EFFECTS OF THE INVASIVE ANNUAL GRASS *Lolium multiflorum* Lam. ON THE GROWTH AND PHYSIOLOGY OF A SOUTHERN AFRICAN MEDITERRANEAN-CLIMATE GEOPHYTE *Tritonia crocata* (L.) Ker. Gawl. UNDER DIFFERENT RESOURCE CONDITIONS

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(B.Sc.)

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2007

Potchefstroom Campus

IN MEMORY OF MY FATHER AND MOTHER

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ABSTRACT

Effects of the invasive annual grass, *Lolium multiflorum* Lam., on the growth and physiology of a South African Mediterranean-climate geophyte, *Tritonia crocata* (L.) Ker. Gawl., under different resource conditions.

Little is known of the physiological and biochemical mechanisms underlying competitive interactions between alien invasive grasses and native taxa, and how these are affected by resource supply. Consequently, this study compared photosystem II (PS II) function, photosynthetic gas and water exchange, enzyme and pigment concentrations, flowering and biomass accumulation in an indigenous geophyte, *Tritonia crocata* (L.) Ker. Gawl., grown in monoculture and admixed with the alien grass, *Lolium multiflorum* Lam., at different levels of water and nutrient supply. Diminished stomatal conductances were the primary cause of reduced net CO_2 assimilation rates, and consequent biomass accumulation in *T. crocata* admixed with *L. multiflorum* at all levels of water and nutrient supply with one exception. These corresponded with decreased soil water contents induced presumably by more efficient competition for water by *L. multiflorum*, whose biomass was inversely correlated with soil water content. Biochemical impairments to photosynthesis were also apparent in *T. crocata* admixed with *L. multiflorum* at low levels of water and nutrient supply. These included a decline in the density of working photosystems (reaction center per chlorophyll RC/ABS), which corresponded with a decreased leaf chlorophyll *a* content and a decreased efficiency of conversion of excitation energy to electron transport (Ψ_0 / 1-Ψ_0), pointing to a reduction in electron transport capacity beyond Q_A^-, a decline in apparent carboxylation efficiency and Rubisco content. At low nutrient levels but high water supply, non-stomatal induced biochemical impairments to

photosynthesis (decreased RC/ABS, chlorophyll *a* and Rubisco content) were apparent in *T. crocata* admixed with *L. multiflorum*. These attributed to a reallocation of fixed carbohydrate reserves to floral production which increased significantly in *T. crocata* under these conditions only and associated with a corresponding reduction in the mass of its underground storage organ (bulb). The results of this study did not support the hypothesis that under conditions of low water and low nutrient supply invasive annual grasses would have a lesser impact on the growth and physiology of native geophytes than under resource enriched conditions that favor growth of these grasses. Unresolved is whether resource limitation and allelopathic mechanisms functioned simultaneously in the inhibition of the native geophyte by the alien grass.

Keywords

Growth, *Lolium multiflorum*, Photosynthesis, Photosystem II, Pigments, Rubisco, *Tritonia crocata*

OPSOMMING

Effekte van die eenjarige indringer gras, *Lolium multiflorum* Lam., op die groei en fisiologie van 'n Suid-Afrikaanse Meditereense-klimaat bolplant, *Tritonia crocata* (L.) Ker. Gawl., onder verskillende hulpbronkondisies.

Daar is tans min inligting beskikbaar oor die fisiologiese en biochemiese meganismes onderliggend aan die kompeterende interaksies tussen uitheemse indringer grasse en inheemse plantspesies, en hoe hulle deur hulpbronvoorsiening geaffekteer word. In hierdie studie is daar 'n vergelyking getref tussen fotosisteem II funksie, fotosintetiese gas- en wateruitruiling, ensiemkonsentrasies, blaarpigmentkonsentrasies, blomvorming en biomassa akkumulasie in die bolplant, *Tritonia crocata* (L.) Ker. Gawl wat as 'n monokultuur of in kombinasie met die indringer gras, *Lolium multiflorum* Lam. onder verskillende kombinasies van water- en voedingstoftoedienings gekweek is.
Behalwe vir een uitsondering was 'n afname in stomageleiding primêr verantwoordelik vir die waargenome verminderding in netto CO_2 opname tempos en die gevolglike afname in biomassa akkumulasie in *T. crocata* in kombinasie met *L. multiflorum* onder alle water- en voedingstofkombinasies.
Hierdie effekte het saamgeval met 'n afname in grondwaterinhoud wat moontlik veroorsaak is deur meer doeltreffende kompetisie vir water deur *L. multiflorum*. Beide stoma- en mesofilbeperking van fotosintese was duidelik waarneembaar in *T. crocata* in kombinasie met *L. multiflorum* onder lae water- en lae voedingstofvoorsiening. Laasgenoemde het 'n afname in die digtheid van aktiewe fotosisteme (reaksiesentrums per chlorofil, RC/ABS), geassosieer met 'n afname in chlorofil *a* inhoud, asook 'n afname in doeltreffendheid van omskakeling van eksiteringsenergie na elektrontransport ($\Psi_0/1-\Psi_0$) ingesluit. Dit is 'n aanduiding van 'n afname in elektrontransportkapasiteit verder as Q_A^-, 'n afname in skynbare karboksileringsdoeltreffendheid en Rubisco inhoud. By lae

voedingstof- en hoë watertoediening was slegs mesofilbeperking van fotosintese (afnames in RC/ABS, chlorofil *a* en Rubisco inhoud) waargeneem in *T. crocata* in kombinasie met *L. multiflorum*. 'n Hertoedeling van koolhidraatreserwes ten gunste van blomvorrning het skynbaar ook plaasgevind aangesien 'n afname in die massa van die ondergrondse stoororgaan van *T. crocata* aangetoon is. Die resultate van hierdie studie het nie die hipotese ondersteun dat onder kondisies van lae water- en lae voedingstoftoediening, uitheemse grasse 'n verminderde impak op die groei en fisiologie van die inheemse bolplante sou hê as onder verrykte hulpbrontoestande wat die groei van die uitheemse grasse bevorder nie. Onbeantwoord is die vraag of hulpbronbeperking en allelopatiese meganismes gelyktydig fungeer tydens die beperking van die inheemse bolplant deur uitheemse grasse.

Sleutelterme

Fotosintese, Fotosisteem II, Groei, *Lolium multiflorum*, Pigmente, Rubisco, *Tritonia crocata*

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Most of all: To God be all the glory for the great things that He has done for me. With Him everything started and will also end.

I hereby declare that this thesis presented for the degree Master of Environmental Sciences, at North-West University (Potchefstroom Campus), is my independent work and has not previously been presented for a degree at any other university or faculty.

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LIST OF ABBREVIATIONS

A	CO_2 assimilation rate at ambient CO_2 concentration (350 µmol mol^{-1})
ABA	Abscisic acid
ABS/CS_M	The phenomenological energy flux (per excited cross section of leaf) for light absorption
ABS/RC	The specific energy flux (per PSII reaction centre) for light absorption
ACE	Apparent carboxylation efficiency (µmol CO_2 m^{-2} s^{-1})
A_{max}	Light saturated rate of photosynthesis (µmol CO_2 assimilated m^{-2} s^{-1})
AQE	Apparent quantum efficiency (µmol CO_2 m^{-2} mol^{-1} PPFD)
ATP	Adenosine tri-phosphate
c_a	Atmospheric CO_2 concentration (µmol mol^{-1})
c_i	Intercellular CO_2 concentration (µmol mol^{-1})
CS	Excited cross section of leaf
DI_0/RC	Dissipation at the level of the antenna chlorophylls
E	Transpiration rate (mmol H_2O m^{-2} s^{-1})
ET	Electron transport
ET_0/CS_M	The phenomenological energy flux (per excited cross section of leaf) for electron transport
ET_0/RC	The specific energy flux (per PSII reaction centre) for electron transport
FNR	Ferredoxin-NADP oxidoreductase
F_V/F_M	The ratio of variable to maximal chlorophyll *a* fluorescence
g_S	Stomatal conductance (mmol m^{-2} s^{-1})
J_{max}	Maximal CO_2 assimilation rate at saturating intercellular CO_2 concentration (µmol CO_2 assimilated m^{-2} s^{-1})
N	Nitrogen
NADPH	β-Nicotinamide adenine dinucleotide

OEC	Oxygen evolving complex
PCR cycle	Photosynthetic carbon reduction cycle
PEA	Plant efficiency analyzer
PPFD	Photosynthetic photon flux density (μmol m^{-2} s^{-1})
PI_{ABS}	Performance index expressed on absorption basis
PQ	Plastoquinone
PSI	Photosystem I
PSII	Photosystem II
Q_A	Primary bound plastoquinone
Q_A^-	Primary bound plastoquinone in reduced state
RC	Photosystem II reaction centre
RC/ABS	The density of active PSII reaction centres on a chlorophyll basis
RC/CS_M	The density of active PSII reaction centres per excited cross section of leaf
R_D	Dark respiration rate (μmol m^{-2} s^{-1})
Rubisco	Ribulose-1,5- bisphosphate carboxylase/oxygenase
RuBP	Ribulose-1,5- bisphosphate
RWC	Relative water content
TR	Trapping of excitation energy
TR_0/CS_M	The phenomenological energy flux (per excited cross section of leaf) for trapping
TR_0/RC	The specific energy flux (per PSII reaction centre) for trapping
WUE_{INT}	Intrinsic Water use efficiency (mmol H_2O μmol CO_2^{-1})
φ_{EO}	Quantum yield of electron transport
ψ_0	Efficiency with which a trapped exciton can move an electron further than Q_A^- into the electron transport chain

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CHAPTER 1: LITERATURE REVIEW

1.1. BIOLOGICAL INVASIONS AND GLOBAL CHANGE

There is general recognition that serious ecological, economic and social consequences result from the invasion of natural ecosystems by foreign biological organisms (Perrings *et al.*, 2000; McNeely *et al.*, 2001). Conservative estimates indicate that the global costs of alien invasive species impacts on natural ecosystems exceed the total economic output of the entire African continent (Pimentel, 2002). Such impacts predicted to intensify in the near future due to global climate change (Mooney & Hobbs, 2000).

Biological invasions form a significant component of global change caused by human movement (Vitousek *et al.*, 1997), and jointly with atmospheric CO_2 concentration, nitrogen deposition and acid rain are believed to be the major drivers of global biodiversity change in terrestrial ecosystems (Sala *et al.*, 2000). Mediterranean ecosystems are especially sensitive to biodiversity loss induced by all drivers of biodiversity change, particularly land use change (Sala *et al.*, 2000), which interacts with other mechanisms of global change to facilitate invasions (D'Antonio & Vitousek, 1992; Richardson *et al.*, 2000; Didham *et al.*, 2005). Biological invasions present one of the most important threats to biological diversity (Vitousek *et al.*, 1997; Dukes & Mooney, 1999; Dukes, 2001; Richardson & van Wilgen, 2004) as they transform ecosystem processes over large areas and feed back to change other components such as climate and land use (Dukes & Mooney, 1999). To minimise these influences, it is important to construct the best available scientific management protocols.

1.2. INVASIVE GRASSES AS A GLOBAL AND REGIONAL PROBLEM

Grasses are one set of invasive species that collectively threaten regional and even global aspects of ecosystem function (D'Antonio & Vitousek, 1992; Knapp, 1996). Their most significant ecological impacts include alteration of fire regimes (van Wilgen & Richardson, 1985; D'Antonio & Vitousek, 1992; Smith & Tunison, 1992; Tunison, 1992) by increasing the abundance of fine fuel thereby accelerating fire frequencies and intensities commonly referred to as the grass/fire cycle (D'Antonio & Vitousek, 1992). In addition, grass invaders are widespread, effective and aggressive competitors with native species (D'Antonio & Vitousek, 1992; Goergen & Daehler, 2001, 2002).

Numerous examples of alien grass invasions are found on all continents. Typical examples include the invasion of coastal salt marshes in Britain by the North America grass *Spartina alterniflora* (D'Antonio, *et al.*, 1992), the invasion of two species of *Ehrharta* (African veldt grass) into the coastal communities in south-western Australia and South America (D'Antonio, *et al.*, 1992) and the displacement of native pasture grasses such as *Trachypogon plumosus* by the alien invasive grass species *Hyparrhenia rufa* (Jaragua) and *Melinis minutiflora* (Molasses grass) (D'Antonio, *et al.*, 1992). However, large-scale invasions are less common in Eurasia and Africa where much of the tropical areas are covered by so-called derived grasslands and savannas (D'Antonio, *et al.*, 1992). These are presumed formerly forested areas in which grasses now dominate as a consequence of intense ungulate grazing and a long history of human activity. This is especially pertinent in Africa where grasses have evolved with hominids for millions of years. Their adaptations to severe grazing, which include rapid growth response to defoliation and subterranean, vegetative propagating organs also confer resistance to fire. Therefore, it is not unexpected that Africa, and to a lesser extent Asia, have been donors rather than recipients of fire-adapted alien grasses. Despite this propensity, there are some examples in Africa of large-scale recipient invasions by alien grasses from other continents, or from other areas within the continent. These include the establishment of several European annual grasses in Mediterranean climate regions of South Africa and the recent spread of perennial grasses of South American, Central and North African origin in southern Africa (Milton, 2004). In southern Africa, invasive grasses are especially prevalent in natural ecosystems along the west coast of South Africa, including waste lands (Bromilow, 2001) and along roadsides (Milton & Dean, 1998; Milton *et al.*, 1998) which can be viewed as conduits for invasion. This is a cause for concern, especially in terms of the wildflower diversity, which forms the basis of a growing lucrative nature-based tourist industry in a Mediterranean-climate region unique in terms of its rich floristic diversity and endemism (Goldblatt & Manning, 2000). Evidence that the natural flora in this region, which is listed among 25, though lately 34 global biodiversity hot spots (Myers *et al.*, 2000; Mittermeier *et al.*, 2004), is under threat from competition by alien grasses is based on an apparent recent increase in the abundance of especially annual grasses on bottomlands and plains (Vlok, 1988; Steinschen *et al.*, 1996).

1.3. SPREAD AND IMPACT OF INVASIVE GRASSES

The advance of annual grasses into natural landscapes is affected from contaminated road verges and agricultural lands particularly, and is facilitated through the transport of their seeds on the hide of grazing animals (Schmida & Ellner, 1983; Knapp, 1996), and in the dung of domestic livestock and wildlife (Davidse, 1986; Malo & Suarez, 1995; Shiponeni, 2003). It is exacerbated by rangeland deterioration caused by ploughing, vegetation clearing and burning, by soil nutrient enrichment from fertilizer run-off and nitrogen-fixing leguminous species (Milton, 2004), and by grazing that tends to be more intensive in small habitat fragments (Kemper *et al.,* 1999; Van Rooyen, 2003). Also, alien grasses are known to impact on ecosystem structure, function and resources by accelerating wild fires, decreasing floral and faunal diversity and forage stability, altering soil food webs, soil water dynamics and decomposition cycles (Vila *et al.,* 2000; Hobbs, 2001; Lenz *et al.,* 2003). To date, there has been little assessment of the ecological drivers and effects of these invasive grass species on the growth and physiology of native species in South Africa (Milton, 2004).

Much of the knowledge on alien invasive grasses has been derived from studies outside South Africa with limited information available on local impacts (Milton, 2004) and practical control strategies (Musil *et al.,* 2005). Also, there is a paucity of information on the effects of the presence of invasive grasses on the physiology and biochemistry of natural taxa (Milton, 2004). Burning and clearing, like other forms of disturbance (Schiffman, 1994; Deregibus *et al.*, 1994; Kotanen, 1995; Hobbs, 2001;), are known to favour the germination and establishment of invasive annual grasses, including *Lolium multiflorum*, and this has been attributed to an increase in the ratio of short- to long-wave radiation reaching seeds on the soil surface (Cowling *et al.,* 1986). Noteworthy, in this regard is that plants with smaller seeds, have greater difficulty in emerging from the dense litter layer produced by invasive annual grasses due to insufficient light and seed resources (Carson & Petersen, 1990; Facelli & Pickett, 1991; Petersen & Facelli, 1992; Facelli *et al.,* 1999). In fact, the dense litter produced by invasive annual grasses is known to inhibit the germination and establishment of native taxa (Facelli & Pickett, 1991; Petersen & Facelli, 1992; Lenz *et al.,* 2003) and alter soil water and temperature regimes that promote fungal pathogens (Facelli, 1994) and other seed and seedling predators (Hoopes & Hall, 2002). Also, fire is known to alter soil mineral levels (DeBano *et al.,* 1998), especially nitrogen and phosphorus in arid regions (Schiffman, 1994), and rates of water infiltration into soils (Osborn *et al.,* 1967; Adams *et al.*, 1970; DeLucia *et*

al., 1989; **Debano**, 2000), these factors possibly contributing to the greater recruitment of invasive annual grasses observed in burnt areas. Indeed, the establishment and growth of invasive annual grasses is promoted by soil nitrogen enrichment (Maron & Connors, 1996) and supplemental water (Cabin *et al.*, 2002).

1.4. CONTROL OF INVASIVE GRASSES

Annual grasses occur for part of their life cycle only as seeds suggesting that the control of such grasses may be effective if their seed banks are completely eliminated (Whelan, 1995). In this regard, it has been reported that *Bromus tectorum* L. is particularly amenable to control by fire prior to seed dispersal, since its soil seed bank can approach zero density at this stage (Pyke & Archer, 1991). However, the effectiveness of fire in controlling other invasive annual grasses has been reported only partial or temporary, with inadequate information regarding seed longevity often leading to incorrect management recommendations (Brooks & Pyke, 2001). For example, fire was initially proposed for the control of the invasive annual grass, *Taeniatherum caput-medusae* (L.) Nevski, (Murphy & Lusk,1961). However, later studies demonstrated that its effectiveness was incomplete (Torell *et al.*, 1963) requiring follow-up treatment with propane weed flamers to destroy individuals that escaped initial fire treatments (Turner *et al.*, 1963), a technique along with herbicide applications and grazing more recently considered for restoration activities in sagebrush annual grassland communities in the Great Basin in the USA (Rasmussen, 1994). Of particular concern is the reported rapid development of multiple herbicide resistance among especially annual hybrids of *Lolium* in South Africa, which means that chemical control measures may become less effective with repeated herbicide use (Gill, 1996; Cairns & Eksteen, 2001).

Grazing by cattle and sheep during flowering and seed set of annual grasses has been used to control annual grass weeds in Australia and South Africa. The advantages are that costs are low. The disadvantages include avoidance by such ungulates of invasive annual grasses with sharp unpalatable seeds, e.g. *Vulpia*, *Hordeum* and *Bromus*, this leading to their increase (Matthews, 1996; Van Rooyen, 2003), while the other more palatable invasive annual grasses, e.g. *Lolium* and *Avena*, decrease (Matthews, 1996), as do palatable perennial grasses indigenous to renosterveld (Cowling, *et al.*, 1986; McDowell, 1994). Another disadvantage is that ungulate grazing intensity needs to be high to reduce seed

production. In fact, intensive ungulate grazing and associated trampling and dunging are considered incompatible with conservation of native plant diversity in renosterveld because this promotes invasive annual plants as well as "weedy" indigenous geophytes (Kemper *et al.*, 1999), such as the cincherinchees (*Ornithogalum conicum* and *O. thyrsioides*), which are particularly toxic to livestock (Watt & Breyer-Brandwijk, 1962). Paradoxically, ungulate grazing has proven a highly effective tool for reducing grass fuel loads and risk of catastrophic fires in other ecosystems (Janzen, 1988; Blackmore & Vitousek, 2000), yet continued grazing inevitably leads to reductions in native species cover and diversity. Indeed the ecological effects of ungulate grazing on global ecosystems continue to inspire fervent debate (Brussard *et al.*, 1994; Fleischner, 1994; Noss, 1994; Brown & McDonald, 1995). Ungulate exclusion from ecosystems that have evolved in their absence has proved an ecologically and economically cost-effective restoration strategy, across large spatial scales and diverse ecological communities. However, there are few reports on the effects of ungulate grazing on native species cover and diversity in renosterveld. Preliminary studies have shown that intensive grazing has little effect on species richness as a whole but alters renosterveld composition by causing a decrease in perennial grasses, and an increase in certain geophytes and Asteraceous shrubs (McDowell, 1994).

Perrenial grasses like *Pennisetum setaceum* (fountain grass) which survive repeated fires and grazing pressure by both vegetative means and by seeding are more difficult to eradicate (Milton, 2004). Persistent creeping perennial grasses, such as *Cynodon dactylon*, are known to exclude the establishment of shrubs (Midoko-Iponga, 2004) and tussock grasses, particularly where grazers and browsers are present (Van Auken, 1994). Their dense root systems intensify below ground competition which restrains growth of some invasive annual grasses, such as *Bromus tectorum* (Yoder & Caldwell, 2002), and inhibits nutrient and water acquisition by native species reducing their growth and reproductive output (Dyer & Rice, 1999; Hamilton *et al.*, 1999; Cabin *et al.*, 2002).

1.5. POTENTIAL PHYSIOLOGICAL IMPACTS OF INVASIVE GRASSES ON NATIVE SPECIES

Invasive annual grasses, such as *Bromus rubens* L., *B. tectorum* L. and *Schismus barbatus* (Loefl. ExL) Thellung, have been reported to compete for soil water resources more effectively and utilize elevated soil N levels more rapidly than native species (Eissentat & Caldwell, 1998; Melgoza & Nowak, 1991) thereby reducing native seedling biomass and

species richness (Brooks, 2000). This more effective competition for nutrient but especially water resources by invasive grasses may lead to physiological modifications and reduced productivity by native species in response to the increased drought and nutrient stress (Taiz & Zeiger, 1998; Chaitanya *et. al.*, 2003).

