Ecophysiological studies of the invasive weed *Chromolaena odorata* (L.) King and Robinson and its control in KwaZulu-Natal

by

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As the candidate's supervisor I have/have not approved this thesis for submission

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Professor G. Naidoo **Date**

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(Co-supervisor)

PREFACE

The experimental work described in this thesis was carried out in the School of Life Sciences, University of KwaZulu-Natal from January 2002 to December 2007 under the supervision of Professor G. Naidoo and Dr. Y. Naidoo.

These studies represent original work by the author and have not otherwise been submitted in any form for any degree or diploma to any tertiary institution. Where use has been made of the work of others, it is duly acknowledged in the text.

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Kubendran Kista Naidoo

DECLARATION 1: PLAGIARISM

I, _____ declare that

1. The research reported in this thesis, except where otherwise indicated, is my original research.
2. This thesis has not been submitted for any degree or examination at any other university.
3. This thesis does not contain other person's data, pictures, graphs or other information, unless specifically acknowledged as been sourced from other persons.
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DECLARATION 2: PUBLICATIONS

Details of contributions to publications that form part and /or include research presented in this thesis:

Publication 1

Naidoo, K. K., Coopoosamy, R. M. & Naidoo, G. 2011. Screening of *Chromolaena odorata* (L.) King and Robinson for antibacterial and antifungal properties. J. Med. Plants Res. 5, 4859-4862.

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Kubendran Kista Naidoo

DEDICATION

To my darling wife, Mayendri Moodley and beloved children, Prishari and Shreyaal.

ACKNOWLEDGEMENTS

I thank Professor G. Naidoo and Dr. Y. Naidoo for supervising this thesis. Thanks also to Nigel Bragg for help with statistics, Dr. Anette Mienie (Director: Research, Mangosuthu University of Technology) for funding and support, National Research Foundation for funding and lastly to my wife, Mayendri Moodley for proofreading this dissertation.

General abstract

Despite increased interest in the control and spread of the alien weed, *Chromolaena odorata*, little is known of its photosynthetic characteristics under field conditions**.** The aim of the study was to obtain a better understanding of the ecophysiological attributes of *C. odorata* that contribute to its invasive success.

Photosynthetic performance of *C. odorata* was evaluated by monitoring diurnal changes in gas exchange, chlorophyll *a* fluorescence and plant water relations. Gas exchange characteristics of plants growing in exposed and shaded environments, as well as seasonal patterns, were evaluated. The response of *C. odorata* to water stress was also determined.

Chromolaena odorata exhibited high CO_2 uptake rates with no light saturation. Shade plants had significantly larger leaf surface areas and greater concentrations of total chlorophyll, total carotenoids and chlorophylls *a* and *b* than sun plants. Relatively high photosynthetic uptake rates in *C. odorata* may allow for greater carbon gain in high light environments thus contributing to increased growth and spread of the species. *Chromolaena odorata* can successfully acclimatise to low photosynthetic photon flux density (PPFD), thus, outcompeting less tolerant species under low light conditions.

Leaf conductance, CO_2 uptake, transpiration and chlorophyll fluorescence parameters in winter were tightly coupled to summer. Plants had higher water use efficiency (WUE) in summer compared to winter, probably to maximise CO_2 uptake and minimise water loss.

There was a progressive decrease in leaf water potential with increase in water stress in water stressed (WS) plants. The leaves of WS plants showed signs of severe wilting 10 days after the onset of stress compared to well watered (WW) plants. Increased proline concentration and leaf wilting probably increase (WUE) and may be an adaptive strategy to protect against dehydration injury.

The effects of the herbicide, glyphosate, on gas exchange and translocation were studied. Glyphosate treatment decreased leaf conductance leading to a reduction in CO_2 uptake and transpiration. Glyphosate is a mobile herbicide that is transported from leaves to roots and caused death of plants within a week of treatment.

The potential antimicrobial properties of the weed were evaluated using selected bacteria and fungi. Crude leaf extracts exhibited some antibacterial and antifungal activity. Extracts from the weed are unlikely to be useful antimicrobial sources due to low concentrations of active compounds.

A co-ordinated strategy, taking into account the high plasticity of the weed, is needed to curtail the spread of *C. odorata*. The ecophysiological responses to environmental conditions should be considered when planning management and control strategies for *C. odorata*.

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1. Literature review

1.1. Plant description

Chromolaena odorata (L.) King and Robinson (Asteraceae, Eupatorieae) is a perennial climbing shrub that forms dense, tangled bushes about 1.5-3.0 m in height (McFadyen, 1991) (Fig. 1A). Several stems can develop from the rootstock and shoots re-sprout rapidly resulting in large plants with 20 or more stems that branch freely from axillary buds (McFadyen & Skarratt, 1996). Although commonly occurring as bushes, the weed can occasionally reach heights of 10 m as a scrambler on trees (Goodall & Erasmus, 1996). Mature stems are brown and fibrous while shoot tips and young stems are green and herbaceous (McFadyen, 1991). The root system is adventitious and usually extends to about 20-35 cm deep in most soils. Plants growing under shaded conditions usually have larger and darker green leaves than those in exposed environments. When crushed, leaves have a distinctive “paraffin” smell (Ambika, 1998). Small white flowers (20-60) are borne in capitula at the tips of stems (McFadyen, 1991) (Fig. 1B). Copious amounts of brown seeds (achenes) are dispersed after flowering, each with a small stiff pappus of white hair (McFadyen, 1991). Seeds are wind dispersed and germinate in moist conditions (Ambika, 1998). The seeds have small barbs which tend to cling to most surfaces.

Under drought conditions, leaves abscise and stems may display signs of dieback. New leaves and shoots develop from the axils of old leaves. In summer, new growth is vigourous in moist conditions. Regeneration can also occur via the rootstock and new stems may be present among older ones. Growth rates of actively growing shoots can exceed 20 mm per day (Ambika, 1998). *Chromolaena odorata* can regenerate from nodes along damaged stems by vegetative propagation (New South Wales Department of Primary Industries, 2013). Plants can withstand fire and water scarcity (McFadyen, 1988).

