeper layers is likely to be an important feature of contrasting seasonal patterns of activity and total productivity between sites. Measuring soil water content and root density at depth is technically challenging in the rocky soils characteristic of the environments in which *P. halepensis* occurs in Israel, particularly when the soil is dry. However, the relative availability of soil water at different depths and the relationship between the relative use of water from the different depths and plant function is clearly important information that will provide a better understanding of *P. halepensis* ecology.

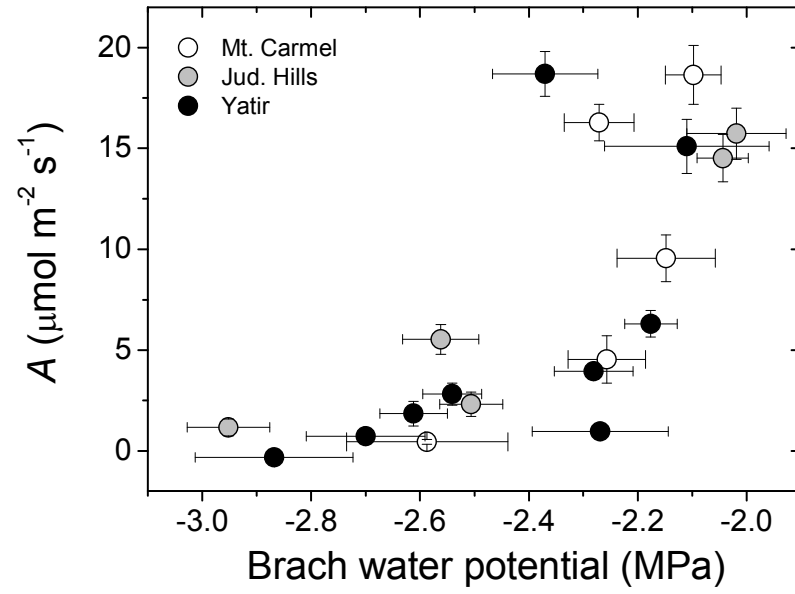


Figure A3. Photosynthetic assimilation rates (A) measured at different branch water potentials at different times of the day in the same samples as in Fig. A1, excluding samples from the irrigation site in Yatir. Measurements of A were made immediately prior to water potential determination.

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