Drought stress is known to impair all three main component processes of photosynthesis, namely stomatal control of CO_2 supply, CO_2 fixation reactions of the photosynthetic carbon reduction (PCR) cycle and photophosphorylation reactions of the thylakoid membrane (Boyer *et al.*, 1997, Lawlor, 2002). The primary cause of reduced rates of photosynthesis under mild or severe conditions of drought stress is stomatal closure (Sharkey, 1990), with metabolic changes involved in stomatal adjustments including phosphorylation state, pH and cytosolic free calcium (Leckie *et al.*, 1998; Grabov & Blatt, 1998). Metabolic impairment of photosynthesis due to water stress may also result from a reduction in the activity or abundance of the photosynthetic enzyme ribulose-1,5-biphosphate carboxylase/oxygenase (Rubisco), or an impairment of phosphorylation reactions, namely ribulose- 1,5- bisphosphate (RuBP) regeneration and the supply of reducing equivalents (ATP and NADPH) for the functioning of the PCR cycle (Lawlor, 2002; Reddy *et al.*, 2004). Reduced Rubisco amounts have been reported in several plants exposed to drought (Parry *et al.,* 2002), with the amount of Rubisco in leaves controlled by the rate of synthesis and degradation (Parry *et al.*, 1999). A rapid decrease in the abundance of Rubisco small subunit (rbcS) transcripts, indicative of reduced synthesis, has been reported in *Arabidopsis* (Williams *et al.*, 1994), rice (Vu *et al.*, 1999) and tomato (Bartholomew *et al.*, 1991) during drought stress. Diminished Rubisco activity under conditions of drought has also been reported in soybean (Majumdar *et al.,* 1991) and tobacco (Parry *et al.,* 1993), in the latter species attributed to the accumulation of tight-binding inhibitors on Rubisco catalytic sites. In contrast, little effect of drought stress on Rubisco has been reported in sunflower (Giménez *et al.*, 1992). With respect to RuBP regeneration, a strong relationship between CO_2 assimilation capacity and RuBP availability was demonstrated in sunflower leaves during drought stress (Giménez *et al.*, 1992) with a general decline in contents of PCR cycle intermediates observed during dehydration. There is also increasing evidence that photosystem II (PS II), the site of photosynthetic electron transport, is the most sensitive component of the thylakoid membrane to drought stress (Golding & Johnson, 2003). Direct damage to PS II has been

reported under conditions of drought stress, this due to the degradation of the D1 reaction centre protein and enhanced phosphorylation of PS II core proteins (Giardi *et al.,* 1996).

The relationship between photosynthesis and nitrogen availability is fundamentally complex, because photosynthesis represents the integrated operation of a series of processes sensitive to environmental factors as well as leaf physiology and structure (Field *et al.*, 1986). Indeed, plants need many different nutrients from their environment to form fully functional organs, including leaves which can photosynthesise effectively. However, nitrogen is needed for the production of leaf area which in combination with the rate of photosynthesis per unit area determines total plant productivity. Reduced nitrogen supply results in lower protein and chlorophyll contents per unit leaf area, thus leading to reduced carboxylation efficiency and RuBP regeneration capacity (Lawlor, 2001).

Nitrogen is a main nutrient and photosynthesis requires a considerable investment of this element because it is a very active metabolic process requiring many protein components. Mineral nutrition (particularly nitrogen), limits those aspects of gas exchange most closely associated with photosynthetic capacity (Field & Mooney, 1986). A high evaporative demand is another potential constraint to maximum photosynthetic capacity, especially during periods of declining soil water content (Flexas, *et al.*, 2002), since water deficits may change aspects of nitrogen assimilation. However, it is still fundamentally unclear as to how drought stress impacts on nitrogen metabolism. There is no evidence supporting a reduced supply of nitrogen (in the form of nitrate, NO_3^-) to plants at low leaf relative water contents, although the flux of NO_3^- to roots is by mass flow, so decreasing transpiration may decrease uptake (Lawlor *et al.*, 2002). The major limitation to nitrogen assimilation under drought stress may result from altered nitrate reductase activity which declines at low leaf relative water contents and rapidly increases in leaves on their re-hydration (Kaiser & Förster, 1989; Ferrario-Méry *et al.*, 1998; Foyer *et al.*, 1998). Also, carbon metabolism is closely linked with NO_3^- assimilation, since increased sucrose and glucose concentrations in leaves stimulate transcription of nitrate reductase genes (Foyer *et al.*, 1998). Therefore, it would appear that nitrogen metabolism under conditions of drought stress is more dependent on mitochondrial metabolism, mostly because it relates to synthesis of amino acids (Morot-Gaundry *et al.*, 2001).

1.6. HYPOTHESES EXPLAINING SUCCESS OF INVASIVE SPECIES

Seven ecological hypotheses have been proposed to explain why alien species are successful invaders of novel environments. The first, the "Preadaptation / Disturbance Hypothesis" presumes pre-adaptation of the invasive species to facets of the novel environment (Sax & Brown, 2000). The second, the "Inherent Superiority Hypothesis" proposes that the invasive species are superior competitors (Sax & Brown, 2000), the third the "Novel Superiority Hypothesis" proposes that the invasive species possess biochemical weapons (allelopathic substances) that native species are susceptible to, the fourth the "Empty Niche Hypothesis" proposes that the invasive species are able to use resources more efficiently than native species in the novel environment, the fifth the "Mutualist Facilitation/ Invasional Meltdown Hypothesis" proposes that for alien species involved in mutualistic relations, e.g. mycorhyiza, to be successful invaders they require concomitant introduction of their mutualists from their native range to their novel range, the sixth the "Biotic Resistance Hypothesis" proposes that reduced competition from native taxa in disturbed natural communities allows the establishment of an invasive species and finally, the "Enemy Release Hypothesis" proposes that the success of an invasive species in its novel range is due to its release from its natural enemies in its native range. An evolutionary corollary of this hypothesis is the "Evolution of Increased Competition Ability Hypothesis", which proposes that when few or no natural enemies of an alien plant species are present, its will direct less energy towards defence mechanisms and more to growth and propagation thereby improving its competitive ability.

1.7. STUDY HYPOTHESIS AND OBJECTIVES

Available data suggest that the "Empty Niche Hypothesis" is often applicable to alien invasive grasses. They are known to be superior competitors for water and nutrient resources (D'Antonio, *et al.*, 1992). Examples include the poor recruitment and growth of oak seedlings reported in California grasslands (Gordon *et al.*, 1989; Danielson *et al.*, 1990) induced by superior competition for soil water resources in the presence of grasses (Danielson *et al.*, 1990) and the observed more rapid decline in soil nitrogen levels in plots planted with alien grasses than native species (Elliott & White, 1989). Also, lower seedling growth rates and tissue nitrogen contents have been reported in native shrubs grown in the presence of invasive grasses after a wildfire in Hawaiian woodland (Hughes *et al.*, 1991).

Most invasive annual grasses do not possess adaptations to low nutrient levels (Brooke, 1999) unlike taxa native to Mediterranean climate ecosystems (Musil *et al.*, 2003) suggesting that in such ecosystems invasive grasses may only be more effective competitors than native taxa under conditions of abnormally high soil nutrient levels arising from fertilizer run-off or nitrogen-fixing alien woody leguminous species (Milton, 2004). Also, many annual grasses possess the C_3 photosynthetic pathway and are particularly responsive to resource enrichment arising from atmospheric CO_2 and soil N enrichment (Richardson *et al.*, 2000) suggesting that they may be more effective competitors than native taxa under conditions of global change.

In view of the above findings, the following hypothesis was tested, namely that under conditions of low water and nutrient supply invasive annual grasses would have a lesser impact on the growth and physiology of native taxa than under opposite conditions. In testing this hypothesis, the main objectives were to increase current understanding of the physiological and biochemical mechanisms underlying competitive interactions between alien invasive grasses and indigenous taxa and how these are affected under different resource supply. In this regard, the following key questions were addressed.

- Which physiological and biochemical mechanisms involved in photosynthetic carbon metabolism and growth is affected in indigenous taxa by competition with alien invasive grasses?
- Does resource supply modify the mechanisms of inhibition or the intensity of the competition?

CHAPTER 2: MATERIALS AND METHODS

2.1. SPECIES SELECTION AND PROPAGULE SOURCES

Geophytes form a major component of renosterveld (Figures 2.1C; 2.1E & 2.2.) broadly categorized as an evergreen, fire-prone, vegetation lacking Proteaceae and Ericaceae which is dominated by small-leafed asteraceous shrubs, especially *Elytropappus rhinocerotis* (L.f.) Lees (renosterbos or rhinoceros bush), with an understory of grasses (Boucher & Moll, 1981; Low & Rebelo, 1996). Post-colonial firewood collection, burning and grazing of vegetation are thought to have shaped modern renosterveld by transforming a woody shrubland-perennial grassland mosaic into a more uniform shrubland in which geophytes form a major component (Boucher & Moll, 1981; Cowling *et al.,* 1986). Consequently, the indigenous geophyte, *Tritonia crocata* (L.) Ker. Gawl., common in renosterveld was selected as the experimental target species. Its bulbs were obtained from a commercial bulb supplier (Hadeco, P.O. Box 7, Maraisburg, 1700, South Africa).

The experimental antagonistic species selected was *Lolium multiflorum* Lam. (rye grass) introduced from Europe as a pasture grass for grazing purposes (Milton, 2004), and proclaimed a weed in South Africa as early as 1659 (Bromilow, 2001). It is presently one of the most widespread alien invasive grasses in South Africa (Figures 2.1B & D), and is common in renosterveld especially adjacent to farmlands disturbed by ploughing, vegetation clearing and burning and grazing by domestic animals (Milton, 2004). Its seeds were obtained from a commercial supplier of agricultural commodities (Pannar Seed (Pty) Ltd, Hildesheim Farm, Main Greytown/Pietermaritzburg Road, Greytown, South Africa)

2.2. EXPERIMENTAL DESIGN AND GROWING CONDITIONS

Bulbs of *T. crocata* and seeds of *L. multiflorum* were sown solely into 30 x 3.5L pots and together into an additional 30 x 3.5L pots which had diameters of 18 cm and depths of 20 cm. Pots were filled with potting media comprising sand from a lowland fynbos site, loam and organic material at a ratio of 3:1:1. The replacement series experimental design comprised 5 replicated blocks located at different positions in a passively ventilated greenhouse with each block comprising 12 pots arranged in 4 rows of 3 pots each. The potted

monocultures and mixtures of *T. crocata* and *L. multiflorum* (Figure 2.3) were thinned 2-weeks after germination to equivalent densities (2 plants per pot).

2.3. TRIAL AND TREATMENTS

There were two nutrient treatments and two water treatments. Nutrient treatments were achieved by additions of different quantities of a granular fertilizer (Ezee Fyngro Super, Ezee Garden Products Pty Ltd, 58 Pondicherry Avenue, Hout Bay, South Africa) to the pots at monthly intervals. The elemental supplements provided in the two nutrient treatments, designated as High Nutrient Treatment (HN) and Low Nutrient Treatment (LN) elevated soil nutrient concentrations by approximately 25% and 100% respectively above those reported for natural and alien-plant infested lowland fynbos soils (Table 2.1.). The two nutrient treatments were combined with two different water treatments, namely a High Water Treatment (HW) and Low Water Treatment (LW) to obtain four different nutrient and water treatment combinations, namely a High Nutrient-High Water treatment (HN-HW), a High Nutrient-Low Water treatment (HN-LW), a Low Nutrient-High Water treatment (LN-HW) and a Low Nutrient-Low Water treatment (LN-LW). The Low- and High- water treatments were attained through additions of 300ml and 600ml of water respectively at weekly intervals to the pots. The 8 different nutrient and water treated monocultures and mixtures of the target species and an additional 4 nutrient and water treated monocultures of the antagonistic species were randomised within the 3 x 4 grids in each of the 5 replicated blocks in the passively ventilated greenhouse (Figure 2.4).

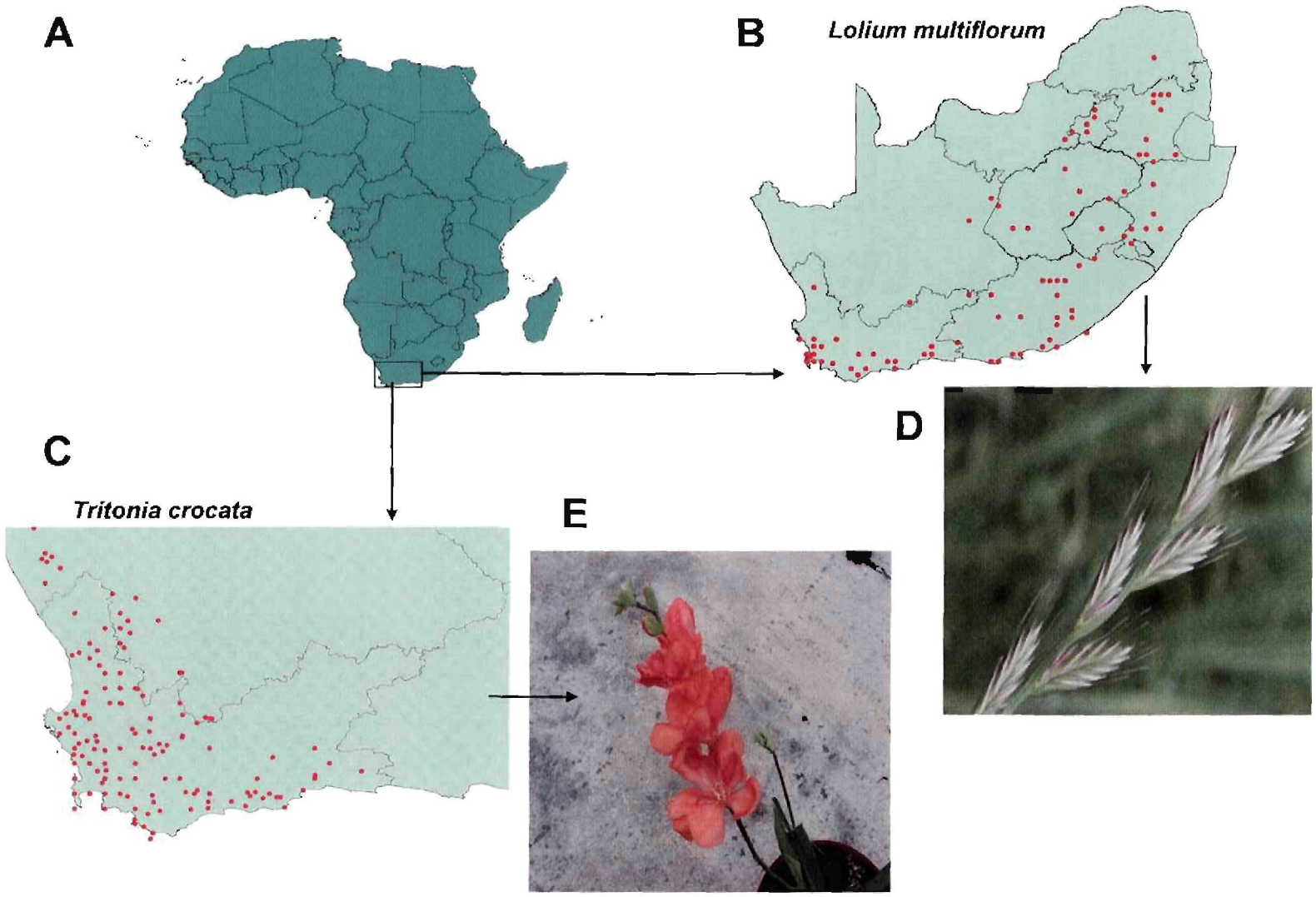


Figure 2.1. A Location of South Africa on the African continent, **B** point localities of the invasive annual grass *Lolium multiflorum* and **C** *T. crocata* in South Africa (PRECIS database) including **D & E** their respective inflorescences.

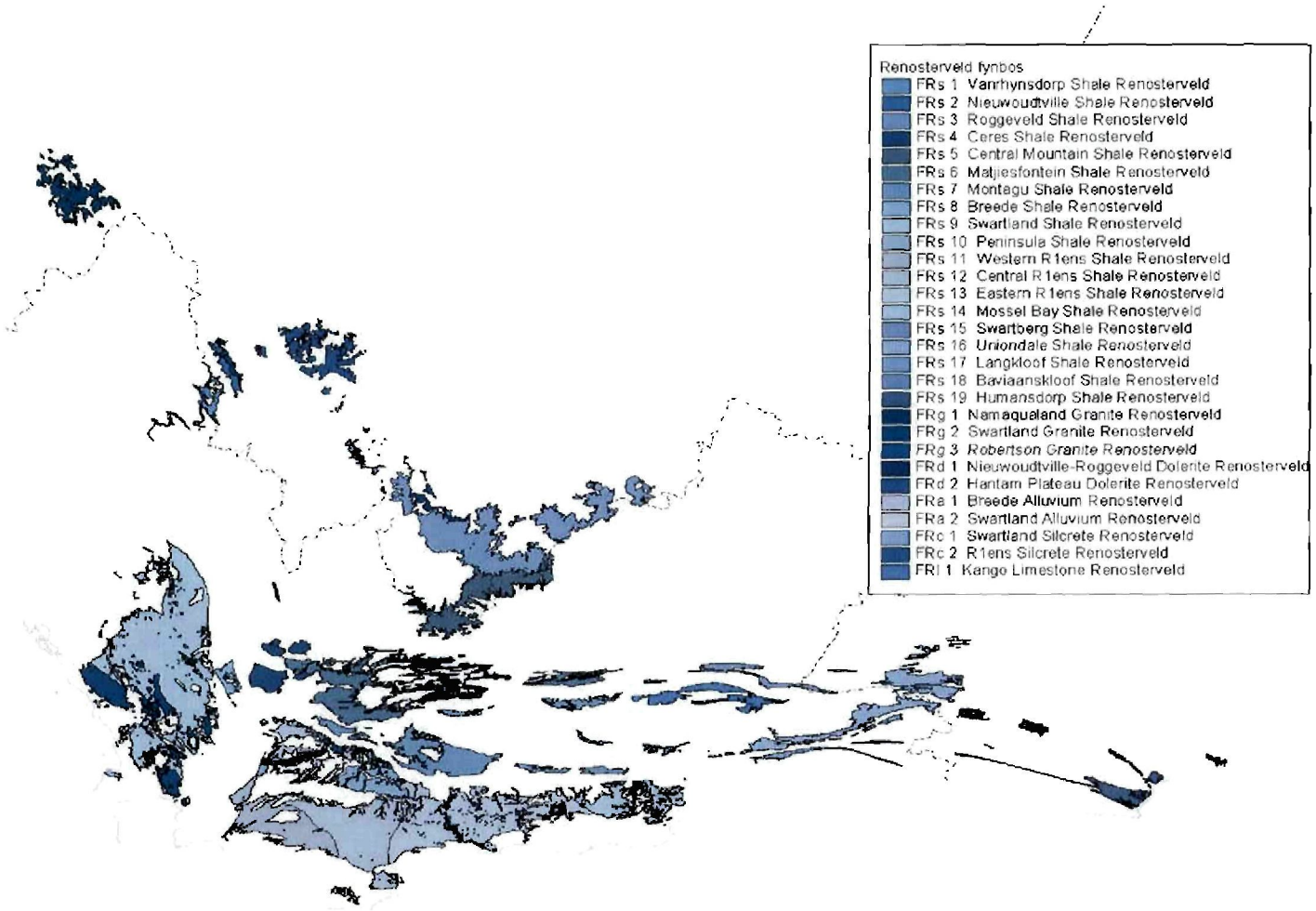


Figure 2.2. Vegetation subunits of renosterveld (adapted from Mucina & Rutherford 2006).