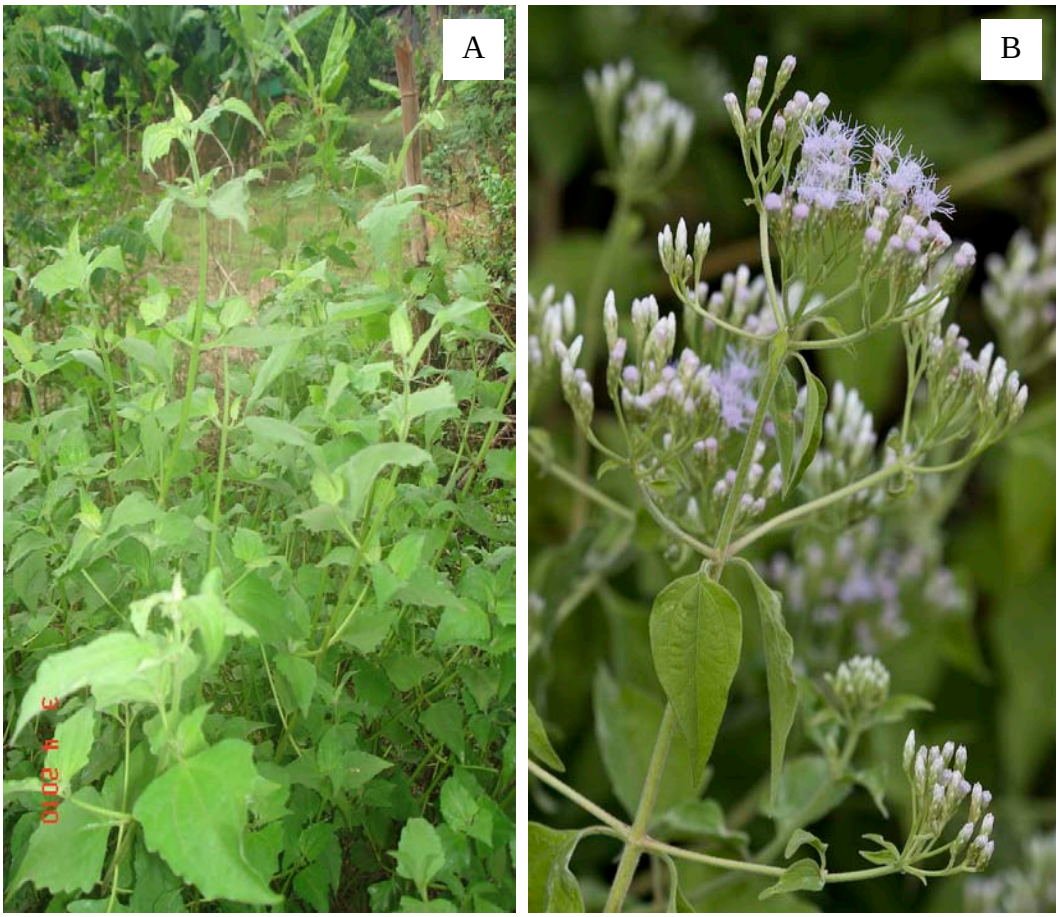

Fig. 1. Vegetative growth of *Chromolaena odorata* showing multi-stem habit (A) and flowering branch showing flowers borne in capitula (B) (courtesy of Pok, 2010 & Bradley, 2008).

1.2. Common names

In South Africa, *C. odorata* is commonly known as “triffid” or “paraffin weed”. “Triffid weed” is derived from its ability to rapidly encroach large areas while “paraffin weed” describes the extraordinary flammability of the plant and the black oil-like smoke that is emitted during burning (Erasmus, 1985). *Chromolaena odorata* has numerous common names in the various countries in which it occurs (Table 1).

Table 1. Common names of *Chromolaena odorata* in various countries (Muniappan, 1992).

COUNTRY	COMMON NAMES
Myanmar	Bizat, Tawbizat, curse of Ceylon, Kal-bun, Kombat-nong-rim, Rel-hlow, Campur grass
Cameroon	Bokassa
Central Africa	Bokassa
China	Feijicao (airplane grass)
Cote d' Ivoire	Sekou Toure
Democratic Republic of Congo	Matapa mbala (the invader), Lantana ngouabi
Laos	Herbe du Laos, French weed
Ghana	Acheampong weed
India	Gandhi gulabi, Communist weed, Sam-solokh, Tongal-lati, Sam-rhabi
Indonesia	Kumpai jepang (Japanese grass), Rumput gol kar (business party grass), Kirinyu, Independent shrub
Malaysia	Siam weed
Mariana Islands	Masiksik
Nepal	Banmara (killer of the forest)
Philippines	Devil weed, Gonoy hagonoy, triffid weed, paraffin weed
Thailand	Saab sua, Yah sua mop
Trinidad	Christmas weed
Vietnam	Co Hoi, Communist weed
South Africa	Armstrong weed, Kingsweed, triffid weed, paraffin weed

1.3. Taxonomy

In the first record of the weed (Plukenet, 1692 in Phytographia), *C. odorata* was illustrated by an etching with the polynomial denomination "*Eupatoria Conzoides Americana*". Browne (1756) gave a brief description of the habitat and scent of the plant. The first botanist to give a binomial name to the species was Linnaeus in 1759 whilst studying plants from Jamaica (Gautier, 1992). Linnaeus named the plant *Eupatorium odoratum* and made reference to the work of Plukenet. A more detailed analysis of the weed was given by Linnaeus in a thesis describing Jamaican plants (Gautier, 1992).

1.4. Classification and nomenclature

1.4.1. *Classification*

The tremendous number of species in the genus *Eupatorium* in the 19th century made it necessary to split it into taxonomically distinct units. The first step towards splitting at the generic level was undertaken by Schultz-Bipontinus in 1866 (Gautier, 1992). In his treatment, the genus *Eupatorium* was split according to the shape of its fruits. He transferred some of the species to the genus *Praxelis* Cass. to create a new genus *Osmia*, which was typified by *Osmia odorata* (L.). In the beginning of the 20th century, the new treatment met with little success as identification of species was difficult. Currently, the genus has about 2000 species that includes 10% of the family Asteraceae (King & Robinson, 1987). For practical reasons and ease of identification, the majority of the species is traditionally placed in the genus *Eupatorium*.

A global revision of the genus was started by R. M. King and H. Robinson in the 1960's (Gautier, 1992) using cytological, chemical and microscopic studies together with phytogeographical distribution (King & Robinson, 1987). The tribe was separated into 18 sub-tribes and 180 genera. The genus *Eupatorium* is restricted to 45 species of arcto-tertiary distribution, mostly concentrated in North America with a single *Eupatorium* species in Europe. Following King & Robinson (1970), *Eupatorium odoratum* was included in the genus *Chromolaena* DC., Prodr. 5:133 (1836) created by De Candolle to include a single species *Chromolaena horminoides* DC.

The current name of the species is *Chromolaena odorata* (L.) King and H. Robinson in Phytologia 20:204 (1970).