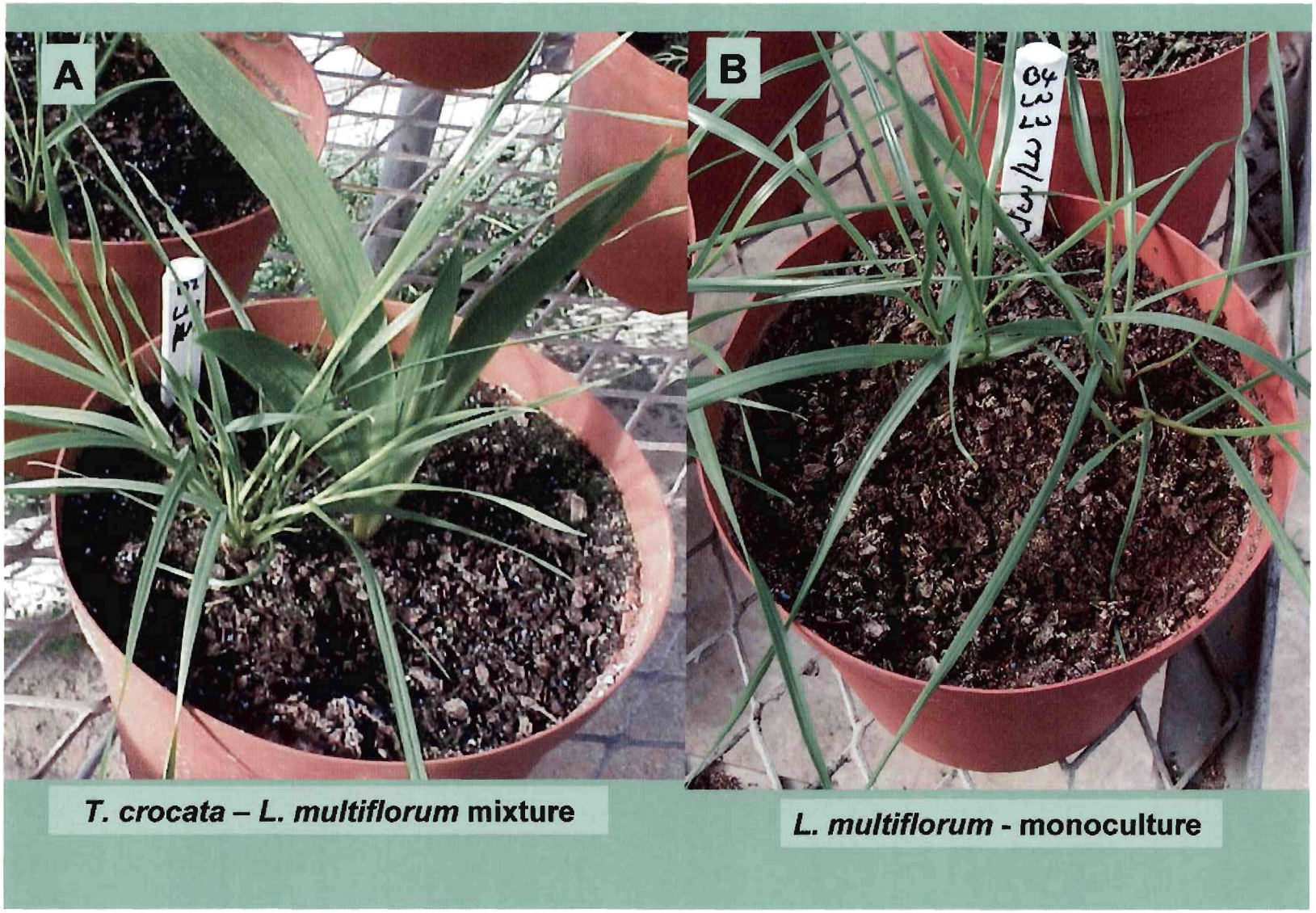
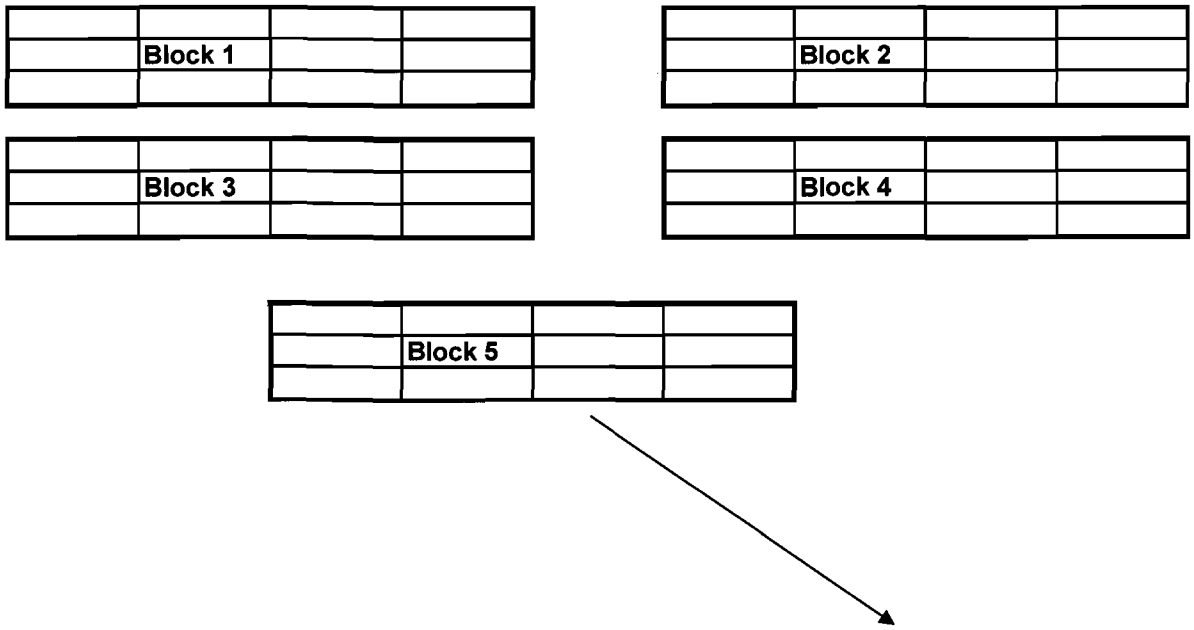


Figure 2.3. Replacement series comprising monocultures and mixtures of indigenous geophyte (*T. crocata*) and alien invasive grass (*L. multiflorum*) at equivalent densities.



Treatments	Block 5					Block 5			
	Ordered					Randomised			
	Mon	Mix	Mon	Mix		1	2	3	4
HN-HW	A	B	C	-	1	A	F	J	E
HN-LW	D	E	F	-	2	I	L	B	H
LW-HN	G	H	I	-	3	C	D	K	G
LW-LN	J	K	L	-					

Figure 2.4. Schematic of experimental design employed in trial. The 12 different treatment combinations (A,B,C,D,E,F,G,H,I,J,K,L) were randomised within 4 x 3 grids within each of 5 blocks in a passively ventilated greenhouse. - = absent, LN = Low Nutrient Treatment, HN = High Nutrient Treatment, LW = Low Water Treatment, HW = High Water Treatment, Mon = monoculture, Mix = mixed culture.

Table 2.1. Concentrations of elemental supplements provided to potting media in low and high nutrient treatments compared with those reported for natural and alien-plant-infested sand plain lowland fynbos soils (Musil, 1993).

Element	Potting media		Sandplain lowland fynbos	
	High nutrient	Low nutrient	Natural	Alien plant-infested
	mmol kg^{-1}	mmol kg^{-1}	mmol kg^{-1}	mmol kg^{-1}
N	24.7109	4.9422	18.348	26.773
P	0.5874	0.1175	0.129	0.160
S	-	-	-	-
K	4.6568	0.9314	0.522	0.736
Ca	-	-	0.6140	7.818
Mg	0.7493	0.1499	0.119	2.699
Fe	0.0571	0.0114	0.705	0.807
Mn	0.0014	0.0003	0.044	0.052
Cu	0.0010	0.0002	0.003	0.003
Zn	0.0058	0.0012	0.008	0.008
Bo	0.0088	0.0018	0.009	0.015
Mo	0.0010	0.0002	-	-

2.4. PHOTOSYNTHETIC PIGMENTS

2.4.1. Introduction

Photosynthetic pigments are isoprenoid plant lipids (Goodwin, 1977; Lichtenthaler, 1977). They comprise the primary photosynthetic pigments chlorophyll *a* and *b*, which absorb light energy in the red and blue regions of the visible spectrum used for photosynthesis (Lichtenthaler *et al.*, 1986), and the secondary photosynthetic pigments which include β-carotene and the xanthophylls. The xanthophylls comprise lutein, violaxanthin, and neoxanthin, which are regular components of photochemically active thylakoids of chloroplasts of higher plants, and other minor xanthophyll species such as antheraxanthin and zeaxanthin. It is thought that neoxanthin and lutein may also function as accessory light harvesting pigments while the main function of β-carotene seems to be the protection of chlorophyll *a* from photo oxidation but it also serves as a light-absorbing pigment (Lichthenthaler, 1987). The xanthophylls primary role is in the dissipation of excess light energy absorbed in the photosynthetic active waveband (Gamon, *et al.*, 1990). This excessive light energy not utilized during photosynthesis occurs as a result of an imbalance between absorbed light energy and rate of photosynthetic dark reactions under stress conditions (Gamon, *et al.*, 1990). It is dissipated by the reversible de-epoxidation of violaxanthin to zeaxanthin via antheraxanthin (Demmig-Adams *et al.*, 1989) and is associated with an increase in the chloroplast thylakoid pH gradient (Yamamoto, 1979; Hager, 1980). Relative amounts of xanthophyll cycle components are directly correlated with the photon yield of photosynthetic electron transport in several species (Thayer & Björkman, 1990) and are sensitive indicators of the efficiency of photosynthetic energy change (Gamon, *et al.*, 1990).

2.4.2. Sampling of leaf material

Fully developed and illuminated apical leaves were randomly selected and harvested from plants in each pot at midday (1300 SAST). Leaves in mature plants were harvested just before flowering. The leaves were used for analysis of chlorophyll *a* and *b* and total carotenoids (β-carotene + xanthophylls). Leaves taken from each plant were frozen in liquid nitrogen for subsequent quantification of ribulose-1,5-biphosphate carboxylase/oxygenase (Rubisco) content.

2.4.3. Analysis of chlorophyll *a* and *b* and total carotenoids

Leaf samples were ground at a low light intensity in 10 ml of 100% methanol at 2°C and extracts centrifuged for 10 minutes at 186 x g at a temperature of 25°C with a bench-top centrifuge (SC-158, Scilab Instruments Co, No.7, Alley 2, Lane 365, Sec. 2, Jhongshan Rd., Jhonghe City, Taipei County 235, Taiwan). Absorbances of the centrifuged extracts were measured at wavelengths of 470, 652.4 and 665.2 nm with a spectrophotometer (Beckman DU 640, Beckman Instruments Inc., Fullerton, USA). Leaf concentrations of chlorophyll *a*, chlorophyll *b* and total carotenoids were computed from the absorbances measured at the above-specified wavelengths applying published formulae (Lichtenthaler, 1987). The leaf residues were dried at 60°C in a forced draft oven, weighed and pigment concentrations expressed as μg mg^{-1} leaf dry mass.

2.5. Chlorophyll Fluorescence

2.5.1. Introduction

The measurement of chlorophyll fluorescence is widely used as a probe of the process of photosynthesis *in vivo* (Krause & Weiss, 1984; Briantais *et al*, 1986; Renger & Schreiber, 1986). Chlorophyll fluorescence is a rapid and non-destructive procedure, which provides information on the inhibition or disruption of electron transfer through photosystem II (PS II). Photosystem II is a sensitive indicator of photoinhibition, and other physiological effects which feed back onto photosynthesis (Bolhăr-Nordenkamp *et al.*, 1989). Laboratory studies have shown that certain aspects of chlorophyll fluorescence relate closely to photosynthetic carbon metabolism and leaf gas exchange, and provide a means for studying the relationships of light and dark reactions within intact leaves (Walker *et al.*, 1983; Ireland, Long & Baker, 1984; Ireland, Baker & Long, 1985). Also, chlorophyll fluorescence is a sensitive tool for comparing the effects of stresses on different genotypes (Smillie & Nott, 1982) and in investigating mechanisms of stress damage to photosynthesis and to the physiology of the plant in general (Schreiber & Bilger, 1987; Baker & Horton, 1988; Strand & Őquist, 1988) resulting from various environmental stresses, such as chilling, freezing, drought and air pollution (e.g. Bilger, Schreiber & Lange, 1987; Őquist, 1987; Baker & Horton, 1988; Lichtenthaler, 1988).

2.5.2. The polyphasic chlorophyll *a* fluorescence transient

Part of the light energy trapped by the chlorophyll antenna of the photosynthetic apparatus of a leaf is re-emitted as red and far-red light (fluorescence). Characteristic changes in the intensity of chlorophyll *a* fluorescence, known as the Kautsky transient, are observed when a dark-adapted leaf is illuminated with a saturated light pulse (Kautsky & Hirsch, 1931). The Kautsky transient shows a fast rise completed in less than one second, with a subsequent slower decline towards a steady state. This rising phase of the transient which reflects the primary reactions of photosynthesis (Krause & Weis, 1991) is polyphasic when plotted on a logarithmic time scale (Figure. 2.5), clearly exhibiting the steps J and I (Strasser & Govindjee, 1992) or I_1 and I_2 (Schreiber & Neubauer, 1987) between the initial O (F_0) and maximum P fluorescence level ($F_P = F_M$).

Upon excitation with a saturated light pulse, there is a rapid initial rise in fluorescence intensity from O to the first intermediate step J within ca. 2 ms. This phase is followed by a further rise to the second intermediate step I within ca. 30 ms and to the final peak P in ca. 200 ms. The O-J-I-P fluorescence transient reflects the filling up of the electron acceptor side of PSII (Q_A, Q_B and PQ pool) with electrons from the donor side of PSII (Papageorgiou, 1975; Lavorel & Etienne, 1977; Strasser & Govindjee, 1992). The relationship of these events to the O-J-I-P fluorescence transient was suggested by Strasser *et al.*, (1995) to be the following: O, minimal chlorofill *a* fluorescence yield (highest yield of photochemistry); O to J, reduction of Q_A to Q_A^- (photochemical phase, light intensity dependent); J to I to P, reduction of the PQ pool (non-photochemical phase). Since the O-J-I-P fluorescence transient reflects the kinetics and heterogeneity involved in the filling up of the PQ pool with electrons, it can be used as a sensitive tool to investigate the photosynthetic apparatus *in vivo* (Strasser *et al.*, 1995). The shape of the O-J-I-P fluorescence transient has been found to be very sensitive to various types of stress (Krüger *et al.*, 1997; Lazár & Ilík, 1997; Tsimilli-Michael *et al.*, 1999; Strauss *et al.*, 2006).

2.5.3. Analysis of chlorophyll *a* fluorescence transients (JIP-test)

The O-J-I-P fluorescence transient is rich in information and can be used to derive a number of parameters by the JIP-test as shown below in Table 2.2. The following data from the original measurements are used by the JIP-test: maximal fluorescence intensity (F_M); fluorescence intensity at 50 μs (considered as F_0); fluorescence intensity at 300 μs ($F_{300\mu s}$) required for calculation of the initial slope (M_0) of the relative variable fluorescence

(V) kinetics; and the fluorescence intensity at 2 ms (the J step) denoted as F_J (Figure 2.5). The JIP-test represents a translation of the original fluorescence data to biophysical parameters that quantify the stepwise flow of energy through PSII at the reaction center (RC) as well as excited cross-section (CS) level (Strasser & Strasser, 1995; Force *et al.*, 2003; Strasser *et al.*, 2004). The parameters which all refer to time zero (onset of fluorescence induction) are: (i) the specific energy fluxes (per reaction centre) for absorption (ABS/RC), trapping (TR_o/RC), dissipation at the level of the antenna chlorophylls (DI_o/RC) and electron transport (ET_o/RC); (ii) the flux ratios or yields, i.e. the maximum quantum yield of primary photochemistry (φ_{Po} = TR_o/ABS = F_V/F_M), the efficiency (ψ_o = ET_o/TR_o) with which a trapped exciton can move an electron into the electron transport chain further than Q_A^-, the quantum yield of electron transport (φ_{Eo} = ET_o/ABS = $\varphi_{Po} \bullet \psi_o$); (iii) the phenomenological energy fluxes (per excited cross section, CS) for absorption (ABS/CS), trapping (TR_o/CS), dissipation (DI_o/CS) and electron transport (ET_o/CS). The fraction of active PSII reaction centres per excited cross section (RC/CS) is also calculated. The formulae presented in Table 2.4 illustrate how each of the above-mentioned biophysical parameters can be calculated from the original fluorescence measurements.

The initial stage of photosynthetic activity of a RC complex is regulated by three functional steps (Figure 2.6) namely absorption of light energy (ABS), trapping of excitation energy (TR) and conversion of excitation energy to electron transport (ET).

Strasser *et al.*, (2000) introduced a multi-parametric expression of these three independent steps contributing to photosynthesis, the so-called performance index (PI_{ABS}):

$$PI_{ABS} = \frac{\gamma}{1-\gamma} \bullet \frac{\varphi_{Po}}{1-\varphi_{Po}} \bullet \frac{\psi_o}{1-\psi_o}$$

where γ is the fraction of reaction centre chlorophyll (Chl_{RC}) per total chlorophyll ($Chl_{RC+Antenna}$). Therefore $\gamma/(1-\gamma)$ = $Chl_{RC}/Chl_{Antenna}$ = RC/ABS. This expression can be de-convoluted into two JIP-test parameters and estimated from the original fluorescence measurements as RC/ABS = $RC/TR_o \bullet TR_o$/ABS = $[(F_{2ms} - F_{50\mu s})/4(F_{300\mu s} - F_{50\mu s})] \bullet F_V/F_M$.

Table 2.2. Summary of the JIP-test formulae using data extracted from the chlorophyll fluorescence transient, O-J-I-P.

Extracted and Technical Fluorescence Parameters

F_0	=	$F_{50\mu s}$, fluorescence intensity at 50μs
$F_{100\mu s}$	=	fluorescence intensity at 100μs
$F_{300\mu s}$	=	fluorescence intensity at 300μs
F_J	=	fluorescence intensity at the J-step (at 2ms)
F_I	=	fluorescence intensity at the I-step (at 30ms)
F_M	=	maximal fluorescence intensity
t_{F_M}	=	time to reach F_M, in ms
V_J	=	relative variable fluorescence at the J-step = $(F_{2ms} - F_0) / (F_M - F_0)$
$(dV / dt)_0 = M_0$	=	fractional rate of PS II reaction centre closure = $4 . (F_{300} - F_0)/(F_M - F_0)$

Quantum Efficiencies or Flux Ratios or yields

$\varphi_{Po} = TR_0 / ABS$	=	$[1 - (F_0 / F_M)] = F_V / F_M$
$\varphi_{Eo} = ET_0 / ABS$	=	$[1 - (F_0 / F_M)] . \Psi_0$
$\Psi_0 = ET_0 / TR_0$	=	$(1 - V_J)$

Specific Fluxes or Specific Activities

ABS / RC	=	$M_0 . (1 / V_J) . (1 / \varphi_{Po})$
TR_0 / RC	=	$M_0 . (1 / V_J)$
ET_0 / RC	=	$M_0 . (1 / V_J) . \Psi_0$
DI_0 / RC	=	$(ABS / RC) - (TR_0 / RC)$

Phenomenological Fluxes or Phenomenological Activities

ABS / CS	=	ABS / CS_{Chl} = Chl / CS or $ABS / CS_0 = F_0$ or $ABS / CS_M = F_M$
TR_0 / CS	=	$\varphi_{Po} . (ABS / CS)$
ET_0 / CS	=	$\varphi_{Po} . \Psi_0 . (ABS / CS)$
DI_0 / CS	=	$(ABS / CS) - (TR_0 / CS)$

Density of Reaction Centres

RC / CS	=	$\varphi_{Po} . (V_J / M_0) . ABS / CS$

Performance Indexes

PI_{ABS}	=	$(RC / ABS) . [\varphi_{Po} / (1 - \varphi_{Po})] . [\Psi_0 / (1 - \Psi_0)]$

ABS, absorption energy flux; CS, excited cross section of leaf sample; DI, dissipation energy flux at the level of the antenna chlorophylls; ET, flux of electrons from Q_A^- into the electron transport chain; φ_{Do}, quantum yield of dissipation; φ_{Eo}, probability that an absorbed photon will move an electron into electron transport further than Q_A^-; φ_{Po}, maximum quantum yield of primary photochemistry; PI_{ABS}, performance index; ψ_o, efficiency by which a trapped exciton, having triggered the reduction of Q_A to Q_A^-, can move an electron further than Q_A^- into the electron transport chain; RC, reaction centre of PSII; RC/CS, fraction of active reaction centres per excited cross section of leaf; TR, excitation energy flux trapped by a RC and utilized for the reduction of Q_A to Q_A^-.

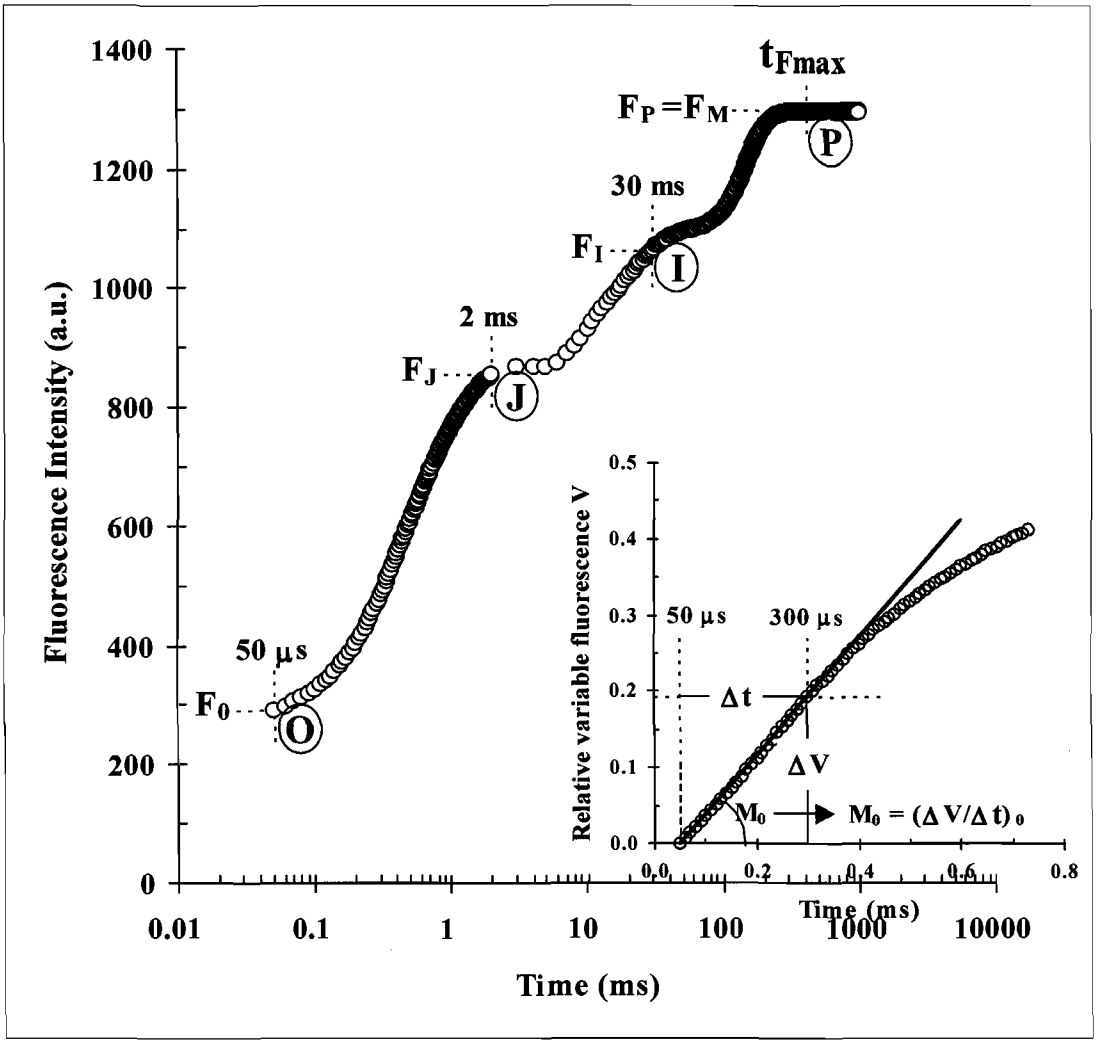


Figure 2.5. A schematic presentation of a typical polyphasic chlorophyll *a* fluorescence transient O-J-I-P emitted by higher plants. The transient is plotted on a logarithmic time scale from 50 µs to 1 s. The labels refer to the fluorescence data used by the JIP-test (see section 2.5.3) for the calculation of various parameters quantifying PSII structure and function. The labels are: the fluorescence intensity F_0 (at 50 µs); the fluorescence intensity F_J (at 2 ms); the fluorescence intensity F_I (at 30 ms) and the maximal fluorescence intensity $F_P = F_M$. The figure insert shows the transient expressed as the relative variable fluorescence, $V = (F - F_0)/(F_M - F_0)$, on a linear time-scale and demonstrates how the initial slope (M_0) is calculated: $M_0 = (D_V/D_t)_0 = (V_{300\mu s})/(0.25\ ms)$. (From Tsimilli-Michael *et al.*, 2001).

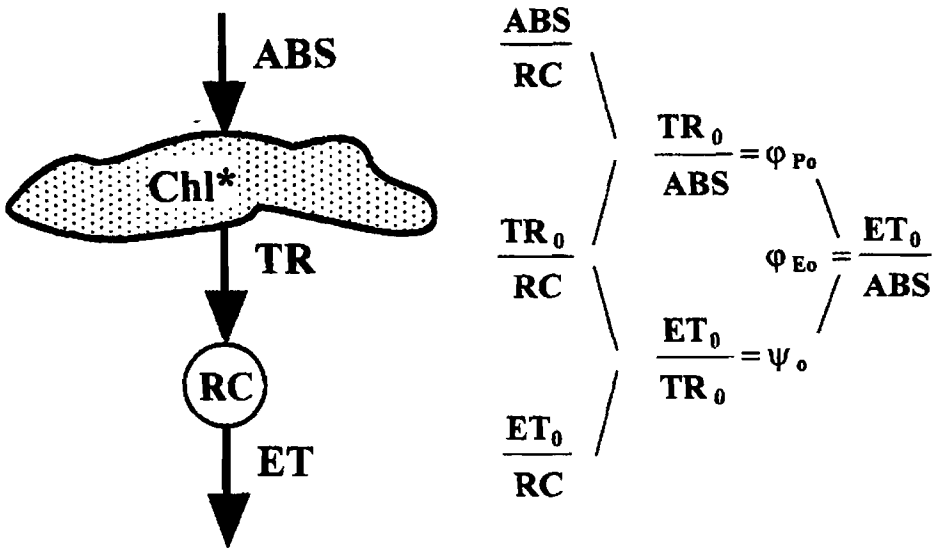


Figure 2.6. Simplified scheme demonstrating the energy cascade from PSII light absorption to electron transport (Strasser & Strasser, 1995).

The expression RC/ABS shows the contribution to the PI_{ABS} due to the RC density on a chlorophyll basis. The contribution of the light reactions for primary photochemistry are estimated according to the JIP-test as $[\varphi_{Po}/(1-\varphi_{Po})] = TR_o/DI_o = k_P/k_N = F_V/F_o$. The contribution of the dark reactions are derived as $[\psi_o/(1-\psi_o)] = ET_o/(TR_o - ET_o) = (F_M - F_{2ms})/(F_{2ms} - F_{50\mu s})$. The JIP-test reveals changes in the behaviour of PSII that cannot be detected by the commonly used $\varphi_{P0} = F_V/F_M$, which is the least sensitive of all parameters.

2.5.4. Measurement of fluorescence transients and calculation of energy fluxes

Chlorophyll fluorescence measurements were performed on fully expanded apical leaves randomly selected from each species in each pot between 08h00 and 10h00 SAST following a 20 min dark adaptation period (Force *et al.*, 2003). Measurements of fluorescence intensity at 50 µs,100 µs, 300 µs, 2 ms and 30 ms intervals following a 1 s saturating light pulse of 3 500 µmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) were obtained with a Plant Efficiency Analyser (PEA, Hansatech Instruments Ltd., King's Lynn, Norfolk, UK). The transients were induced by a red light (peak at 650 nm) of 3500 µmol m^{-2} s^{-1} (sufficient excitation intensity to ensure closure of all PSII reaction centers to obtain a true fluorescence intensity of F_M) provided by the PEA through an array of six light-emitting diodes. All measurements were conducted on fully dark-adapted attached leaves.

The recorded OJIP transients were subsequently analysed by the JIP test (explained fully in section 2.5.3) and translated into biophysical parameters that quantify energy flow through photosystem II (PSII) from which a multi-parametric expression, designated that photochemical performance index (PI_{ABS}) was computed (Strasser *et al.*, 2000). The three partial responses of PI_{ABS} contributing to photosynthesis included the density of working photosystems (reaction center per chlorophyll, RC/ABS), the efficiency of primary photochemistry (trapping) (φ_{P0} / 1-φ_{P0}) and the efficiency of conversion of excitation energy to electron transport (Ψ_0 / 1-Ψ_0) (Strauss *et al.*, 2006).

2.6. PHOTOSYNTHESIS

2.6.1. Introduction

Limitations to the rate of photosynthesis can be broadly classified into three general classes, namely: (1) the supply or utilization of light, (2) the supply or utilization of CO_2 and (3) the supply or utilization of phosphate (Sharkey, 1985).

The first photosynthetic rate limitation is examined by determining the quantum requirements of photosynthesis, given by the relationship between net CO_2 assimilation rate (A) and photosynthetic photon flux density (PPFD) (Figure 2.7). The initial slope of the A/PPFD curve expresses the apparent quantum yield of photosynthetic utilisation of CO_2 and is a measure of photochemical efficiency. The second photosynthetic rate limitation is most readily examined by determining how the CO_2 assimilation rate varies with the partial pressure of CO_2 inside the leaf, given by the relationship between net CO_2 assimilation rate (A) and leaf internal CO_2 concentration (C_i) (Figure 2.8). The initial slope of the A/C_i curve expresses the photosynthetic utilization of CO_2 and is the measure of the activity and content of the carboxylation enzyme, Rubisco, which corresponds to the efficiency of C_3 photosynthetic type concentrating mechanisms (Von Caemmerer, 2000). The initial slope of the A/C_i response curve is not a wholly external variable, determined independent of environment or investment in leaves versus roots (Ghannoum *et al.*, 1998; von Caemmerer & Furbank, 1999).

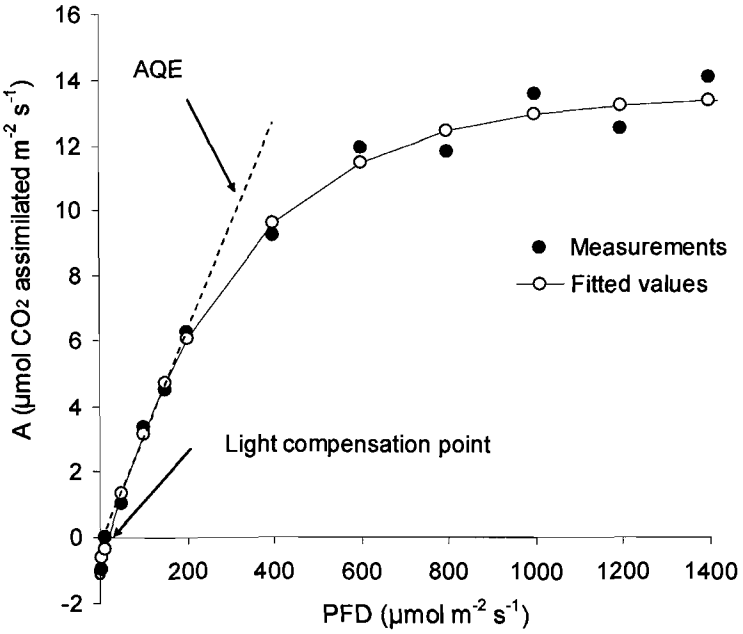


Figure 2.7. Response of net CO_2 assimilation rate (A) to photosynthetic photon flux density (PPFD). AQE is the apparent quantum efficiency and a measure of the amount of CO_2 assimilated or oxygen evolved per photon utilized.

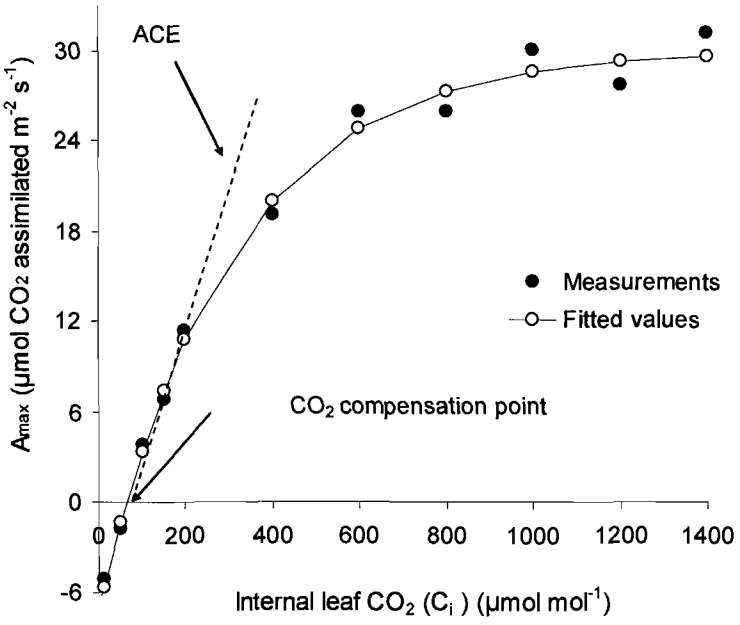


Figure 2.8. Response of light saturated net CO_2 assimilation rate (A_{max}) to leaf internal CO_2 concentration (C_i). ACE is the apparent carboxylation efficiency and indicative of Rubisco activity.

Leaf nitrogen content affects the initial slope of the A/C_i response curve (Long, 1985; Sage *et al.*, 1999). Because leaf nitrogen content varies considerably between species and soil fertility, it follows then that the initial slope of the A/C_i response curve will invariably also vary with these factors. For photosynthetic capacity, the amount and activity of photosynthetic machinery per unit leaf area (Condon, *et al.*, 2002), two of the most critical aspects will be factors related to mineral nutrition and to water acquisition (Ehleringer, 1995). Gas exchange parameters that are usually used to describe the response of plants to changing soil water content and atmospheric vapor pressure deficit, Rubisco content and activity conditions are maximum CO_2 assimilation (A_{max}) and maximum stomatal conductance (g_{smax}) at saturating PPFD.

The main role of stomata is to maximise CO_2 assimilation while limiting water loss (Farquhar & Sharkey, 1982). The slope of the relationship between net CO_2 assimilation rate (A) and transpiration rate (E) provides a measure of overall water use efficiency (WUE). Intrinsic water use efficiency (WUE_{INT}), also defined as the ratio of carbon gain to water loss (Hetherington & Woodward, 2003) is given by the slope of the relationship between A and stomatal conductance (g_s). High WUE_{INT}, can be achieved through high photosynthetic rates or low transpiration rates or both (Condon, 2002; Polley, *et al.*, 2002). Both processes are regulated by the opening or closing of stomata. WUE_{INT} and the ratio of external to internal leaf CO_2 concentrations (C_i/C_a ratio) are two parameters that are usually used to measure the relationship between photosynthetic activity and water loss (Beale *et al.,* 1999). The C_i/C_a value is determined by the balance between stomatal conductance (that is, supply of CO_2 to the leaf interior), and photosynthetic capacity, *i.e.* the demand for CO_2 (Ehleringer, 1995). Leaf A/g_s is positively related to C_a and negatively related to C_i/C_a as $A/g = C_a (1- C_i/C_a)/ 1.6$. The C_i/C_a ratio reflects the changes in the relationship between stomatal and biochemical capacity for photosynthesis.

2.6.2. Measurement of photosynthetic gas and water exchange

Measurements were performed with a portable photosynthesis system (Li-Cor 6400, Lincoln, NE, USA) on attached apical leaves of predetermined surface area randomly selected from mature plants in each pot. Readings of net CO_2 assimilation rate (A), intercellular CO_2 concentration (C_i), transpiration rate (E) and stomatal conductance (g_S) were taken at different photosynthetic photon flux densities (PPFD) of 0, 50, 100, 250, 500, 1000,

1500 to 2000 μmol m^{-2} s^{-1} provided by an LED array (LI-6400-02) at an atmospheric CO_2 concentration (c_a) of 350 μmol mol^{-1} and also at 12 different c_a levels ranging between 0 - 1500 μmol mol^{-1} at a saturating PPFD of 1500 μmol m^{-2} s^{-1} to ensure full activation of Rubisco. Leaf temperatures in the cuvette were maintained at 28.13 ± 0.02 °C and vapor pressure deficits at 2.13 ± 0.01 kPa. Gas and water vapor exchange parameters were computed according to published equations (Von Caemmerer & Farquhar 1981; Harley & Sharkey 1991).

Responses of A to PPFD and A to C_i were fitted with the aid of interative non-linear regressions to a monomolecular function (Causton & Dale, 1990), which provides a better fit to such data than the rectangular hyperbola model (Olsson & Leverenz, 1994). The equation of the monomolecular function is given by:

$$y = a(1- e^{b-cx}) \qquad 1.$$

Where:

y is the net CO_2 assimilation rate (A)

x is the light intensity (PPFD) or intercellular CO_2 concentration (c_i)

a is the upper asymptotic maximum of the light saturated photosynthetic rates (A_{max}) or CO_2 saturated photosynthetic rate (J_{max})

b/c is the light compensation point or CO_2 compensation point

ace^b is the apparent quantum efficiency (AQE) or carboxylation efficiency (ACE) given by the initial slope of the curves of A versus PPFD or A versus c_i respectively, and $a(1-e^b)$ is the dark respiration rate (R_D)

2.6.3. Analysis of Rubisco content

2.6.3.1. Extraction of total soluble proteins

Samples of frozen leaf powder (80 mg) were extracted with 3ml ice-cold extraction buffer containing 50 mM Tris-HCl (pH 7.8), 1 mM EDTA, 1 mM EGTA, 1 mM PMSF, 10 μM E-64, 1 μM Pepstatin-A, 2 mM Benzamidine, 2 mM Aminocaproic acid, 3 mM DTT, and 20 mg insoluble PVPP in a pre-chilled mortar and pestle. The crude extract was transferred to a pre-cooled micro centrifuge tube and centrifuged at 10 000 x g at 4°C for 15 minutes. Soluble

protein content of the supernatant was determined according to the method of Bradford (1976). A 10µg bovine serum albumin standard was included in the assay for protein quantification.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer (250 µl) was then added to 750 µl clarified supernatant. The SDS-PAGE sample buffer contained 25 ml of 0.5 M Tris-HCl (pH 6.8), 20 ml glycerol, 2 g SDS, 0.001 g bromophenol blue, β-mercapto-ethanol (40 µl/ml) and ultrapure water in a final volume of 50 ml. The samples were boiled for 5 minutes and used for SDS-PAGE after brief cooling on ice.

2.6.3.2. Separation of proteins and protein subunits

SDS-PAGE was used for the separation of proteins and protein subunits. According to this technique the proteins are denatured with SDS, β-mercapto-ethanol and boiling prior to electrophoresis. Proteins were separated by SDS-PAGE with a mini gel system (Bio-Rad Laboratories Ltd., Bio-Rad House, Hertfordshire HP2 7TD England). The 10% resolving gel solution contained 2.5 ml 40% (w/v) acrylamide/bisacrylamide solution, 2.5 ml 1.5 M Tris-HCl (pH 8.8) and 4.8 ml ultra pure water and was degassed prior to casting. The gel was casted between the glass plates after addition of 100 µl 10% (w/v) SDS, 100 µl 10% (w/v) ammonium persulphate and 10 µl TEMED. The gel solution was overlaid with a thin layer of water-saturated butanol. Polymerization of the gel took ± 45 minutes.

The 4% stacking gel solution contained 0.2 ml 40% (w/v) acrylamide/bisacrylamide solution, 0.5 ml 0.5 M Tris-HCl (pH 6.8) and 1.27 ml ultra pure water and was degassed prior to casting. The stacking gel solution was casted on top of the polymerized resolving gel after addition of 20 µl 10% (w/v) SDS, 12.5 µl 10% (w/v) ammonium persulphate and 2.5 µl TEMED. A plastic gel comb was immediately inserted into the stacking gel solution to form 10 sample wells in which the protein extracts and marker proteins were loaded. Polymerization took ± 1 hour. After the stacking gel had polymerized, the gel comb was carefully removed and the sample wells rinsed thoroughly with SDS-PAGE running buffer containing 25 mM Tris, 192 mM Glycine, and 0.5% SDS. Protein samples (10 µg total soluble protein/lane) and 10 µl Bio-Rad pre-stained molecular weight markers (Bio-Rad Laboratories Ltd., Bio-Rad House, Hertfordshire HP2 7TD England) were loaded in the sample wells. The inner buffer tank (formed by the gel plates) was completely filled with SDS-PAGE running buffer while the outer buffer tank was also filled with the same buffer until the glass plates

were nearly completely submerged. Proteins were separated at room temperature for 1h30 – 1h45 at 100 volts. The gels were either stained overnight in Coomassie Brilliant Blue (CBB) R250 stain to verify equal protein loadings or immediately used for protein transfer to nitrocellulose membranes.

2.6.3. Protein transfer to membrane

After separation with SDS-PAGE, proteins were transferred from the resolving gel to a Hybond C-extra nitrocellulose membrane (Amersham Life Science, Little Chalfont, United Kingdom). The high binding capacity, high mechanical strength and chemical resistance of the Hybond membrane makes it especially useful for protein transfer applications. Prior to protein transfer the resolving gel, membrane, sheets of filter paper and blotting foam discs were incubated for 30 minutes in protein transfer buffer containing 25 mM Tris, 192mM Glycine, 0.1% SDS and 20% (v/v) HPLC-grade methanol. The blot sandwich containing the gel, membrane, filter paper and foam discs was assembled according to manufacturer instructions. Transfer of separated proteins from the resolving gel to the membrane was conducted at 100 V for 30-40 minutes (with cooling) using a Trans-Blot Electrophoretic Transfer Cell (Bio-Rad Laboratories Ltd., Bio-Rad House, Hertfordshire HP2 7TD England).

2.6.4. Western blot immuno-detection of Rubisco

The nitrocellulose membrane containing the transferred proteins was incubated in blocking buffer (2.42 g Tris, 1.82 g EDTA, 8.77 g NaCl and 50 g skimmed milk powder in a total volume of one litre) for 2 – 3 hours with gentle shaking. The membrane was then probed overnight with primary antibody (raised in rabbit) specific to the large subunit (LSU) of Rubisco at a dilution ratio of 1:250 in blocking buffer. The following morning the membrane was rinsed (3 x 10 min) with blocking buffer to remove unbound primary antibody. The membrane was then probed with anti-rabbit IgG Horse Radish Peroxidase-linked secondary antibody (1:1000 dilution in blocking buffer) for 2 hours. The membrane was rinsed (3 x 10 min) with blocking buffer to remove unbound secondary antibody before detection of labelled proteins. A detection reagent containing 40 µg chloro-naphtol (dissolved in 500µl absolute ethanol before addition) and 50 µl 30% (w/v) H_2O_2 in 100 ml 50 mM Tris buffer (pH 7.6) was used for the detection of labelled Rubisco LSU on the membrane.

2.6.5. Quantification of Rubisco polypeptide content

The labelling of the large subunits of Rubisco with a specific antibody allowed quantification of Rubisco content from measured intensities of the antibody reaction. The labelled Rubisco LSU in the 8 different lanes representing treatments were photographed with a digital camera and their intensities (numbers of pixels per cm^2) quantified applying image analysis software (Image-J, National Institute of Health, USA - http://rsb.info.nih.gov/ij/).

2.7. GROWTH AND REPRODUCTION

2.7.1. Flowering, reproductive and vegetative biomass

Approximately 5 months after commencement of each trial, plants were harvested from the pots. The inflorescences and above-ground material were separated and dried to a constant mass and weighed.