1.4.2. *Nomenclature*

The Asteraceae is more widespread in North and South America (McFayden, 1991). Many species are herbs or shrubs while trees are not common. *Chromolaena* is a very large, well defined and highly evolved genus within the Asteraceae. There are comparatively few crop plants but include many ornamentals (King & Robinson, 1977). Therefore, the economic value is low (Toelken, 1983). The genus has 165 species, all from the Americas and the West

Indies (King & Robinson, 1987). Two well known species, *C. waefolia* and *C. laevigata*, are abundant and occasionally invasive in the Americas while *C. odorata* has spread beyond the New World (McFayden, 1988). *Chromolaena odorata* is the most recognised species because of its abundance in the tropics and in places where it is considered an exotic invader. It is often referred to by its former name *Eupatorium odoratum* or by common names such as triffid weed (Lanaudi *et al.*, 1991).

The biotype of *C. odorata* growing in South Africa closely resembles, yet is morphologically different from the Asian and West African forms.

1.5. Origin of *C. odorata*

Flowering time, septoria fungal inoculations, isoenzyme studies and scanning electron microscopy of leaf trichomes and glands of the South African form indicate that it originated in the northern region of South America or the West Indies (Kluge & Caldwell, 1994). The West Indies was suggested as a country of origin due to the high similarity of specimens from this region with identical inflorescences compared to the South African biotype (Gareeb *et al.*, 2002). Evidence shows that the South African biotype of *C. odorata* originates from one or more of the islands in the northern Caribbean region which include Jamaica, Puerto Rico, Cuba or the Bahamas. Unfortunately, the origin of the South African biotype could not be determined using deoxyribonucleic acid (DNA) analysis (von Senger, 2002). However, recent inter-simple sequence repeats (ISSR) have shown that the South African biotype probably originates in Jamaica or Cuba (Paterson & Zachariades, 2013).

1.6. Habitat and distribution

1.6.1. *World*

Chromolaena odorata thrives in hot and humid areas, within latitudes 30^{o} north and south and at an altitude of approximately 1000 m (Ambika, 1998). It proliferates in regions with rainfall greater than 200 mm per annum and where temperatures range from 20^{o}C to 37^{o}C

(Ambika & Jayachandra, 1990). The weed is indigenous to rainforest areas of the Americas from southern Florida in the United States to Mexico, the West Indies, Brazil and Bolivia (McFadyen, 1989) (Fig. 2).

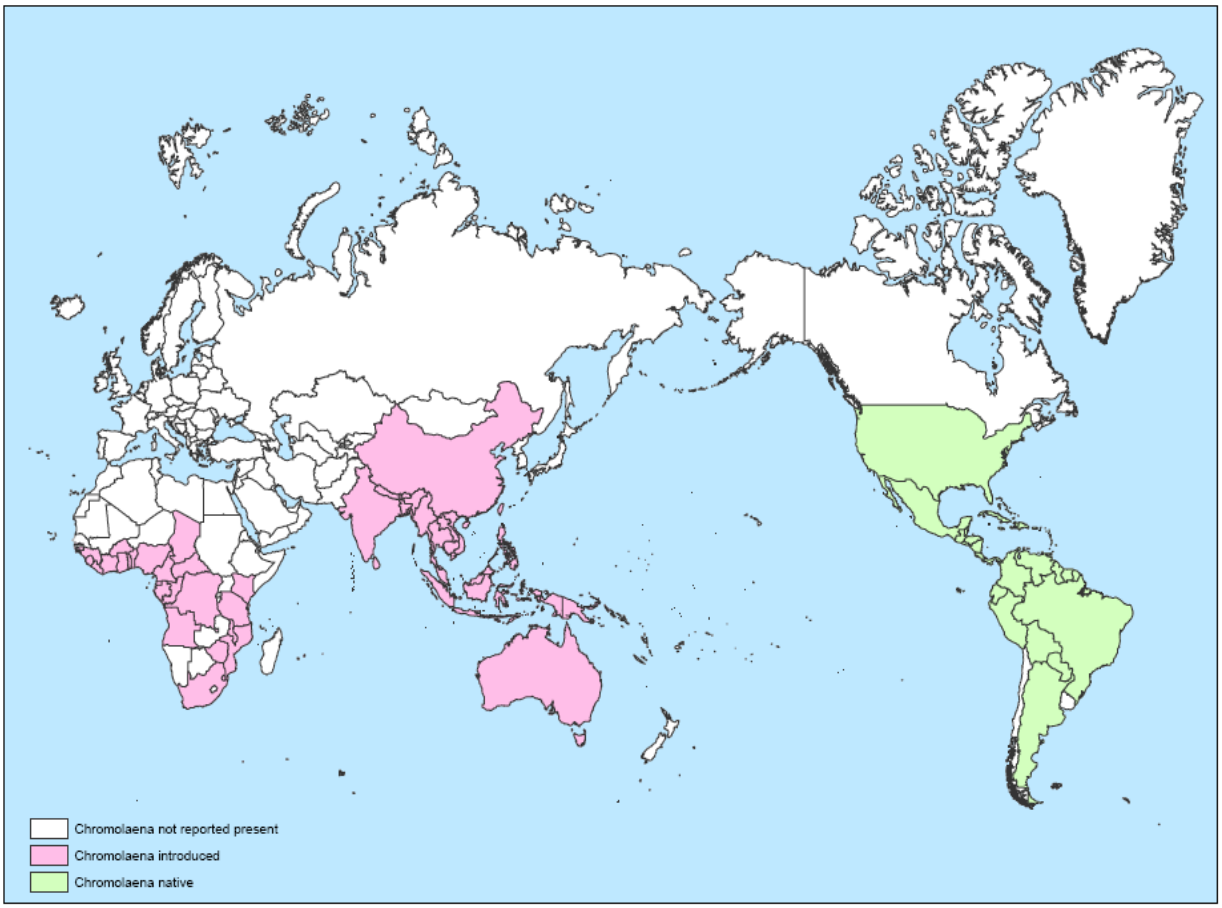

Fig. 2. Global distribution of *Chromolaena odorata* (Zachariades *et al*., 2009 modified from Jimaima Le Grand).

Originally, it was probably an invader of forest clearings and river banks, establishing as a pioneer and rapidly forming dense stands and then slowly disappearing, as rainforest succession proceeded to climax (McFadyen, 1989). Increased clearing of the forest for timber production and crop cultivation has favoured the proliferation of the weed. In native habitats it is widely distributed and abundant along stream banks, in agriculture and pastures, forest clearings and in abandoned waste lands (McFadyen, 1991).

Chromolaena odorata was planted as an ornamental in India in the 1940's (McFadyen, 1989). Voigt (1845) suggested that *C. odorata* was suitable for gardens in the Calcutta area leading to the establishment of the weed in the Ganges flood plain in the 1970's. From

Calcutta, the plant spread east into Myanmar and then gradually east and south-east through Indochina and Indonesia (McFadyen, 1989). It was first propagated as an ornamental in West Africa in the 1930's and has since spread from Mauritania in the west to the Central African Republic and Zaire in the east (Prasad *et al*., 1996). The first infestation of *C. odorata* in Australia was found in the Tully river catchment, south of Cairns in 1994 (Waterhouse, 1994).

1.6.2. *South Africa*

Chromolaena odorata is a successful pioneer of various habitats. It can grow on tree trunks, various substrates containing minimal amounts of soil and even in nutrient poor soils (Ambika, 1998). The weed is one of the most widespread alien invaders of disturbed habitats in KwaZulu-Natal. The seeds are wind dispersed and the plants are capable of extremely rapid growth to form impenetrable stands which eventually shade out indigenous vegetation. As a result, it has become a prolific weed in riverine ecosystems, crop plantations, grasslands and natural forests (Goodall & Erasmus, 1996). *Chromolaena odorata* occurs in numerous veld types described by Acocks (1988), particularly in the Dune and Coastal types that extend from Port Edward to Kosi Bay. However, its neotropical origin confines it to frost free areas along the east coast of South Africa (Goodall & Erasmus, 1996) (Fig. 3).

Chromolaena odorata has also invaded drier regions such as the Zululand Thornveld, the Lowveld Sour Bushveld and the Lowveld. This suggests that *C. odorata* is capable of growing under diverse habitats, from forests (annual rainfall > 1500 mm) to savanna and arid Bushveld (annual rainfall < 500 mm). Like many invasive alien plants, *C. odorata* is commonly found in parts of land where some form of disturbance has occurred e.g. timber plantations, road verges and squatter settlement sites. Ploughing or trampling by cattle has also created ideal conditions for establishment.

Chromolaena odorata was first recorded in South Africa in 1947 near Ndwedwe, KwaZulu-Natal (Hilliard, 1977). Since its introduction, the species has increased rapidly making it one of the sub region's worst weeds (Goodall & Erasmus, 1996). It has spread rapidly along the entire east coast of KwaZulu-Natal as far south as Port St. Johns in the Eastern Cape and as far north as Manguzi on the South African-Mozambique border (Liggitt, 1983; MacDonald, 1984). Between 1960 and 1962, *Chromolaena* was present between Port Shepstone in the

south to Gingindlovu in the north of the province (Egberink & Pickworth, 1969), probably aided by the prevailing north/south wind direction (Erasmus, 1988). By 1970, the weed was present in the Hluhluwe-Umfolozi Game Reserve (Pickworth, 1976). In 1982, the weed occurred from Port Edward to Mtunzini along the KwaZulu-Natal east coast and inland, as far as Pietermaritzburg (Liggitt, 1983). A small infestation was recorded near Tzaneen in 1984 (MacDonald, 1984). By 1987, *Chromolaena* was present along the entire KwaZulu-Natal coast and as far inland as Pietermaritzburg (Henderson, 1989). Since then, weed infestations have spread into two other provinces, Limpopo and Mpumalanga (Fig. 3).

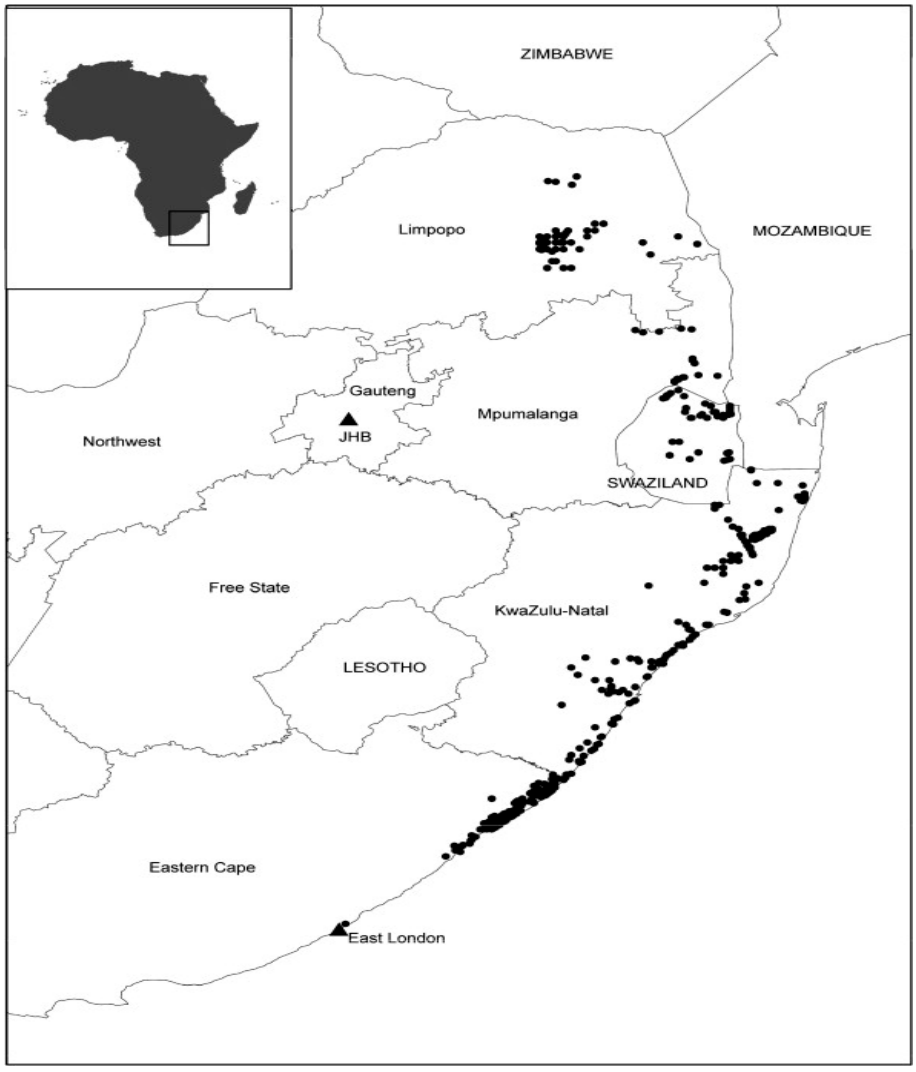

Fig. 3. Distribution of *Chromolaena odorata* in South Africa (Robertson *et al*., 2008).