2.8. GREENHOUSE ENVIRONMENT

2.8.1. Photosynthetic photon flux density

The net assimilation rate of many sun and shade plants is linearly related to the logarithm of the light intensity up to maximum daylight (Blackman & Wilson, 1951). Since the light intensity in a greenhouse may be reduced and its quality affected according to the alignment of the greenhouse and the type and cleanliness of the glaze, it was measured at different positions in the greenhouse. Measurements of PPFD were taken at solar noon (13h00 SAST) in the greenhouse at its northern, central, southern, eastern and western extremities and in the outdoor environment with a quantum sensor (LiCor 189, Li-COR, Lincoln, NE, USA).

2.8.2. Air temperatures

Photosynthesis and growth of plants are affected by the air temperature (Chabot, 1977). Since air temperatures may vary at different positions in a greenhouse, these were monitored daily in the greenhouse at its southern, central and northern extremities and in the outdoor environment with maximum and minimum thermometers suspended at the heights of the experimental plants.

2.8.3. Soil water content

Soil water content affect plant growth and photosynthesis (Zavitkowski & Ferrell, 1968). Since these formed one of the experimental treatments, they were measured weekly in each pot just prior to water applications with a Theta probe (Type ML2x, Delta-T Devices, Cambridge England).

2.9. DATA SYNTHESIS AND STATISTICAL ANALYSES

A multiple factor analysis of variance applying a generalized linear model (GLM) tested for significant differences between culture type (monocultures versus mixtures), level of water and nutrient supply and their interactions on measured physiological, biochemical and growth parameters in the experimental target and antagonistic species. Product moment (Pearson) correlations tested for significant correspondence between measured plant and environmental variables.

CHAPTER 3: RESULTS

3.1. GREENHOUSE ENVIRONMENT

3.1.1. Photosynthetic photon flux density

Photosynthetic flux densities (PPFD) showed no significant ($P \geq 0.05$) differences between the southern and northern extremities of the greenhouse and at its center. However, significantly ($P \leq 0.05$) lower PPFD's were recorded inside the greenhouse than outdoors. Those measured inside the greenhouse averaged 70% of the PPFD's measured outdoors in the ambient environment (Figure 3.1A), but did approximate PPFD's required for maximal net CO_2 assimilation rates in both the target species (Figure 3.1 B) and the antagonistic species (Figure 3.1C).

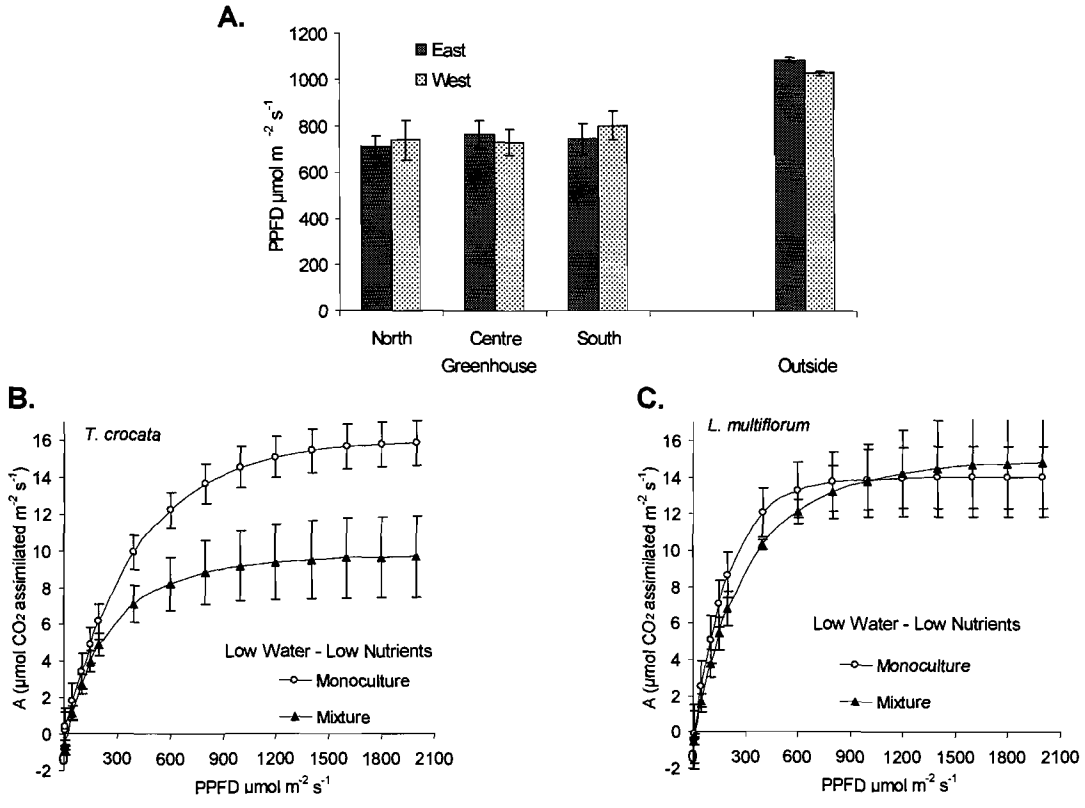


Figure 3.1. (A) Photosynthetic photon flux densities (PPFD) at different positions in the greenhouse and outdoors and responses of net CO_2 assimilation rates in (B) the target species (*T. crocata*) and (C) the antagonistic species (*L. multiflorum*) to different PPFD's.

3.1.2. Air temperatures

Minimum daily air temperatures recorded at the centre of the greenhouse and at its northern extremity did not differ significantly ($P \geq 0.05$) from those recorded outside the greenhouse. However, minimum daily temperatures recorded at the southern extremity of the greenhouse did differ significantly ($P \leq 0.05$) from those recorded outside the greenhouse. They were on average 1.2°C lower at this position in the greenhouse than outdoors (Figure 3.2A).

Maximum daily air temperatures recorded at the centre of the greenhouse also did not differ significantly ($P \geq 0.05$) from those recorded outside the greenhouse. However, those recorded at the northern and southern extremities of the greenhouse did differ significantly ($P \leq 0.05$) from maximum daily air temperatures recorded outside the greenhouse. At the northern extremity of the greenhouse, maximum daily temperatures were on average 1.8°C higher than those outdoors but at the southern extremity of the greenhouse they were on average 2.9°C lower than those outdoors (Figure 3.2B).

Only the 24-hour mean daily air temperatures at the southern extremity of the greenhouse differed significantly ($P \geq 0.05$) from those outdoors. These temperatures were on average 1.6°C lower at this position in the greenhouse than outdoors (Figure 3.2C).

3.1.3. Soil water content

Soil water contents in the potting media were significantly ($P \leq 0.001$) altered by the level of water supplied and culture type (Table 3.1). They were reduced on average by 36% in potting media receiving the low water treatments compared with those receiving the high water treatments and by 36% also in potting media comprising mixtures of *T.crocata* and *L. multiflorum* compared with those containing *T. crocata* monocultures. Also, there was a significant ($P \leq 0.05$) 2-way interaction between culture type and level of water supplied on measured soil moisture contents in the potting media (Table 3.1). Compared with *T. crocata* monocultures, soil moisture contents in potting media comprising mixtures of *T.crocata* and *L. multiflorum* decreased on average by 52% at low levels of water supply and on average by 23% only at high levels of water supply (Figure 3.2D). A significant ($P \leq 0.001$) positive correlation was found between above-ground biomass of *T. crocata* and corresponding soil water contents in the potting media with the converse apparent with respect to above-ground biomass of *L. multiflorum* (Table 3.2).

Table 3.1. Statistics for the effects of culture type, nutrient supply and water level on soil water contents in the potting media. Values in bold indicate significantly differences at ***P ≤ 0.001; **P ≤ 0.01; *P ≤ 0.05.

Main Effects			2-way Interactions		3-way Interactions
Water	Nutrients	Culture type	Water x Culture type	Nutrients x Culture type	Nutrients x Water x Culture type
$F_{1,119}$ = 62.2* **	$F_{1,119}$ = 1.1	**$F_{1,119}$ = 62.5*** **	**$F_{1,119}$ = 4.2***	$F_{1,119}$ = 3.7	$F_{1,119}$ = 0.8

Table 3.2. Pearson correlation coefficients (r), t-statistics (t) and probability levels (P) derived from statistical comparisons between *T. crocata* and *L. multiflorum* above-ground biomass and corresponding soil water contents in the potting media.

Species	R	$t_{1, 38}$	P
T. crocata	0.3617	2.3917	0.0109
L. multiflorum	-0.3332	-2.1786	0.0178

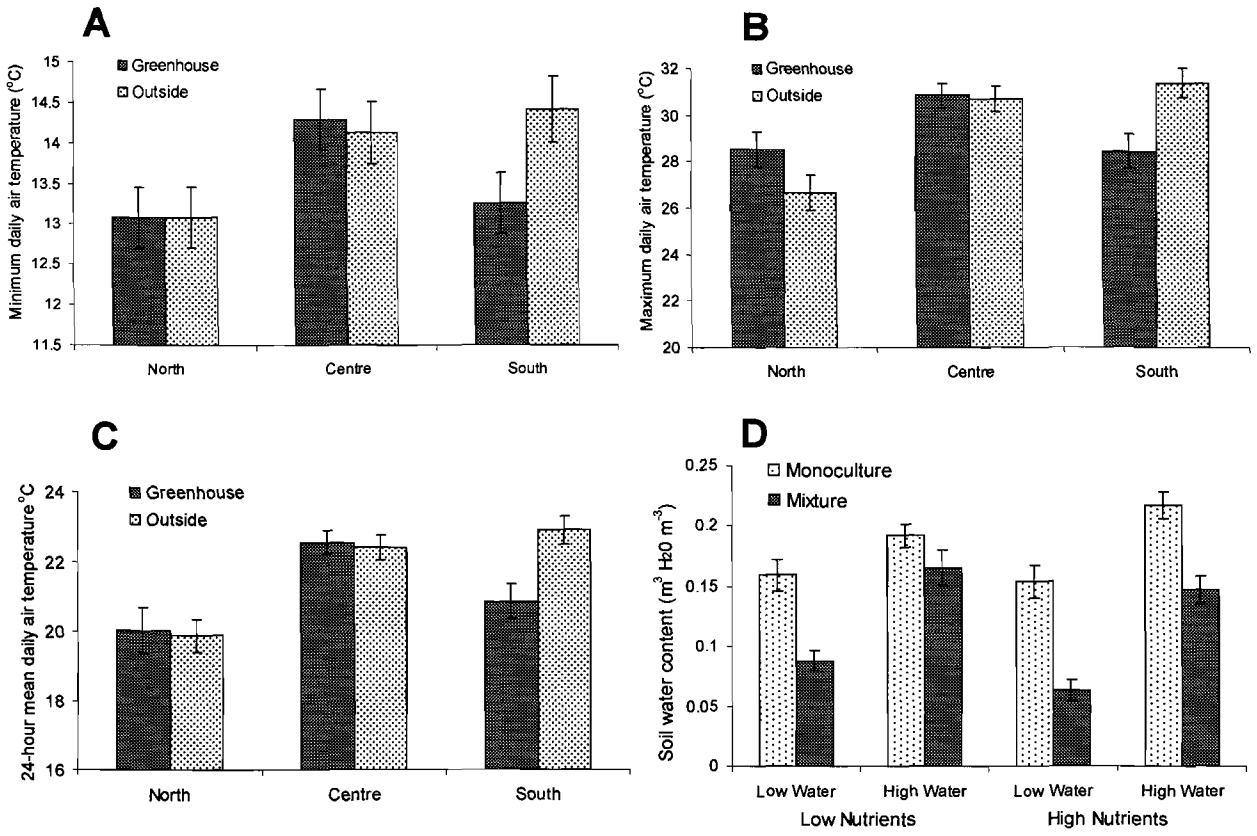


Figure 3.2. Average minimum (A), maximum (B) and 24-hour daily mean (C) air temperatures at different positions inside the greenhouse and outdoors in the ambient environment as well as average soil moisture levels (D) measured in different culture types at different levels of water and nutrient supply.

3.2. PLANT PHYSIOLOGY AND GROWTH

3.2.1. PHOTOSYNTHETIC PIGMENTS

3.2.1.1. Main Effects

3.2.1.1.1. T.crocata

Foliar concentrations of chlorophyll *a* (Figure 3.3A) and total carotenoids (Figure 3.3E) were significantly ($P \leq 0.05$) altered in *T. crocata* by both level of nutrient supply and culture type, but not by level of water supplied (Table 3.3). Chlorophyll *a* concentrations declined on average by 26% and total carotenoid concentrations on average by 31% in this species at low levels of nutrient supply, compared with high levels of nutrient supply. They also declined on average by 18% and 24% respectively in *T. crocata* admixed with *L. multiflorum* compared with *T. crocata* monocultures.

Table 3.3. Statistics for the effects of culture type, nutrient supply and water level and their interactions on foliar pigment concentrations of *T. crocata and L. multiflorum.* Values in bold indicate significant differences at ***$P \leq 0.001$; **$P \leq 0.01$; *$P \leq 0.05$.

Parameter	Competing Species	Main Effects			2-way Interactions		3-way Interactions
		Water	Nutrients	Culture type	Water x Culture type	Nutrients x Culture type	Nutrients x Water x Culture type
Chlorophyll *a*	*T. crocata*	$F_{1,39} = 0.0$	**$F_{1,39} = 9.8^{**}$**	**$F_{1,39} = 4.2^{*}$**	$F_{1,39} = 0.1$	**$F_{1,39} = 4.3^{*}$**	$F_{1,39} = 0$
	L.multiflorum	$F_{1,59} = 0.4$	**$F_{1,59} = 30.95^{***}$**	**$F_{1,59} = 4.4^{*}$**	$F_{1,59} = 1.5$	$F_{1,59} = 0$	$F_{1,59} = 0.2$
Chlorophyll *b*	*T. crocata*	$F_{1,39} = 0.4$	$F_{1,39} = 2.6$	$F_{1,39} = 0.3$	$F_{1,39} = 0.1$	$F_{1,39} = 0.8$	$F_{1,39} = 0.4$
	L.multiflorum	$F_{1,59} = 0.8$	**$F_{1,59} = 11.9^{*}$**	**$F_{1,59} = 17.7^{***}$**	$F_{1,59} = 0.5$	$F_{1,59} = 0.8$	$F_{1,59} = 03$
Carotenoids	*T. crocata*	$F_{1,39} = 0.0$	**$F_{1,39} = 13.4^{***}$**	**$F_{1,39} = 7.5^{**}$**	$F_{1,39} = 0$	**$F_{1,39} = 4.4^{*}$**	$F_{1,39} = 0.5$
	L.multiflorum	$F_{1,59} = 0.2$	$F_{1,59} = $ **37.2^{***}**	$F_{1,59} = 0.9$	$F_{1,59} = 1.9$	$F_{1,59} = 0.5$	$F_{1,59} = 0.7$

3.2.1.1.2. L. multiflorum

Foliar concentrations of chlorophyll *a* and *b* (Figure 3.3B & D), and total carotenoids (Figure 3.3F), were all significantly ($P \leq 0.05$) altered in *L. mutiflorum* by level of nutrient supply, but not by level of water supplied (Table 3.3). These concentrations decreased on average by 54%, 43% and 51% respectively in *L. multiflorum* at low levels of nutrient supply compared with high levels of nutrient supply. Culture type also significantly ($P \leq 0.05$) affected foliar concentrations of chlorophyll *a* and chlorophyll *b* in *L. multiflorum* (Table 3.3). Compared with *L. multiflorum* monocultures, chlorophyll *a* and chlorophyll *b* concentrations increased on average by 31% and 78% respectively in *L. multiflorum* admixed with *T. crocata*.

3.2.1.2. Interactions

3.2.1.2.1. T.crocata

There were significant ($P \leq 0.05$) 2-way interactions between culture type and level of nutrient supply on chlorophyll *a* (Figure 3.3A) and total carotenoid (Figure 3.3E) concentrations in *T. crocata* (Table 3.3). Compared with *T. crocata* monocultures, chlorophyll *a* and total carotenoid concentrations in *T. crocata* admixed with *L. multiflorum* decreased on average by 37% and 46% respectively at low nutrient supply but only by insignificant 0.2% and 5% respectively at high nutrient supply (Figures 3.3A & E).

3.2.1.2.2. L. multiflorum

There were no significant ($P \geq 0.05$) 2-way interactions between culture type and levels of nutrient and water supply on any of the photosynthetic pigment concentrations in *L. mutiflorum* (Table 3.3).

3.2.2. PHOTOSYSTEM II (PS II) FUNCTION

3.2.2.1. Main Effects

3.2.2.1.1. T.crocata

Excitation of dark-adapted leaves with a saturated light pulse induced typical O-J-I-P fluorescence transients in plants of all treatments. As an example, the normalized (between the two fluorescence extremes O and P) O-J-I-P transients recorded in leaves of *T. crocata* grown in monocultures and admixed with *L. multiflorum* under conditions of low nutrient and water supply are presented in Figure 3.4. Upon excitation, there was an initial rapid rise in fluorescence intensity from O (F_0) to the first intermediate step J, at 2 ms, representing the

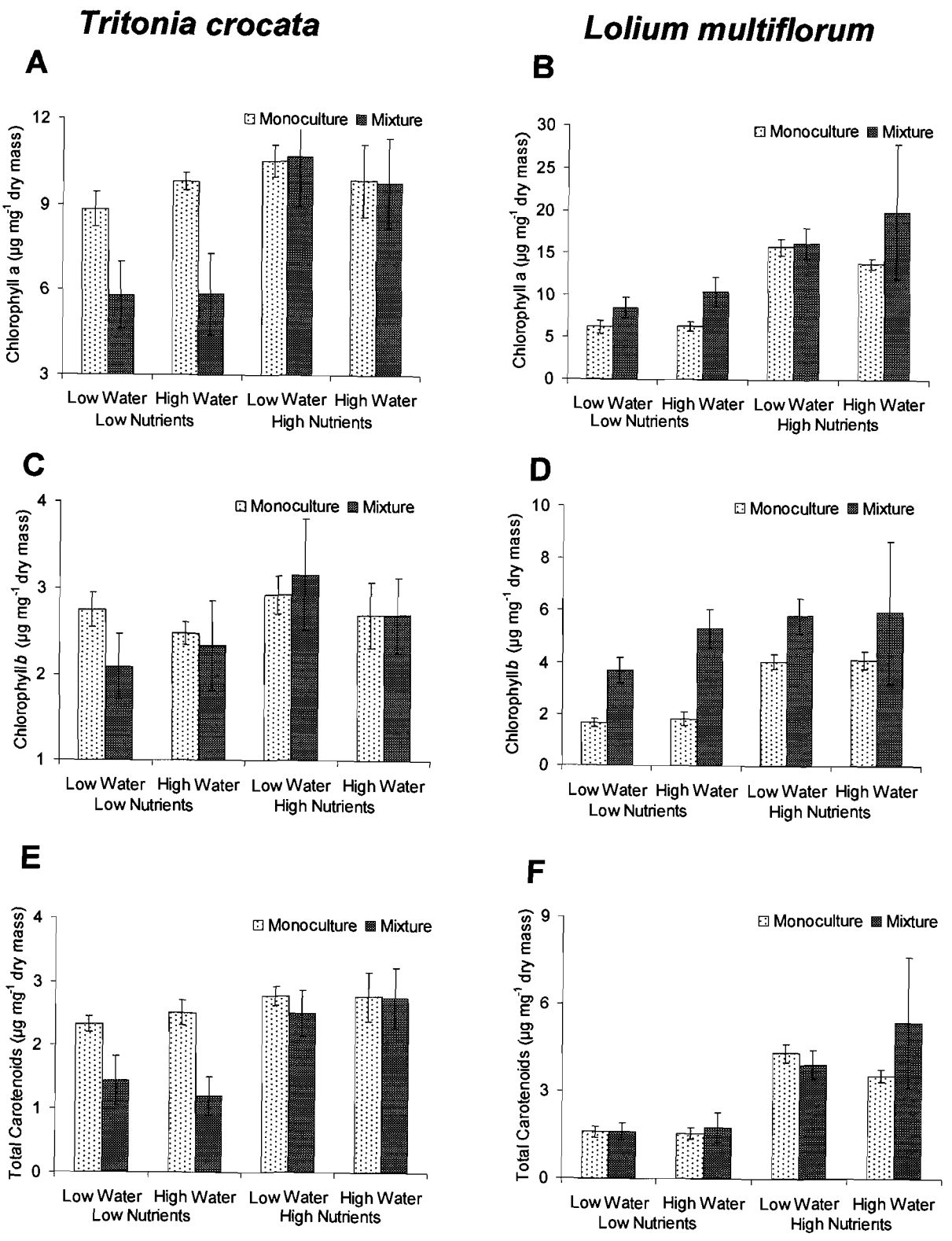


Figure 3.3. Effects of nutrient supply and water level on foliar pigment concentrations of *T. crocata and L. multiflorum* grown in monocultures and mixtures.

single turn over events with respect to Q_A reduction (reviewed in Lazár, 2006). A further rise then followed to the second intermediate step, I, at 30 ms, finally reaching P (F_M) at ca. 300 ms. During this J to I to P phase, mainly light intensity-independent multiple turnover redox events (reduction of the PQ pool) occur (reviewed in Lazár, 2006). In terms of actual fluorescence intensities and kinetics, differences between *T. crocata* monocultures and mixtures with *L. multiflorum* were clearly visible with elevated fluorescence values evident over nearly the entire time range between O and P in mixed cultures compared to monocultures (Figure 3.4).

The photochemical performance index (PI_{ABS}), in *T. crocata* was significantly ($P \leq 0.05$) altered by the level of water supplied (Table 3.4) with an average reduction of 19% in PI_{ABS}, measured in this species at low water levels compared with high water levels (Figure 3.5G). This decline in PI_{ABS} at low water levels was accompanied by significant ($P \leq 0.05$) reductions in two of its three partial responses (Table 3.4). These included the density of working photosystems (reaction center per chlorophyll, RC/ABS), which displayed an average reduction of 12% at low water levels compared with high water levels (Figure 3.5A), and the efficiency of primary photochemistry (trapping) (φ_{P0} / 1-φ_{P0}), which exhibited an average reduction of 11% at low water levels compared with high water levels (Figure 3.5C). Also, PI_{ABS} was significantly ($P \leq 0.05$) altered by culture type with an average reduction of 16% in PI_{ABS} measured in *T. crocata* admixed with *L. multiflorum* compared with *T. crocata* monocultures. This decline in PI_{ABS} was accompanied by significant ($P \leq 0.05$) reductions in all three of its partial responses (Table 3.4), namely RC/ABS, φ_{P0} / 1-φ_{P0} as well as the efficiency of conversion of excitation energy to electron transport (Ψ_0 / 1-Ψ_0) which declined on average by 5%, 6% and 12% respectively in *T. crocata* admixed with *L. multiflorum* compared with *T. crocata* monocultures (Figure 3.5E). In contrast, nutrient supply had no significant ($P \geq 0.05$) effect on PI_{ABS} in *T. crocata,* but all three of its partial responses were significantly ($P \geq 0.01$) altered by nutrient supply (Table 3.4). RC/ABS and φ_{P0} / 1-φ_{P0} displayed average increases of 8% and 6% respectively in *T. crocata* at low nutrient supply compared with high nutrient supply. In contrast Ψ_0 / 1-Ψ_0, exhibited an average decrease of 11% in this species at low nutrient supply compared with high nutrient supply (Figures 3.5 A, C &E).

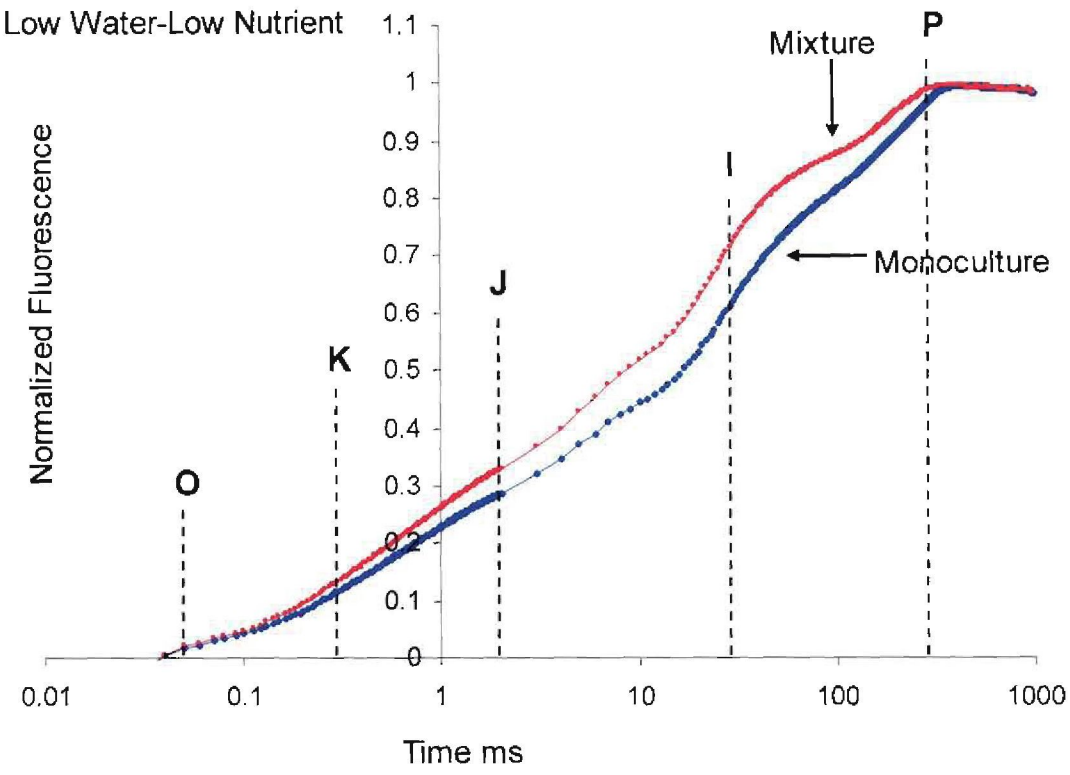


Figure 3.4. O-J-I-P fluorescence transients recorded in leaves of *T. crocata* grown in monocultures and admixed with *L. multiflorum* under conditions of low nutrient and water supply. Each transient represents the average of measurements obtained from 5 plants per treatment. The position of the main steps (O, J, I and P) is indicated.

3.2.2.1.2. L. multiflorum

Only nutrient supply had a significant ($P \leq 0.05$) effect on PI_{ABS} in *L. multiflorum* (Table 3.4) with an average increase of 21% in PI_{ABS} measured in this species at low nutrient supply compared with high nutrient supply (Figure 3.5H). Also, two of the three partial responses of PI_{ABS}, namely RC/ABS and φ_{P0} / 1-φ_{P0} were significantly ($P \leq 0.05$) altered by nutrient supply and displayed average increases of 10% and 13% respectively in *L. multiflorum* at low nutrient supply compared with high nutrient supply (Figure 3.5B & D). Also, φ_{P0} / 1-φ_{P0} was significantly ($P \leq 0.05$) altered by water level (Table 3.4) with an average decrease in φ_{P0} / 1-φ_{P0} of 8% measured in *L. multiflorum* at low water levels compared with high water levels (Figure 3.5B).

Table 3.4. Statistics for the effects of culture type, nutrient supply and water level and their interactions on the photochemical performance index (PI_{ABS}) and its three partial responses, namely RC/ABS, φ_{P0} / 1-φ_{P0} and Ψ_0 / 1-Ψ_0, in *T crocata and L. multiflorum*. Values in bold indicate significant differences at ***P ≤ 0.001; **P ≤ 0.01; *P ≤ 0.05.

		Main Effects			2-way Interactions		3-way Interactions
Parameter	Competing Species	Water	Nutrients	Culture type	Water x Culture type	Nutrients x Culture type	Nutrients x Water x Culture type
RC / ABS	*T. crocata*	**$F_{1,132}$=46.8*** **	**$F_{1,132}$=17.5*** **	**$F_{1,132}$ = 8.1** **	$F_{1,132}$ = 0.7	**$F_{1,132}$=12.7*** **	$F_{1,132}$ = 3.2
	L.multiflorum	$F_{1,88}$ = 0.1	**$F_{1,88}$ = 7.4** **	$F_{1,88}$ = 3.3	$F_{1,88}$ = 2.3	$F_{1,88}$ = 0.0	**$F_{1,88}$ = 3.2***
φ_{P0} / 1-φ_{P0}	*T. crocata*	**$F_{1,132}$=28.0*** **	**$F_{1,132}$ = 7.3** **	**$F_{1,132}$ = 9.2** **	$F_{1,132}$ = 0.2	$F_{1,132}$ = 0.1	$F_{1,132}$ = 2.3
	L.multiflorum	**$F_{1,88}$ = 4.1***	**$F_{1,88}$ =10.9*** **	$F_{1,88}$ = 0.1	$F_{1,88}$ = 2.4	$F_{1,88}$ = 3.0	$F_{1,88}$ = 0.2
Ψ_0 / 1-Ψ_0	*T. crocata*	$F_{1,132}$ = 0.2	**$F_{1,132}$ = 8.5** **	**$F_{1,132}$=10.3** **	**$F_{1,132}$=7.7** **	$F_{1,132}$ =0.2	**$F_{1,132}$ = 6.2***
	L.multiflorum	$F_{1,88}$ =1.3	$F_{1,88}$ = 0.1	$F_{1,88}$ = 5.0	$F_{1,88}$ = 0.2	$F_{1,88}$ = 0.0	**$F_{1,88}$ = 3.2***
PI_{ABS}	*T. crocata*	**$F_{1,132}$=13.1*** **	$F_{1,132}$ = 0.1	**$F_{1,132}$=10.1** **	$F_{1,132}$ = 1.8	$F_{1,132}$ = 2.4	$F_{1,132}$ = 2.8
	L.multiflorum	$F_{1,88}$ = 2.3	**$F_{1,88}$ = 7.7** **	$F_{1,88}$ = 3.8	**$F_{1,88}$ = 4.3***	$F_{1,88}$ = 2.4	**$F_{1,88}$ = 3.3***

3.2.2.2. Interactions

3.2.2.2.1. T.crocata

There was a significant ($P \leq 0.05$) 2-way interaction between water level and culture type on Ψ_0 / 1-Ψ_0, and between nutrient supply and culture type on RC/ABS in *T. crocata* (Table 3.4). Compared with *T. crocata* monocultures, RC/ABS and Ψ_0 / 1-Ψ_0 declined in *T. crocata* admixed with *L. multiflorum* on average by 12% and 21% respectively at low water levels, but only by insignificant 1.2% and 2% respectively at high water levels (Figure 3.5E).

A significant ($P \leq 0.05$) 3-way interaction between culture type, water level and nutrient supply, was apparent in one of the three partial responses of PI_{ABS}, namely Ψ_0 / 1-Ψ_0 (Table 3.4). Compared with *T. crocata* monocultures, Ψ_0 / 1-Ψ_0 displayed a 31% reduction in *T. crocata* admixed with *L. multiflorum* at a low water level and low nutrient supply, followed by a 12% reduction at a low water level and high nutrient supply, a 9% reduction at a high water level and high nutrient supply with an opposing 8% increase evident at a high water level and low nutrient supply (Figure 3.5E).

3.2.2.2.2. L. multiflorum

There was a significant ($P \leq 0.05$) 2-way interaction between culture type and water supply on PI_{ABS} (Table 3.4) in *L. multiflorum*. Compared with *L. multiflorum* monocultures, PI_{ABS} increased in *L. multiflorum* admixed with *T. crocata* on average by 32% at a low water level but only by 2% at a high water level. These differential responses of PI_{ABS} in *L. multiflorum* admixed with *T. crocata* at different levels of water supply, were also modified by level of nutrient supply. This corroborated by the significant ($P \leq 0.05$) 3-way interaction found between culture type, water level and nutrient supply for PI_{ABS} *in L. multiflorum,* and also for two of its three partial responses, namely RC/ABS and Ψ_0 / 1-Ψ_0, (Table 3.4). Compared with *L. multiflorum* monocultures, PI_{ABS}, RC/ABS and Ψ_0 / 1-Ψ_0 displayed 79%, 9% and 91% increases respectively in *L. multiflorum* admixed with *T. crocata* at a low water level and high nutrient supply; 5%, 20% and 96% increases respectively at a high water level and low nutrient supply; 1%, 9% and 8% increases respectively at a low water level and low nutrient supply and 7%, 6% and 1% decreases respectively at a high water level and high nutrient supply (Figure 3.5B, D & H).

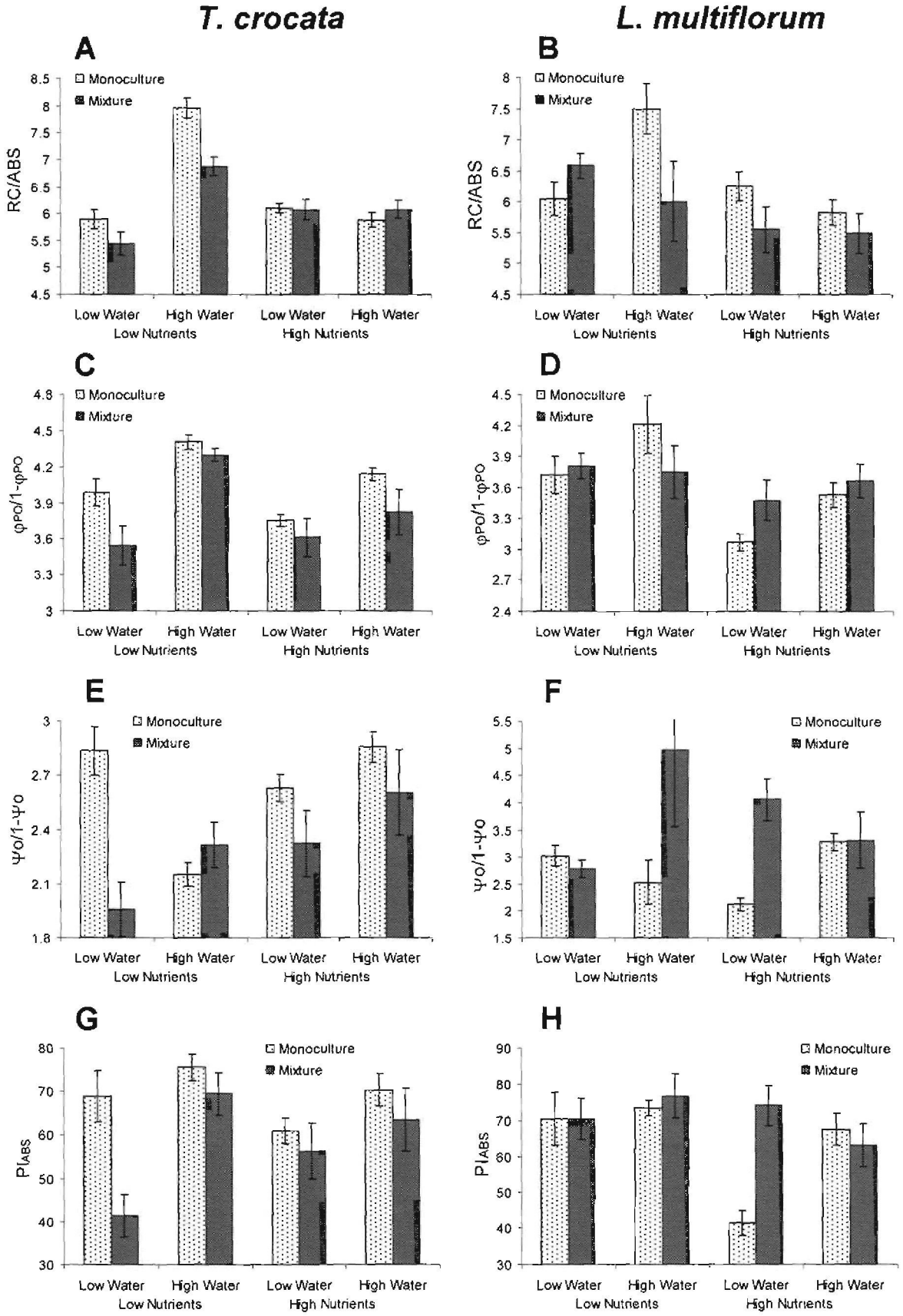


Figure 3.5. Effects of nutrient supply and water level on the photochemical performance index (PI_{ABS}) and its three partial responses, namely RC/ABS, φ_{P0} / 1-φ_{P0} and Ψ_0 / 1-Ψ_0 in *T crocata and L. multiflorum* grown in monocultures and mixtures.

3.2.3. PHOTOSYNTHETIC GAS AND VAPOUR EXCHANGE

3.2.3.1. Main Effects

3.2.3.1.1. T. crocata

Water level significantly ($P \leq 0.05$) affected transpiration (E) and respiration (R_D) rates in *T. crocata* (Table 3.5). Transpiration rates declined on average by 31% and R_D increased on average by 56% in this species at low water levels compared with high water levels (Figure 3.6A & B). Nutrient supply significantly ($P \leq 0.05$) altered E, stomatal conductance (g_s), light saturated rate of photosynthesis (A_{max}), apparent carboxylation efficiency (ACE) and intrinsic water use efficiency (WUE_{INT}) in *T. crocata* (Table 3.5). Transpiration rates and g_s decreased on average by 38% and 53% respectively *in T. crocata* at low nutrient supply compared with high nutrient supply (Figures 3.6B & C). In contrast, A_{max}, ACE and WUE_{INT} increased on average by 30%, 36% and 37% respectively *in T. crocata* at low nutrient supply compared with high nutrient supply (Figures 3.6D, E & H). Culture type significantly ($P \leq 0.05$) affected WUE_{INT}, E, g_s, A_{max}, J_{max} and ACE (Table 3.5). Compared with *T. crocata* monocultures WUE_{INT} increased on average by 31.9% in *T.crocata* admixed with *L. multiflorum,* whereas E declined on average by 38%, g_s by 42%, A_{max} by 37%, J_{max} by 23% and ACE by 22% in *T. crocata* admixed with *L. multiflorum* (Figures 3.6 B, C, D, E & F). In fact, the significantly lower values of A_{max} and J_{max} apparent in all nutrient and water treatments in *T. crocata* admixed with *L. multiflorum* were clearly evident in the light and CO_2 response curves (Figure 3.7).

3.2.3.1.2 L. multiflorum

Only ACE was significantly ($P \leq 0.05$) altered in *L. multiflorum* by culture type (Table 3.5), which in comparison with *L. multiflorum* monocultures increased on average by 47% in *L. multiflorum* admixed with *T. crocata* (Figure 3.6H).

3.2.3.2. Interactions

3.2.3.2.1. T. crocata

There were significant ($P \leq 0.05$) 2-way interactions between culture type and nutrient supply and between culture type and water level for E and WUE_{INT} in *T. crocata* (Table 3.5). Compared with *T. crocata* monocultures, E decreased on average by 60% and WUE_{INT} increased on average by 55% in *T. crocata* admixed with *L. multiflorum* at low water supply but at high water supply E decreased only by 17% and WUE_{INT} increased only by 10% (Figures 3.6B & D). The converse was apparent with respect to nutrient supply. Compared with *T. crocata* monocultures, E declined on average by 17% and

WUE_{INT} increased on average by less than 1% in *T. crocata* admixed with *L. multiflorum* at low nutrient supply but at high nutrient supply E decreased by 60% and WUE_{INT} increased by 62% (Figures 3.6B & D). Also, there was a significant ($P \leq 0.05$) 2-way interaction between culture type and level of water supply for g_s (Table 3.5) which in comparison with *T. crocata* monocultures decreased on average by 65% in *T. crocata* admixed with *L. multiflorum* at low water supply, but only by 17% at high water supply (Figure 3.6C).

Significant ($P \leq 0.05$) 3-way interactions between culture type, nutrient supply, and water level were apparent for R_D, E and g_S (Table 3.5); compared with *T. crocata* monocultures the following percentage changes in R_D, E and g_S were observed in *T. crocata* admixed with *L. multiflorum* at the different levels of water and nutrient supply: At low nutrient and low water supply a 33%, 63% and 68% decrease in R_D, E and g_S respectively (Figures 3.6A, B & C). At low nutrient and high water supply a 31% decrease in R_D, a 20% and 28% increase in E and g_S respectively (Figures 3.6A, B & C). At low water and high nutrient a 25%, 58% and 62% decrease in R_D, E and g_S respectively (Figures 3.6A, B & C). At high water and high nutrient supply an only 8% decrease in R_D, a 63% decline in E and a 68% decrease in g_S (Figures 3.6A, B & C).

3.2.3.2.2. L. multiflorum

No significant ($P \geq 0.05$) 2-way or 3-way interactions were computed for *L. multiflorum* as gas and water exchange measurements were restricted to the most extreme resource limiting conditions, *i.e.* low levels of water and low nutrient supply (Table 3.5), where this invasive grass was expected to have the least competitive advantage over *T. crocata* (Figures 3.6A-H).

3.2.4. RUBISCO CONTENT

3.2.4.1. Main Effects

3.2.4.1.1. T. crocata

Visual examination of the SDS-Page gels of *T. crocata* did not reveal any distinct changes in protein profiles in the different lanes representing the various treatments (results not shown). There was no visible evidence of the appearance of new polypeptide bands or disappearance of existing polypeptide bands in the different lanes. However, the measured intensities of the labeled Rubisco large subunit in the different lanes of the Western blots of *T. crocata* were significantly ($P \leq 0.01$) altered by nutrient supply only.

Table 3.5. Statistics for the effects of culture type, nutrient supply and water level and their interactions on photosynthetic gas and water vapour exchange in *T. crocata and L. multiflorum*. Values in bold significantly different at ***P ≤ 0.001; **P ≤ 0.01; *P ≤ 0.05.