Although invasions are mono-specific and extensive in frost free areas with adequate rainfall, *C. odorata* will only become abundant if water is not limiting during summer (Goodall &

Erasmus, 1996). The occurrence of the weed into the cool, moist midland regions of KwaZulu-Natal has been hampered by the presence of frost, thus limiting its distribution in the Pietermaritzburg area and further north into the interior of the country (Erasmus, 1988).

1.6.3. *Potential to spread*

McFadyen & Skarratt (1996) developed a CLIMEX model to predict the potential distribution, seasonal growth and stress periods of *C. odorata* based on climate data and the species' response to variables such as temperature and moisture (Taylor & Kumar, 2013). This model showed that the potential distribution of *C. odorata* in West Africa coincided with the area currently invaded, suggesting that the weed had reached its climatic limits. In Central and East Africa, however, the weed is not yet present in certain areas and could pose a serious invasion threat (McFadyen & Skarratt, 1996). The ability of *C. odorata* to replace grasslands in humid areas with monoculture thickets must receive management priority in view of the importance of wildlife reserves and ecotourism to the economy of those countries (MacDonald, 1983) and eradication measures must be undertaken, where possible (Goodall & Erasmus, 1996). The potential to spread in Australia and Oceania includes most of the eastern and northern coasts of Australia and all the Pacific islands including North Island in New Zealand (McFadyen & Skarratt, 1996). The weed is also spreading in southern China where it has not reached its climatic limits (McFadyen & Skarratt, 1996).

1.7. Weediness in South Africa

1.7.1. *History of invasion*

Chromolaena odorata is thought to have been unintentionally introduced to KwaZulu-Natal in seed contaminated packing material offloaded at Durban Harbour during World War II (Pickworth, 1976) or less likely as an ornamental for gardens in Durban (Byford-Jones, 1989).

1.7.2. *Current status and legislation*

Chromolaena odorata is not considered an invasive weed in its natural habitat in the Americas. Seedlings seldom become established on extensively cultivated land. In fields and along road verges, growth is usually slashed at three or four monthly intervals (McFadyen, 1991). It is a plant of "secondary succession" and invades habitats and persists until shaded out by the overgrowth of trees and large shrubs (Leslie, 2000). In citrus or cocoa plantations, the weed is present only at the fringes where they are easily controlled by annual or bi-annual cutting (McFadyen, 1991).

Chromolaena is also controlled by various other factors including attack by indigenous insects and diseases, together with competition with co-occurring plants. *Chromolaena odorata* is confined to the tropical zone in the Americas and has not spread into the sub-tropical humid areas as it has done in the Old World, notably in northern India and Nepal, southern China and in southern Africa (McFadyen, 1991). This difference may be due to competition with many other *Eupatorium* species and to the effects of attack by herbivores and diseases found in its natural but not introduced habitat (McFadyen, 1989). Thus, the absence of these specific factors has resulted in naturalisation in parts of Africa, India, Indochina, Malaysia and Indonesia where it has become a highly successful, noxious invader (McFadyen, 1991).

Chromolaena odorata was declared an exotic invader in South Africa according to the Conservation of Agricultural Resources Act (Act 43 of 1983) therefore the presence of the weed on land constitutes an offence (Erasmus, 1985). Major efforts are been undertaken by the Working for Water Program, funded by the Department of Environmental Affairs, to remove this species from sensitive catchment areas in KwaZulu-Natal.

1.7.3. *Habitat destruction*

Chromolaena odorata is a fire hazard, even when actively growing (MacDonald, 1983; MacDonald, 1984) and its flammable nature promotes the spread of veld fires in forests (MacDonald, 1983; MacDonald, 1984; Le Roux, 1991). In Cote d' Ivoire and Sumatra, *C. odorata* invades oil palm and rubber plantations (Holm *et al*., 1977). Young saplings are competitively excluded by the weed and in mature plantations, the abundance of mature

stems is a fire risk in the dry season (Walton & Waterhouse, 1996). *Chromolaena odorata* is not killed by fire but rather regenerates even more vigorously from the rootstock after burning.

Disturbance such as fires are also contributory factors facilitating weed invasions. To this end, the expansion of forest plantations and urban land use has compounded the problem allowing the weed to colonise new "habitats". Species diversity and the carrying capacity of both grassland and forest habitats are therefore compromised (Erasmus, 1985; Erasmus & van Staden, 1986a). The weed readily colonises extensive grassland, especially in areas that have been overgrazed, resulting in a loss of grazing material (Field, 1991).

The weed is allelopathic and phytotoxins are released through the rain-wash of leaves, exudation from roots and from decomposing plant residues (Ambika, 1998). Leaf material is considered toxic to animals (Sajise *et al.*, 1974). However, goats have been noted to browse on *Chromolaena* thickets when alternative food material is limited (Goodall *et al.*, 1994; Hai *et al*., 2013). *Chromolaena odorata* is known to have caused deaths, abortions and has reduced cattle herd numbers in the Philippines (Sajise *et al.*, 1974). Although it is not considered poisonous in some countries, livestock have been observed to avoid it due to the bitter taste of its leaves (Walton & Waterhouse, 1996).

The weed decreases the biological diversity and carrying capacity in both forest and grassland habitats and poses a serious threat to commercial crops. In Kerala, India, *C. odorata* is an invasive weed of crops such as rubber, coffee, coconut, cocoa and cashew (Joy *et al*., 1993). *Chromolaena odorata* can rapidly degrade coastal forest habitats occupying niches created by dying trees. The weed is able to rapidly invade suitable habitats thus preventing occupation from pioneer indigenous species (Goodall & Erasmus, 1996). Once established in a suitable habitat, the weed impedes human related activities such as domestic and commercial farming and the movement of livestock.

The major problem of *C. odorata* in countries other than South Africa is the infestation of plantations resulting in suppression of crop seedling growth, increased fire hazard and the formation of obstacles to silvicultural operation (Erasmus, 1985). In South Africa, it is mainly seen as a threat to wildlife conservation and also has major impacts on indigenous forests and

crop plantations. Ezemvelo KZN-Wildlife considers the weed to be one of the biggest problems threatening biodiversity in game and nature reserves in the province. Many reserves, such as Hluhluwe-Umfolozi have special units that are responsible for clearing *Chromolaena* infestations.