		Main Effects			2-way Interactions		3-way Interactions
Parameter	Competing Species	Water	Nutrients	Culture type	Water x Culture type	Nutrients x Culture type	Nutrients x Water x Culture type
R_d	*T. crocata*	**$F_{1,28}$ = 4.4***	$F_{1,28}$ = 3.5	$F_{1,28}$ = 1.4	$F_{1,28}$ = 0.1	$F_{1,28}$ = 1.7	**$F_{1,28}$ = 5.5***
	L. multiflorum	-	-	$F_{1,7}$ = 0.1	-	-	-
E	*T. crocata*	**$F_{1,28}$ = 9.1****	**$F_{1,28}$ =11.0****	**$F_{1,28}$=24.4*****	**$F_{1,28}$ = 7.2***	**$F_{1,28}$ = 6.4***	**$F_{1,28}$ = 6.4***
	L. multiflorum	-	-	$F_{1,7}$ = 0.3	-	-	-
g_s	*T. crocata*	$F_{1,28}$ = 2.8	**$F_{1,28}$ = 9.3****	**$F_{1,28}$=15.8****	**$F_{1,28}$ = 5.9***	$F_{1,28}$ = 3.2	**$F_{1,28}$ = 6.3***
	L. multiflorum	-	-	$F_{1,7}$ = 0.5	-	-	-
WUE_{INT}	*T. crocata*	$F_{1,28}$ = 1.0	**$F_{1,28}$ =14.5****	**$F_{1,28}$ =10.9****	**$F_{1,28}$ = 4.8***	**$F_{1,28}$ =11.5****	$F_{1,28}$ = 3.2
	L. multiflorum	-	-	$F_{1,7}$ = 0.1	-	-	-
A_{max}	*T. crocata*	$F_{1,28}$ = 0.1	**$F_{1,28}$ = 5.2***	**$F_{1,28}$=20.0*****	$F_{1,28}$ = 0.2	$F_{1,28}$ = 0.3	$F_{1,28}$ = 1.4
	L. multiflorum	-	-	$F_{1,7}$ = 0.2	-	-	-
J_{max}	*T. crocata*	$F_{1,28}$ = 2.5	$F_{1,28}$ = 0.1	**$F_{1,28}$ =11.2****	$F_{1,28}$ = 0.1	$F_{1,28}$ = 0.1	$F_{1,28}$ = 0.1
	L. multiflorum	-	-	$F_{1,7}$ = 1.0	-	-	-
AQE	*T. crocata*	$F_{1,28}$ = 0.1	$F_{1,28}$ = 0.1	$F_{1,28}$ = 0.5	$F_{1,28}$ = 1.4	$F_{1,28}$ = 0.1	$F_{1,28}$ = 0.3
	L. multiflorum	-	-	$F_{1,7}$ = 3.2	-	-	-
ACE	*T. crocata*	$F_{1,28}$ = 0.9	**$F_{1,28}$ = 6.4***	**$F_{1,28}$=17.7*****	$F_{1,28}$ = 1.3	$F_{1,28}$ = 1.0	$F_{1,28}$ = 3.5
	L. multiflorum	-	-	**$F_{1,7}$ = 6.4***	-	-	-

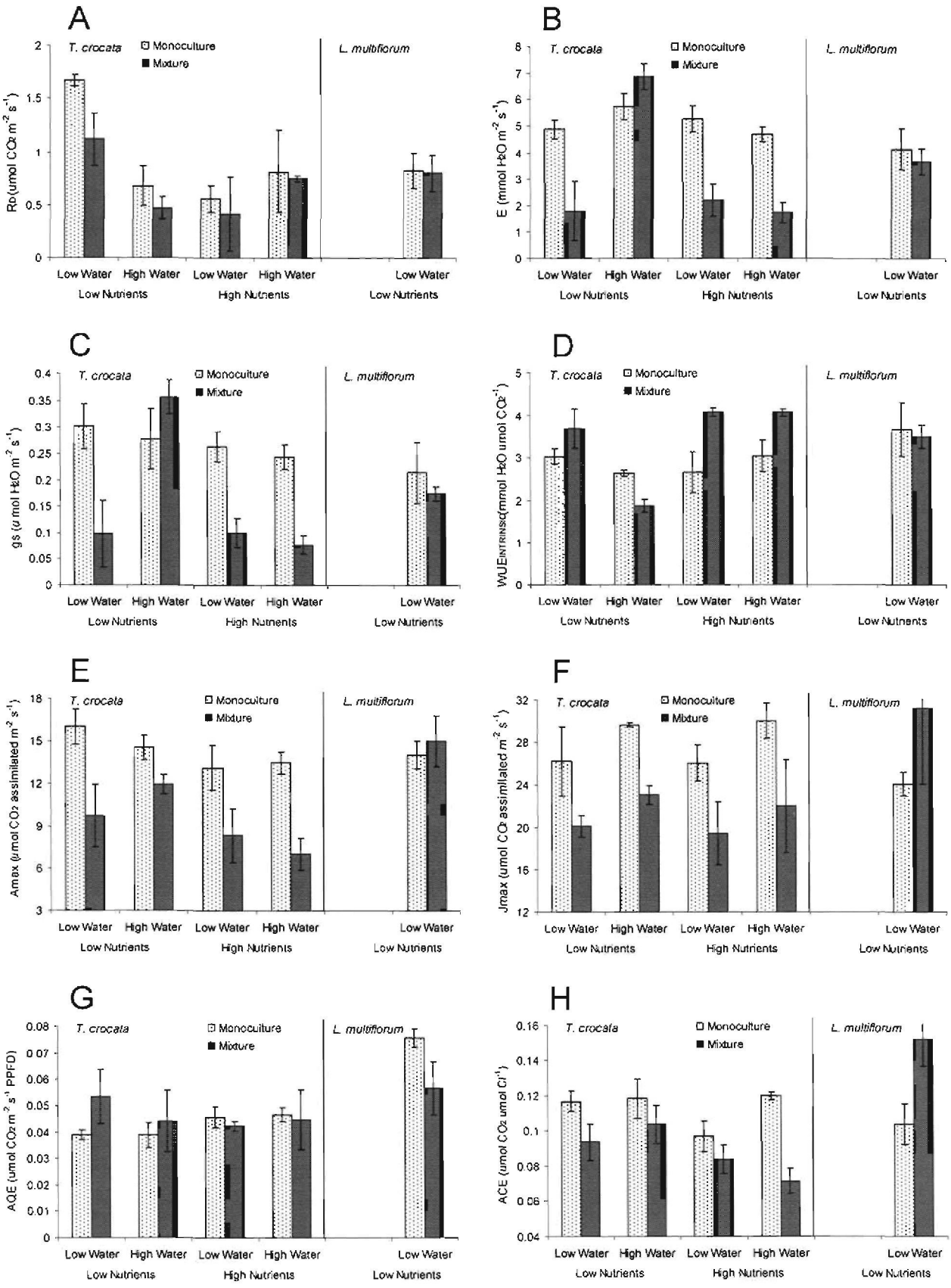

A
T. crocata
Monoculture
Mixture
L. multiflorum
Rb (umol CO2 m-2 s-1)
Low Water
High Water
Low Nutrients
High Nutrients
B
E (mmol H2O m-2 s-1)
C
gs (u mol H2O m-2 s-1)
D
WUEINTRINSIC (mmol H2O umol CO2-1)
E
Amax (umol CO2 assimilated m-2 s-1)
F
Jmax (umol CO2 assimilated m-2 s-1)
G
AQE (umol CO2 m-2 s-1 PPFD)
H
ACE (umol CO2 umol Cl-1)

Figure 3.6. Effects of nutrient supply and water level on photosynthetic gas and water vapour exchange and intrinsic water use efficiency of *T. crocata and L. multiflorum* grown in monocultures and mixtures.

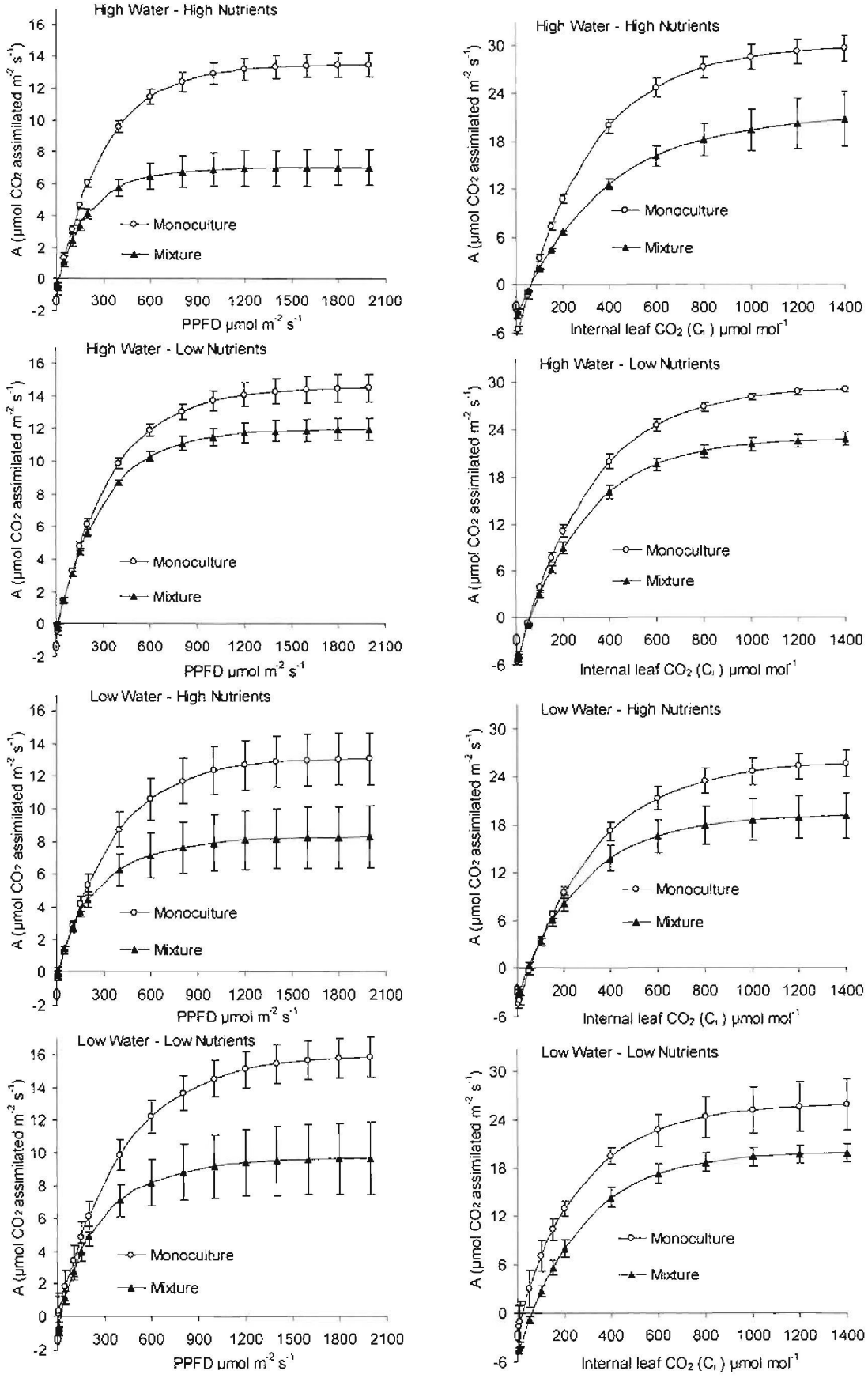


Figure 3.7. Relationships between net CO_2 assimilation rates, photosynthetic photon flux densities and internal leaf CO_2 concentrations for *T. crocata* grown in monocultures and in mixtures with *L. multiflorum* at different water and nutrient levels.

These intensities declined in *T. crocata* on average by 12% at low nutrient supply compared with high nutrient supply (Figure 3.8).

3.2.4.1.2. L. multiflorum

Effects of water level, nutrient supply and culture type on Rubisco content were not determined in *L. multiflorum*, as there was no evidence of any photochemical or photosynthetic inhibition in this alien invasive species in mixtures with *T. crocata*.

3.2.4.2.2. Interactions

3.2.4.2.2.1. T. crocata

Significant ($P \leq 0.05$) 2-way interactions (Table 3.6) between the culture type and water level and between culture type and nutrient supply were observed on the measured intensities of the labeled Rubisco large subunits in the different lanes of the Western blots of *T. crocata*. Compared with *T. crocata* monocultures, these intensities decreased on average by 27% in *T. crocata* admixed with *L. multiflorum* at low nutrient supply, but displayed an opposing 17% increase at high nutrient supply (Figure 3.9). With respect to water supply, these intensities in comparison with *T crocata* monocultures increased by an insignificant 3% in *T. crocata* admixed with *L. multiflorum* at low water supply but decreased by 15% at high water supply.

3.2.5. GROWTH AND REPRODUCTION

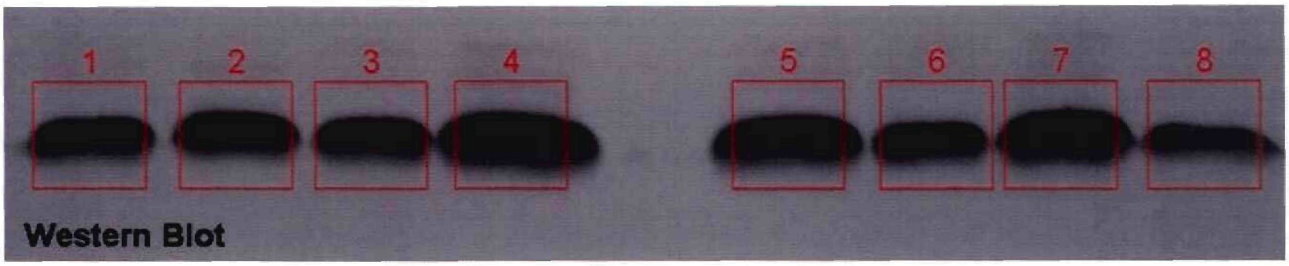
3.2.5.1. Main Effects

3.2.5.1.1. T.crocata

The above-ground biomass but not the below-ground biomass of *T. crocata* was significantly ($P \leq 0.05$) affected by culture type (Table 3.7) which in comparison with *T crocata* monocultures decreased on average by 143% in *T. crocata* admixed with *L. multiflorum* (Figure 3.10A).

3.2.5.1.2. L. multiflorum

The above-ground biomass of *L. multiflorum* was significantly ($P \leq 0.05$) altered by nutrient supply, water level and culture type (Table 3.7). It decreased on average by 70% at low nutrient supply compared with high nutrient supply, by 16% at low water levels compared with high water levels and in comparison with *L. multiflorum* monocultures increased on average by 128% in *L. multiflorum* admixed with *T. crocata* (Figure 3.10D).



Mon Mix Mon Mix Mon Mix Mon Mix

HW-HN LW-HN LW-LN HW-LN

Figure 3.8. Western Blot comprising labeled Rubisco large subunits within different treatments (lanes). HW = High Water, LW = Low Water, HN = High Nutrient, LN = Low Nutrient, Mon = monoculture and Mix = mixture.

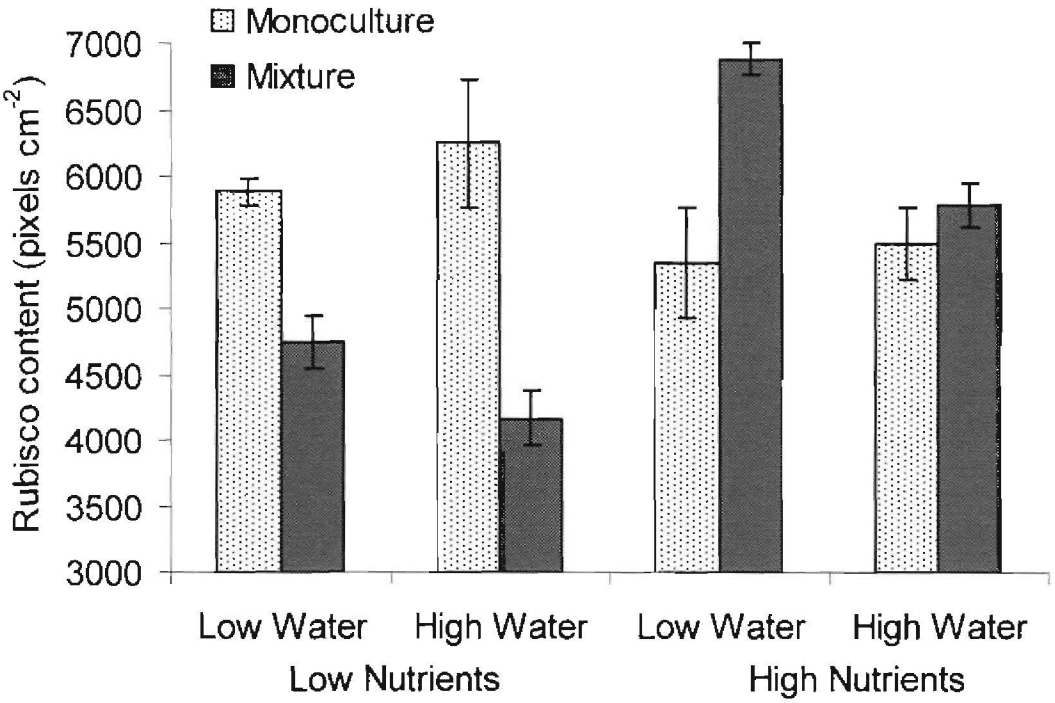


Figure 3.9. Rubisco contents detected on western blots in *T. crocata* grown in monocultures and admixed with *L. multiflorum* at different water levels and nutrient supply.

Table 3.6. Statistics for the effects of culture type, nutrient supply and water level and their interactions on Rubisco content in *T. crocata.* Values in bold indicate significant differences at ***P ≤ 0.001; **P ≤ 0.01; *P ≤ 0.05.

Parameter	Competing Species	Main Effects			2-way Interactions		3-way Interactions
		Water	Nutrients	Culture type	Water x Culture type	Nutrients x Culture type	Nutrients x Water x Culture type
Rubisco	*T. crocata*	$F_{1,31}$ =2.1	**$F_{1,31}$ = 9.8****	$F_{1,31}$ = 3.2	**$F_{1,31}$ = 7.7****	**$F_{1,31}$=41.2*****	$F_{1,31}$ = 0.5
	L. multiflorum	-	-	-	-	-	-

Table 3.7. Statistics for the effects of culture type, nutrient supply and water level and their interactions on floral production and above- and below-ground biomass of *T. crocata and L. multiflorum*. Values in bold indicate significantly different at ***P ≤ 0.001; **P ≤ 0.01; *P ≤ 0.05.

Parameter	Competing Species	Main Effects			2-way Interactions		3-way Interactions
		Water	Nutrients	Culture type	Water x Culture type	Nutrients x Culture type	Nutrients x Water x Culture type
Floral numbers							
	T. crocata	$F_{1,39}$ = 0	$F_{1,39}$ = 2.9	$F_{1,39}$ = 0	**$F_{1,39}$=15.4*****	$F_{1,39}$ = 1.0	$F_{1,39}$ = 3.8
	L.multiflorum	-	-	-	-	-	-
Above-ground biomass							
	T. crocata	$F_{1,39}$ = 0.5	$F_{1,39}$ = 1.1	**$F_{1,39}$ = 17.1*****	$F_{1,39}$ = 2.1	$F_{1,39}$ = 1.2	$F_{1,39}$ = 0.3
	L.multiflorum	**$F_{1,39}$=28.4*****	**$F_{1,39}$=1081.6*****	**$F_{1,39}$=573.5*****	**$F_{1,39}$=5.5***	**$F_{1,39}$=166.0*****	**$F_{1,39}$=20.2*****
Below-ground biomass							
	T. crocata	$F_{1,39}$ = 0.3	$F_{1,39}$ = 0.2.4	$F_{1,39}$ = 0	$F_{1,39}$ = 0.3	**$F_{1,39}$ = 8.8****	$F_{1,39}$ = 1.2
	L.multiflorum	-	-	-	-	-	-

3.2.5.2. Interactions

3.2.5.2.1. T.crocata

There was a significant ($P \leq 0.05$) 2-way interaction (Table 3.7) between the culture type and water level on floral production in *T. crocata*. Compared with *T. crocata* monocultures, floral production increased on average by 47% in *T. crocata* admixed with *L. multiflorum* at low water levels but decreased by 89% at high water levels (Figure 3.10C). Also, there was a significant ($P \leq 0.01$) 2-way interaction between nutrient supply and culture type (Table 3.7) on the below-ground biomass of *T. crocata*, which in comparison with *T. crocata* monocultures increased on average by 48% in *T. crocata* admixed with *L. multiflorum* at high nutrient supply, but displayed an opposing 43% decrease at low nutrient supply (Figure 3.10B).

3.2.5.2.2. L. multiflorum

There were significant ($P \leq 0.05$) 2-way interactions between culture type and water level and between culture type and level of nutrient supply (Table 3.7) on the above-ground biomass of *L. multiflorum*. Compared with monocultures the above-ground biomass of *L. multiflorum* increased in mixed cultures on average by 125% and 127% respectively at low water levels and low nutrient supply and by 130% and 128% at high water levels and high nutrient supply (Figure 3.10D). Also, there was a significant ($P \leq 0.05$) 3-way interaction between the culture type, water level and nutrient supply on the above-ground biomass of *L. multiflorum* (Figure 3.10D), which in comparison with *L. multiflorum* monocultures displayed 132% increase in *L. multiflorum* admixed with *T. crocata* at a low water level and low nutrient supply, a 123% increase at a high water level and low nutrient supply, a 124% increase at a low water level and high nutrient supply and a 132% increase at a high water level and high nutrient supply (Figure 3.10D).