1.8. Methods of control

1.8.1. *Mechanical*

Mechanical control has been routinely implemented for the management of *Chromolaena* (Ambika, 1998). Young plants can be easily hand-pulled. Hoes, machetes and bush knives are commonly used to eradicate small stands (Muniappan & Marutani, 1991). Weed control methods include labour intensive hand weeding, digging, uprooting and use of machinery such as brush cutters, mowers, tillers, ploughs and other tractor drawn equipment (Muniappan & Marutani, 1991). In timber plantations, manual slashing is usually performed annually (Erasmus, 1988). In conservation areas, mature stands are first slashed followed by uprooting. However, the use of motorised brush cutters and tractor-drawn mowers can be restricted by the accessibility of the terrain and the prevalence of desirable plants.

In general, slashing and burning measures are taken to curtail the growth of the weed in agricultural land and prevent its spread. Ploughing to a depth of 20 cm or more is known to successfully limit weed infestation and this may account for its absence in mechanical holdings (M'Boob, 1991).

1.8.2. *Chemical*

Most chemical control studies on *Chromolaena* were conducted in the Philippines, Indonesia, India, West and South Africa. These include 2,4-dichlorophenoxyacetic acid (2,4-D), 2-methyl-4-chlorophenoxyacetic acid (MCPA), glyphosate, 2,4,5 trichlorophenoxy-acetic acid (2,4,5-T), trichlopyr, 3,6 dichloropicolinic acid (3,6-D), paraquat and paraquat-based mixtures with diuron, atrazine, terbuthylazine, ioxynil and fluorodifen (M'Boob, 1991). Experiments were mostly conducted in cocoa, coffee and rubber plantations (Table 2).

Table 2. Experiments conducted to measure chemical efficacy of *Chromolaena odorata* (adapted from M'Boob, 1991).

COUNTRY	HABITAT	CHEMICAL	REFERENCE
Philippines	Seedlings	2,4-D	Madrid (1974)
	Mature stands	2,4-D combined with 2,4,5-T, picloram or Dicamba	
		Gramoxone (Paraquat 20%)	Tumaliuan & Halos (1979)
		Tordon 101	Castillo *et al*. (1980)
Indonesia	Pastures	Picloram (1kg/ha)	Risdiono (1975)
		Picloram	Soerjani *et al*. (1975)
		MCPA and 2,4-D	
Java	Rubber plantations	2,4-D, triclopyr or picloram	Tjitrosemito *et al*. (1986)
India	Rubber plantations	Gramoxone (0.5kg/acre)	George (1968)
		Gramoxone + 2,4-D	
		Paraquat and Fennoxone	Rai (1976)
		2,4-D	Matthew *et al*. (1977), Borthakur (1977)
West Africa	Rubber plantations	2,4-D	Martin (1977), Parker (1978), Sheldrick (1968)
	Cocoa plantations	Glyphosate	Delorme (1979), Ivens (1974)
	Coffee plantations	Dowco 290	Parker (1978)
		Tordon 101 and Roundup	Parker (1978)
		2,4-D + 2,4,5-T in ester	Durfour *et al*. (1979)
		2,4,5-T, picloram or 3,6-DCP, glyphosate	Ivens (1974), Delorme (1979), Quencez & Dufour (1982)
South Africa	Grassland, Forest	Triclopyr	Erasmus & van Staden (1986b, 1987)

In South Africa a total of 16 herbicides; 11 for foliar application, two for cut stump application and three for soil application were registered by 1995 (Table 3) (Vermeulen *et al*., 1996).

Table 3. Herbicide treatments registered for the control of *Chromolaena odorata* in South Africa (Vermeulen *et al*., 1996).

TRADE NAME	ACTIVE INGREDIENT	SITE OF APPLICATION	HERBICIDE MIXTURE	REMARKS
Clear Out Enviro Glyphosate 360 Glyphosate 360 Mamba 360 SL Profit 360 Roundup Sunup 360 SL	Glyphosate 359 g/L	Foliage	1 L per 100 L water	Non-selective, broad spectrum weed-killer
Garlon 4	Triclopyr 480 g/L	Foliage	375 mL per 100 L water	Selective broadleaf woody weed-killer
Garlon 4	Triclopyr 480 g/L	Stumps	1 L per 100 L diesel	Selective broadleaf weed-killer
Brush-Off 600 DF Escort 600 DF	Metsulfuron methyl 600 g/kg	Foliage	25 g per 100 L water	Selective broadleaf weed-killer
Touchdown	Sulfosate 720 g/L	Foliage	0.67 L per 100 L water	Non-selective, broad spectrum weed-killer
Chopper	Imazapyr 100 g/L	Stumps	2 L per 100 L water	Do not apply near crops, orchards plantations
Graslan 20P Spike 20P	Tebuthiuron 200 g/kg	Soil	1 g per m^{-2}	Do not apply near crops, orchards, plantations natural forests
Reclaim WP	Tebuthiuron 752 g/kg	Soil	1kg per 10 L water	Do not apply next to or in crops, orchards, plantations, natural forests

The effectiveness of various herbicides sprayed onto actively growing foliage 0.5-1.0 m tall was evaluated by Erasmus & van Staden (1987) and Erasmus (1988). The cost of chemicals

to limit the spread of *Chromolaena* can be reduced by the utilisation of herbicides to shorten regrowth (Erasmus, 1988). Furthermore, herbicide trials revealed that the stage of regrowth after slashing was not critical when using triclopyr, glyphosate and picloram (Erasmus, 1988). The slashed plants exhibited three stages of re-growth i.e. plants up to 0.5 m tall, 0.5-1.0 m tall and plants 1.0-1.5 m tall.

Table 4. Management recommendations for controlling the spread of *Chromolaena odorata* in various habitats (Erasmus, 1988; Goodall, 1992; Goodall, 1995).

HABITAT	HEIGHT OF PLANTS	DENSITY	STRATEGY
Grassland	≤ 1.5 m	Sparse	Complete control is achieved by burning.
		Dense	Foliage, if accessible, is sprayed with Garlon. Inaccessible foliage is cut and knee high coppice sprayed with Garlon. Burning should be considered as an option.
	≥ 1.5 m	Sparse	Complete control achieved by burning.
		Dense	If plants have been burnt/ cut previously and are multi-stem, cut again and spray knee high coppice with Garlon. If there are a few stems apply Chopper freely to all cut surfaces.
Indigenous forest	≤ 1.5 m	Sparse	Loosen and hand pull plants growing in compacted soil. Foliage is sprayed with Garlon or glyphosate.
		Dense	Foliage is sprayed, if accessible, with Garlon or glyphosate
	≥ 1.5 m	Sparse	Chopper is applied to freshly cut surface of all stems on stump or cut and uproot.
		Dense	If plants have been burnt/ cut previously and are multi-stem, cut again and spray knee high coppice with Garlon. If there are a few stems apply Chopper freely to all cut surfaces.
Plantations	≤ 1.5 m	Sparse	Spot spray foliage with Garlon or glyphosate.
		Dense	Spray foliage, if accessible, with Garlon. Inaccessible foliage is cut and knee high coppice sprayed with Garlon or glyphosate.
	≥ 1.5 m	Sparse	Cut and spot spray knee-high coppice with Garlon or glyphosate.
		Dense	Cut and spray knee-high coppice with Garlon or glyphosate.
Gardens	All	All	Slash foliage, loosen soil and uproot.
Public spaces	≤ 1.5 m	Sparse	Spot spray foliage with Garlon.
		Dense	Foliage, if accessible, is sprayed with Garlon. Inaccessible foliage is cut and knee high coppice sprayed with Garlon.