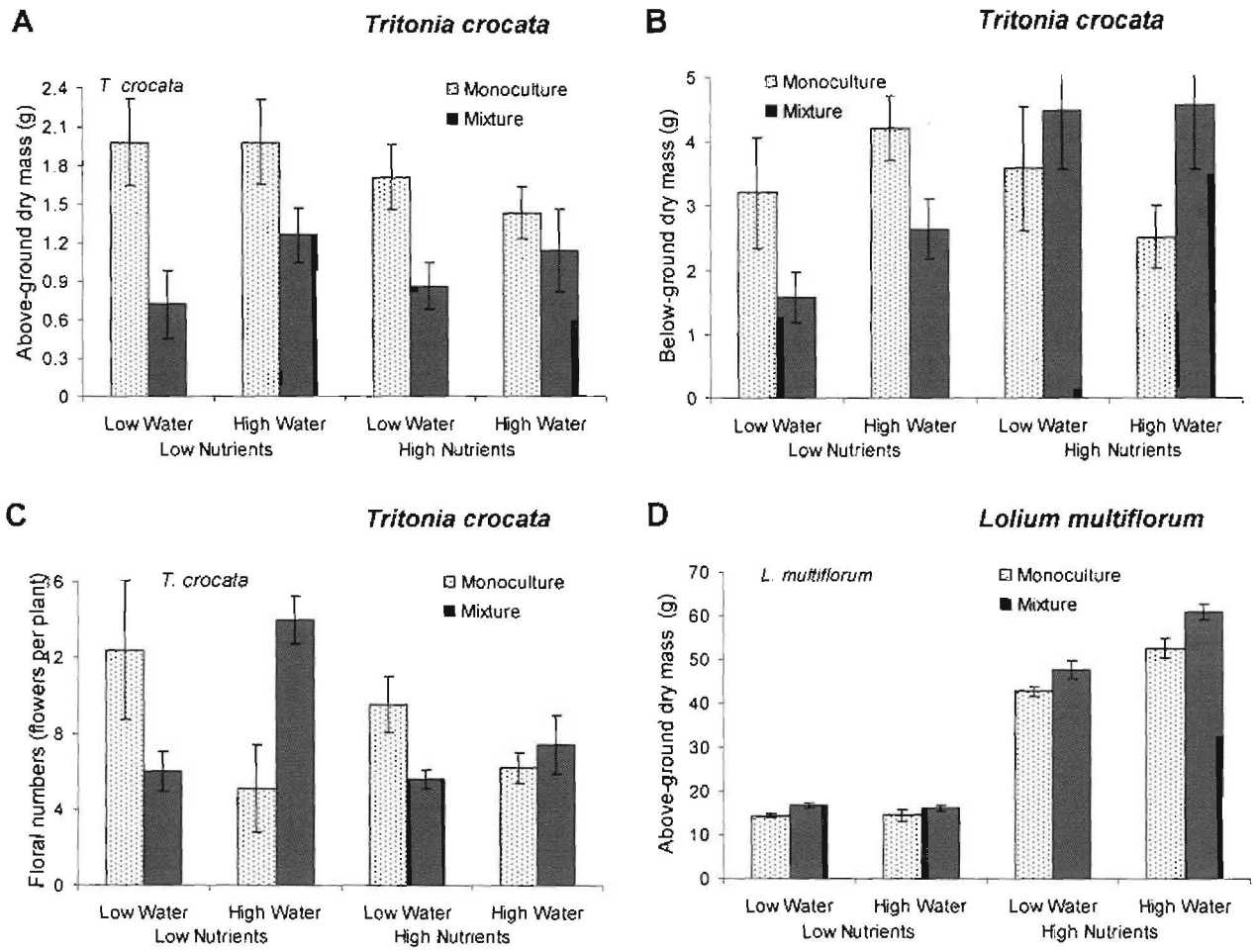


Figure 3.10. Effects of nutrient supply and water level on biomass accumulation (A, B & D) and floral production (C) of *T. crocata* and *L. multiflorum* grown in monocultures and mixtures.

CHAPTER 4: DISCUSSION

4.1. GREENHOUSE ENVIRONMENT

4.1.1. Photosynthetic photon flux density

Light limits the photosynthetic productivity of all crops (Wilson *et al.*, 1992) and is the most important variable affecting productivity in the greenhouse (Wilson *et al.*, 1992, Papadopoulos & Pararajasingham, 1997). The control of light in the greenhouse which is received from the sun is out of normal control (Stanghellini & Van Meurs, 1992; Van Meurs & Stanghellini, 1992) and the use of supplementary lighting to increase yield during low light periods was avoided in this study due to associated temperature increases and high costs of their operation and installation (Papadopoulos & Pararajasingham, 1997). Greenhouses intercept a percentage of light falling on them allowing a maximum of 80% of the light to reach the plants around solar noon, with an overall average of 68% over the day (Wilson *et al.*, 1992). This 32% attenuation of visible light on average in a greenhouse compared well with the average 30% attenuation of photosynthetically active radiation measured in the greenhouse in this study (Figure 3.1A). Despite this attenuation, measured levels of photosynthetically active radiation (PPFD range: 700 to 800 μmol m^{-2} s^{-1}) in the greenhouse during the course of the experimental treatments were close to those required for optimum net CO_2 assimilation in both the target and antagonistic species (Figure 3.1 B&C).

4.1.2 Air temperatures

The ambient temperature in the greenhouse has effects on both photosynthesis and transpiration of plants (Stanghellini & Van Meurs, 1992; Van Meurs & Stanghellini, 1992) with the optimum temperature determined by the processes involved in the utilization of assimilate products of photosynthesis, i.e., distribution of dry matter to shoots, leaves, roots and fruit (De Koning, 1996). Average temperatures over one or several days are generally considered more important than the day/night temperature differences (Bakker, 1989; De Koning, 1996). This average temperature is also referred to as the 24-hour average temperature or 24-hour mean temperature (Bakker, 1989; Portree, 1996). Greenhouse plants show a very close relationship between growth, yield and the 24-hour mean temperature

(Bakker, 1989, Portree, 1996). Optimum photosynthesis occurs between 21 to 22°C (Portree, 1996), with this temperature range serving as the target for managing temperatures in greenhouses. Indeed, in this study the 24-hour mean temperature measured in the greenhouse (range: 20.1°C to 22.6°C) was in the range of 21°C to 23°C reported for optimum photosynthesis and vegetative growth of vegetable crops (Bakker, 1989). However, the slight 24-hour mean temperature anomaly (up to 2°C) between the southern and northern extremities of the greenhouse (Figure 3.2C) on photosynthesis and growth of the experimental plants was minimized by the randomization of the experimental treatments throughout the greenhouse.

4.1.3. Soil water content

Stomatal behaviour and photosynthesis is affected primarily by soil water content rather than leaf water status (Turner *et al.*, 1985), and in this study an overall reduction of 36% in soil water content was measured in the low water treatments which received half the quantities of water supplied in the high water treatments. However, this reduction was exacerbated by the presence of *L. multiflorum* with soil water contents measured in pots that comprised mixtures of both *L. multiflorum* and *T. croctata* 52% lower in the low water treatments though only 23% lower in the high water treatments than in *T. crocata* monocultures. This was presumably the result of more efficient competition for available water resources by *L. multiflorum* whose above-ground biomass was inversely correlated with soil water content (Table 3.2).

4.2. Plant Physiology and Growth

4.2.1. Photosynthetic pigments

The decreased concentrations of chlorophyll *a* and total carotenoids which were only observed in *T. crocata* admixed with *L. multiflorum* under conditions of low nutrient supply were seemingly a response to nutrient limitation. This presumably exacerbated by more efficient competition for this resource by the alien grass whose biomass was enhanced by nutrient supply. Decreased chlorophyll contents have been reported in wheat under nitrogen limiting conditions (Shanguan *et al.*, 2000) and likewise, increased chlorophyll contents have been reported in maize, sorghum and apple in response to inorganic and organic fertilizer

additions (Sotiropoulos *et al.*, 2005; Amujoyegbe *et al.*, 2007). Water stress may also lead to decreased chlorophyll and carotenoid concentrations as reported in maize (Sanchez *et al.*, 1982) and *Picea abies* seedlings (Pawe *et al.*, 2005). However, there was no evidence of water limitation on photosynthetic pigment concentrations in *T. crocata* admixed with *L. multiflorum* in this study as these concentrations were unaffected by level of water added at both low and high nutrient supply. *L. multiflorum* displayed contrasting changes in concentrations of its primary photosynthetic pigments in mixtures with *T. crocata*. Concentrations of chlorophyll *a* and *b* in this species admixed with *T. crocata* increased in the low nutrient treatment, the increases greater at high water levels than at low water levels. Noteworthy in this regard is that one study which examined the interactive effects of nutrient supply and soil water content in the dune plant, *Cakile edentula,* did report that high nutrient supply tended to lower leaf chlorophyll concentration with this effect more pronounced under high than low soil water contents (Ögren & Öquist, 1985). Despite this anomaly in chlorophyll concentrations between *L. multiflorum* monocultures and mixtures, concentrations in this species however did also display and overall decrease with reduced nutrient and water supply.

4.2.2. PHOTOSYSTEM II (PS II) FUNCTION

Both laboratory and field studies have confirmed that PI_{ABS} is a sensitive indicator of the physiological status of plants during water stress (van Heerden *et al.*, 2007) with several other JIP-test parameters also reported affected in water stressed plants (Christen *et al.*, 2007). This contrasts with generally reported insignificant changes in the Fv/Fm ratio in water stress-stressed plants (Cornic & Briantais, 1991; Epron *et al.*, 1993; Liang *et al.*, 1997; Lima *et al.*, 2002; Kocheva et al., 2004). Indeed, contradictory reports exist in the literature as to the direct effects of water stress on PSII functionality where the Fv/Fm ratio has been applied. This has led various authors to suggest that PSII photochemistry is not affected by mild water stress (Cornic *et al.*, 1989; Epron & Dreyer, 1992; Flexas & Medrano, 2002; Morales *et al.*, 2004), though under extreme water stress it has been shown that both PSI and PSII are severely affected (Genty *et al.*, 1987; Meyer & De Kouchkovsky, 1993; Colom & Vazzana, 2003). Other studies have suggested that changes to PSII activity under water stress are related to photoinhibition rather than to a direct damage to PSII (Baker & Bowyer, 1994). Nevertheless the consensus is that the Fv/Fm ratio by itself provides only limited information

about overall PSII function with its insensitivity to water stress well established (Bukhov & Carpentier, 2004).

In this study, PI_{ABS} declined significantly in *T. crocata* admixed with *L. multiflorum* with two of its three partial responses, namely the density of working photosystems (reaction center per chlorophyll, RC/ABS) and the efficiency of conversion of excitation energy to electron transport (Ψ_0 / 1-Ψ_0) displaying significant reductions under conditions of low nutrient supply. The observed decline in RC/ABS was associated primarily with low nutrient conditions as it occurred at both low and high water levels and corresponded with equivalent reductions in chlorophyll *a* and total carotenoids under these conditions. In contrast, the observed decline in Ψ_0 / 1-Ψ_0 was associated primarily with low water supply as it occurred only under low water conditions but at both low and high nutrient levels. It appeared a response to soil water deficits presumably induced by the large demand for water by the alien grass which displayed only an insignificant (8%) reduction in Ψ_0 / 1-Ψ_0 under conditions of low water supply. Reductions in Ψ_0 / 1-Ψ_0 point to a reduction in electron transport capacity beyond Q_A^- (Strauss *et al.*, 2006). Reductions in electron flow subsequent to photosystem II have also been inferred from disparate changes in induced and constant yield fluorescence measured in water stressed *Pinus radiata* (Conroy, 1986). Also, severe water stress has been reported to negatively effect several chlorophyll *a* fluorescence parameters, including PSII activity (Fv/Fm) and the vitality index, in spruce needles, this also suggesting an inhibition of photosynthetic electron transport (Pawe *et al.*, 2005). It has been argued that down regulation of photosynthetic electron transport in water stressed plants is essential for balancing the supply of, and demand for, reducing equivalents by the Calvin-Benson cycle, since limitation of CO_2 assimilation often precedes inactivation of electron transfer reactions resulting in the generation of an excess of reducing equivalents (Lawlor, 1995). Consequently, to avoid an imbalance between the rate of reducing power supply through photochemistry and the utilization of reducing equivalents by reductive carbon metabolism, a combined down regulation of all components of the photosynthetic process is required under water stressed conditions (Medrano *et al.*, 2002). It is thought that PSII, which catalyses the oxidation of water into oxygen and initiates photosynthetic electron transport, is essential for this regulation (Golding & Johnson, 2003). In fact, recent studies indicate that PSII reaction centers are functionally altered under stress conditions so that that the free energy gap between $S_2Q_A^-$ and $S_2Q_B^-$ is reduced contributing to the photoprotection of PSII through

reaction center quenching (Ivanov *et al.*, 2006), *i.e.* a decrease in active reaction centers per excited cross section (RC/CS), the largest (31%) reduction in RC/CS measured in *T. crocata* admixed with *L. multiflorum* under conditions of low water and low nutrient supply.

An examination of the OJIP transients revealed a significantly greater increase in fluorescence intensity in *T. crocata* admixed with *L. multiflorum* over nearly the entire time range between O and P under conditions of low nutrient and low water supply. The slight increase in fluorescence intensity in the region of the K-step (300 μs) point towards uncoupling of the oxygen-evolving complex (OEC) and electron transport reactions between pheophytin and the primary electron acceptor Q_A, (Strasser *et al.*, 2000; Lazár, 2006), a typical symptom of foliar N-deficiency (Strasser *et al.*, 2004) that might be expected under conditions of both low nutrient and water supply. Low nutrient supply will directly affect foliar N levels, whereas low water supply may do so indirectly through reduction in nitrate reductase activity (Fresneau *et al.*, 2007), an enzyme very sensitive to reduced intercellular CO_2 concentrations induced by stomatal closure (Kaiser & Förster, 1989). The reduction in Rubisco content further supports the presence of foliar N-limitation in these treatments (Fig 3.9).

The first intermediate step J at 2ms is thought to represent single turn over events with respect to Q_A reduction and the observed large increase at this step was indicative of blockage of electron transport beyond Q_A^- (Strauss *et al.*, 2006), and is the main factor that determines the magnitude of reduction in PI_{ABS}. Mainly light intensity-independent multiple turnover redox events (reduction of the PQ pool) occur at the subsequent steps I and P (Lazár, 2006). An increase in fluorescence intensity in the I-P phase is apparently caused by PSI-associated limitations (Munday & Govindjee, 1969). This is thought to result from the accumulation of the reduced Q_A^- and PQ pools due to obstruction of electron flow further down the photosynthetic electron transport pathway (Schreiber & Neubauer, 1987; Strasser *et al.*, 2000), particularly as it has been observed that there is an involvement of cyclic electron transport in PSI in regulating PSII activity (Katona *et al.*, 1992). Inactivation of ferredoxin-NADP+ oxidoreductase (FNR) has also been suggested as a factor that could contribute towards this phenomenon (Schansker *et al.*, 2003).

4.2.3. PHOTOSYNTHESIS AND GROWTH

It is generally assumed that reduced photosynthesis at least during mild water stress is due to stomatal closure which decreases CO_2 supply to the mesophyll, rather than to any direct effect of water stress on the capacity of the photosynthetic apparatus (Chaves, 1991; Flexas *et al.*, 2004; Flexas & Medrano, 2002). In addition to stomatal conductance, another potential diffusive photosynthetic limitation is decreased mesophyll conductance to CO_2 which reduces its concentration at the site of carboxylation within the chloroplast stroma (Massacci & Loreto, 2001; Flexas *et al.*, 2004) with both stomatal and mesophyll resistances presumed contributing to photosynthetic rate limitation under water stress conditions (Flexas *et al.*, 2004) Also, stomatal responses are considered more closely related to soil moisture content than to leaf water status (Chaves *et al.*, 2002) which has led to suggestions that stomata are responding to chemical signals, such as ABA (Socias *et al.*, 1997), produced by the dehydrating roots (Reddy *et al.*, 2004) although recent novel evidence in support of a hydraulic signal instead, now challenges this idea (Christmann *et al.*, 2007).

In this study, diminished stomatal conductances were observed in *T. crocata* admixed with *L. multiflorum* in all treatments except the low nutrient and high water treatment where an anomaly was apparent. These diminished stomatal conductances were associated with decreased net CO_2 assimilation rates (A_{max}) and a corresponding reduction in above ground biomass of *T. crocata*. They were attributed to more efficient competition for soil water resources by *L. multiflorum,* whose above ground biomass, in contrast to that of *T. crocata,* was inversely correlated with soil water content. The decline in net CO_2 assimilation rates at saturating intracellular CO_2 concentrations (J_{max}) also observed in *T. crocata* admixed with *L. multiflorum* in all nutrient and water treatments pointed to reduced RuBP regeneration capacity and according to the model of photosynthetic gas exchange diminished photochemical activity (Farquhar *et al.*, 1980). However photochemical reactions are normally rather tolerant of mild water stress with J_{max} usually reduced to a much greater extent than electron transport, though under severe water stress conditions decreased ATP synthesis, through ATP-synthase impairment, may lead to diminished RuBP regeneration capacity (Tezara *et al.*, 1999). Indeed, evidence of diminished photochemical activity in *T. crocata* admixed with *L. multiflorum* was apparent from the observed decline in P_{IABS} and two of its three partial responses under conditions of low water and nutrient supply (See section 4.2.2).

Ecophysiological responses to changes in the external environment may not necessarily elicit clear cut relationships between parameters, e.g. A_{max} and g_s, due to variability in physiological thresholds, transient or acclamatory response of species (Gill *et al.*, 2002). Indeed, under conditions of low nutrient and high water supply, only metabolic impairments to photosynthesis (decreased RC/ABS, chlorophyll *a* and Rubisco content) were apparent in *T. crocata* admixed with *L. multiflorum*, the observed decline in A_{max} associated with an increase in g_s and also Ψ_0 / 1-Ψ_0 which pointed to enhanced electron transport capacity beyond Q_A^- (Strauss *et al.*, 2006). Increases in g_s and electron transport rate have been reported in mango trees receiving extra irrigation capacity, but unlike this study these were also associated with increases in A_{max} (Gonzalez *et al.*, 2006). The decline in A_{max} observed in this study suggested a down regulation of photosynthesis due to reallocation of fixed carbohydrate reserves to floral production which increased significantly in *T. crocata* admixed with *L. multiflorum* under conditions of low nutrient and high water supply only and corresponded with a decline in the mass of its underground storage organ (bulb). Under conditions of low nutrient and low water supply where both stomatal and metabolic limitations to photosynthesis were apparent, the observed decline in both floral production and underground storage organ mass in *T. crocata* admixed with *L. multiflorum* seemed mainly due to decreased amounts of carbohydrates fixed leading to reduced amounts translocated to the underground storage organ and available for floral production.

Loss of Rubisco activity has been reported in several plant species under conditions of water stress (Parry *et al.,* 2002) and increased Rubisco concentrations have also been reported in *Solanum tuberosum L.* (Millard & Catt, 1988) and *Triticum aestivum L.* (Farage *et al.*, 1998) in response to N applications. However, there exist conflicting reports on short-term responses of Rubisco to water stress. Gimenéz *et al.*, (1992) and Gunasekera & Berkowitz (1993) found little effect of water stress on Rubisco whereas Majumdar *et al.*, (1991) observed rapid loss of Rubisco during water stress in soybean. However, increasing severity and duration of water stress have been reported to reduce both Rubisco activity (Tezara & Lawlor, 1995) and its protein content (Kicheva *et al.*, 1994) in sunflower and wheat respectively.

In this study, the decline in apparent carboxylation efficiencies observed in *T. crocata* admixed with *L. multiflorum* in all water and nutrient treatments also implied decreased Rubisco activity (Medrano *et al.*, 1997). However, this contrasted with actual measured

amounts of Rubisco in *T. crocata* admixed with *L. multiflorum* which only declined under conditions of low nutrient supply irrespective of levels of water supplied. In this regard, it has been demonstrated that when Rubisco is not fully activated *in vivo*, it is the amount of activated Rubisco that controls the rate of CO_2 assimilation, rather than the total amount of Rubisco present (Cheng & Fuchigami, 2000). Diminished Rubisco activation state during water stress, due to the accumulation of tight-binding inhibitors on Rubisco catalytic sites, has been reported previously (Parry, *et al.*, 1993). Noteworthy, also was that respiration rates displayed much greater reductions in *T. crocata* admixed with *L. multiflorum* under conditions of low than high nutrient supply, this apparent at both low and high water levels. As a consequence, an accumulation of leaf carbohydrates could potentially occur. This has been correlated with a decrease in ACE (McKee & Woodward, 1994), and may be the signal that brings about a decrease in Rubisco levels (Stitt, 1991), the underlying mechanism believed to operate via the repression of photosynthetic gene expression (Webber *et al.*, 1994; Koch, 1996; Drake *et al.*, 1997).

4.3. CONCLUSIONS

In conclusion, the results did not support the original hypothesis that invasive annual grasses would have a lesser impact on the growth and physiology of native geophytes under resource limiting conditions. In fact, the stomatal and metabolic constraints induced by *L. multiflorum* on *T. crocata* under conditions of low water and nutrient supply suggest that resource limitation mechanisms (Suding *et al.*, 2004) may not provide a suitable means of altering competitive dynamics in favor of native geophytes adapted to semi-arid and nutrient impoverished environments. This is of particular concern since both mechanical (fire and ungulate grazing) and chemical (herbicide) control measures applied for controlling alien grasses have logistic and economic constraints and in some cases adverse effects on native biodiversity (Milton, 2004; Musil *et al.*, 2005). This leaves biological control measures as the only suitable alternative which requires further investigation. Unresolved also, was the possible simultaneous function of resource limitation and allelopathic mechanisms in the inhibition of the native geophyte by the alien grass. Indeed, it has been suggested that the chemical suppression of plants by competitors may be enhanced in poor-resource environments since many allelochemicals which are products of secondary metabolism also increase under low resource conditions (Whittaker & Feeny, 1971; del Moral, 1972; Rice,

1974; Robinson, 1974; Tang *et al.*, 1995; Hierro & Callaway, 2003). This possibility merits further investigation.

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