Glyphosate and triclopyr were tested in a subsequent foliar spray trial aimed at reducing herbicide volume without sacrificing efficacy (Erasmus & van Staden, 1987). Triclopyr was found to be better at preventing re-growth, whereas glyphosate was deemed not to be as

effective. However, further tests revealed that glyphosate resulted in 87% reduction compared to 70% reduction in regrowth using trichlopyr (Utulu, 1994). Table 4 indicates management recommendations for controlling the spread of *C. odorata* growing in different habitats (Erasmus, 1988; Goodall, 1992; Goodall, 1995).

In instances where *C. odorata* populations are less than two plants per square meter and with stems at least 15 mm in diameter, application of herbicide to freshly cut stump is most successful (Erasmus & Noel, 1989). It was shown that using imazapyr on treated stumps resulted in more than 80% mortality. The cut stump method of herbicide application thus provides a cost effective alternative to mechanical control. Herbicide can be applied directly on the target and application can take place in windy conditions when foliar application is unsuitable. It is important to maintain the veld with routine burning every second year in order to prevent re-infestation (Erasmus, 1991b).

Application by mist-blower in inaccessible areas is more rapid than by knapsack spraying (Erasmus, 1988). Goodall (1992) found that low concentrations of triclopyr applied by a sprayer mounted onto a tractor was an effective, less labour intensive and quick method of treating stands of 2-3 m high and 5 m wide. However, this type of treatment required two phases since only 80% of the foliage was reached following initial application. Foliar applications are considered risky where the weed grows in close proximity to desirable species (Erasmus & van Staden, 1987).

Besides synthetic herbicides, many plants and microorganisms produce phytotoxins which can be used as herbicides to effectively manage weeds invading sensitive ecosystems or agricultural land (Flamini, 2012). These chemicals are more environmentally friendly and safer to use than synthetic herbicides.

1.8.3. *Biocontrol*

The invasive growth of *C. odorata* may be attributed partly to the absence of natural herbivores which feed on the weed in its country of origin. In Trinidad, for example, it is a common plant but not considered an invader and control by co-evolved herbivores is probably a reason for this (Cruttwell, 1968).

Biocontrol of exotic invaders is viewed as an alternative option for large undeveloped areas such as parks, reserves, regional forests and vast grassland biomes (Centre *et al.*, 1995). However, the use of biocontrol agents is controversial amid environmental risks associated with deliberate introductions of non-indigenous species (Louda *et al.*, 1997). Biological control of *Chromolaena odorata* has spanned over 45 years; the emphasis has largely centred on the use of arthropod agents (McFadyen, 1991). Less attention has been paid to using fungal pathogens as a control measure (Ooi *et al*., 1991). The exotic characteristic of the weed, together with its taxonomic isolation from crop plants, makes the species a suitable target for biocontrol using fungal pathogens (Evans & Ellison, 1990).

Except for a few species, relatively little information on the potential use of phytophagous insects as biological agents, is known (Cock, 1984). The use of biological agents to control the spread of *C. odorata* in countries where it has been declared a weed has not been a success, despite a wealth of information on insects recorded in its country of origin (McFadyen, 1988). *Chromolaena odorata* serves as a host for approximately 240 arthropods in Trinidad and Central and South America (Cruttwell, 1974).

The first biological control insect was *Pareuchaetes pseudoinsulata* (Bennet & Rao, 1968; Cruttwell, 1968). The establishment of this agent was not successful in many countries (Cock & Halloway, 1982) except in Guam and three other islands in the Marianas (Seibert, 1989). Failure to establish in West Africa and India was attributed to a prolonged dry winter whereas the insect was adapted to a less severe season in its native Trinidad (Cock & Halloway, 1982; Cock, 1984). However, the successful establishment of this species in Sri Lanka was due to the climate being similar to that of Trinidad. It was recommended that *P. pseudoinsulata* and other species be harvested from climatic zones matching those of proposed target zones in order to be effective in controlling *Chromolaena* (Cock & Halloway, 1982). The importance of an integrated attack by arthropods and fungi to reduce weed vigour became abundantly clear after the accidental introduction of *Cercospora eupatorii* into Australia (CAB International Institute of Biological Control, 1998; Emery & Doran, 2013). Integrated control is therefore more effective in reducing weed vigour than a single biological agent. The full range of oligophagous species recommended by Cock (1984) is shown in Table 5. No information exists on the occurrence of bacterial, viral or nematode pathogens on *C. odorata*. Pathogenic fungi that occur on *C. odorata* (Evans, 1987) are summarised in Table 6.

Table 5. Oligophagous arthropods with potential for control of *Chromolaena odorata* (Cock, 1984).

ORDER	FAMILY	SPECIES
Acari	Eriophyidae	*Acalitus adoratus* Keifer
Coleoptera	Curculionidae	*Apion brunneonigrum* B.B. *Rhodobaenus* spp.
	Cemmbycidae	*Aerenica hirticornis* Klug.
	Chrysomelidae	*Pentispa explanata* (Chap.) *Aulocochlamys* sp. *Chlamisus insularis* (Jac.)
Diptera	Cecidomyfidae	*Neolasiopteraf rugivora* Gagne *Asphondylia corbulae* Mohn *Contarinia* sp. *Perasphondylia reticulate* Mohn *Clinodiplosis* sp.
Lepidoptera	Tephritidae	*Cecidochares fluminensis* (Lima)
	Agromyzidae	*Melanagromyza eupatoriellae* Spencer
	Arctiidae	*Pareuchaetes pseudoinsulata* Rego Barros
	Pymlidae	*Mescinia parvula* (Zeller)
	Nymphalidae	*Actinote anteas* Doubleday
	Riodinidae	*Calephelis laverna* Godman & Saivin

Table 6. Pathogenic fungi occurring on *Chromolaena odorata* (Evans, 1987).

DIVISION	FAMILY	SPECIES
Ascomycotina	Dothidiales	*Guignardia eupatorii* Punithalingarn
Basidiomycotina	Uredinales	*Cionothrix praelonga* (Wint.) Arthur
Deuteromycotina	Hyphomycetes	*Cercospora eupatorii* Peck
		C. eupatoriicola Govindu & Thirumalachar
		C. eupatorii-odoratii Yan
		Pseudocercospora eupatorii-formosani (Sawada) Yen
		Phomopsis eupatoriicola Petrak
		Phyllosticia eupatoriicola Kab. & Bub.

Although the majority of attempts at classical biological control of weeds concentrated on arthropods, fungal pathogens have been successfully used to control invader plants in many countries including South Africa, Australia and the Americas (Day *et al*., 2013). *Entyloma ageratinae* Barreto & Evans has been used to control *Ageratina riparia* (Regel) R. M. King & H. Rob. in Hawaii, whilst *A. adenophora* (Sprengel) R. King & H. Robinson has been partially controlled in Hawaii and Australia by *Procecidochares utilis* Stone and *Cercospora eupatorii* Peck (Dodd, 1961).

In some cases, successful biological control has been achieved by the introduction of a single control agent (Myers, 1986) however it is more likely to succeed by using a range of natural herbivores (Harris, 1986). In the past, the chosen agent has often been favoured because it was easier to manipulate and breed rather than being known to be more effective (Myers, 1986). A vast range of natural herbivores are known and greater attention should be given to the utilisation of a truly representative agent for long term biological control of *C. odorata* (Harris, 1986). Mass rearing of the Arundo wasp, *Tetramesa romana* Walker was recently shown to limit growth of *Arundo donax* L., an invasive perennial grass (Moran *et al*., 2014). These researchers concluded that mass rearing of biological agents could also be applied to other weed species, including *C. odorata*.

1.8.4. *Cultural*

Re-sprouting plants have the ability to exclude and suppress weeds in shifting agricultural systems (Delvaux, 1985; Aweto, 1981; Zinke *et al*., 1978). *Chromolaena odorata* is controlled in Nigeria by weeding and by using naturally occurring plant communities which shade the weed (Aweto, 1981). *Tephrosia purpurea* (Linn.) Pers., planted as a cover crop in coconut plantations in Sri Lanka, prevented the establishment of *C. odorata* (Salgado, 1972). Rai (1976) recommended *Pueraria phaseolides* (Roxb.) Benth. as a cover crop in rubber plantations in India. Planting of *Leucaena leucocephala* (Lam.) de Wit in pastures in the Philippines reduced *C. odorata* populations. Planting of *Panicum maximum* Jaqu., *Chloris guayana* Kunth, *Cynodon dactylon* (L.) Oers (K11) and *Setaria megaphylla* Stapf ex Stapf and C.E. Hubb suppressed the growth of *C. odorata* seedlings (Erasmus & van Staden, 1986a) after initial mechanical control. Signal grass (*Brachiaria decumbens* Stapf.) successfully competes and reduces the incidence of *C. odorata* in pastures in China (Wu & Xu, 1991).

Legumes such as *Calopogonium mucunodes* Desv., *Centrosema pubescens* Benth. and the grass *Setaria* species, when cultivated in burnt fields, are effective in preventing regrowth of *C. odorata* (Torres & Paller, 1989). Muniappan & Marutani (1988) suggested that mulching around the bases of trees effectively suppresses *C. odorata* in plantation crops. However, the high labour cost and availability of mulching materials may be problematic.

It has been suggested that *Chromolaena* is fire sensitive rather than being highly flammable (Goodall & Erasmus, 1996). These researchers suggest that the reluctance of farmers to use fire in the grasslands along the east coast of South Africa was promoting the establishment of the weed. Similar trends were suggested to be occurring in the savannas of Central Africa (Gautier, 1992). *Chromolaena odorata* plants that were slashed at any period of the year and then left to dry became highly flammable (Moni & Subramoniam, 1960). Fire could, therefore, be used as a cost effective control strategy in eradicating *Chromolaena* infestations in grasslands.

1.8.5. *Integrated*

Integrated control involves a combination of different methods which are most effective, economical and acceptable, followed by managerial practices aimed at minimising re-infestation. Combining different control methods are usually more effective than applying one method. *Chromolaena odorata* is susceptible to damage at different times during its life cycle. Whilst physical removal or slashing may not be the most effective method during the dry winter months, the application of herbicides is considered more effective at the beginning of summer when plants are actively growing (Liggit, 1983).

Fire has been used to suppress *Chromolaena* seedlings (Erasmus, 1988; 1991a). It was shown that over-sowing with grass after burning reduced re-infestation significantly as a result of achene and seedling mortality. However, minimal weed re-establishment occurred in unburnt plots where grass colonisation was successful. Therefore, it is likely that veld re-infestations can be managed by the incorporation of a suitable fire programme which excludes grazing in order to hasten grass recovery. The use of naturally occurring plant species should be used to rehabilitate weed dominated communities (Erasmus, 1988). Further research is needed to understand the role of fire in the establishment of this weed in various ecosystems. Research

should focus on the impact of fire, the process of weed establishment, vegetative change and the time scale of the invasive process in savannas and forests (Erasmus, 1988). This may lead to the development of a more practical integrated approach to weed control.

Although biological control methods may help to reduce the vigour and competitiveness of *C. odorata*, a combination of physical and chemical methods remain more effective in the clearing of infestations (Liggit, 1983).

1.9. Economic importance

1.9.1. *Effects on flora*

Chromolaena affects coconut plantations in Sri Lanka (Muniappan & Marutani, 1988). It reduces food and commercial crop yield by competing for light, moisture and nutrients and, to some extent, by allelopathic effects (Table 7) (M'Boob, 1991). One of the most important effects of the weed on production is the high cost of labour required for initial clearing and subsequent frequent post-planting weeding (Muniappan & Marutani, 1988).

Table 7. Major crops in which *Chromolaena odorata* is a problem (Holm *et al*., 1977).

COUNTRY	CROP
Cameroon	Oil palm, rubber, cocoa
Cote d'Ivoire	Oil palm, rubber, cocoa
Ghana	Oil palm, rubber, cocoa
Guam	Unspecified
India	Rubber, teak, tea, citrus, vegetables
Myanmar	Unspecified
Indonesia	Rubber, rice
Malaysia	Rubber, abaca, oil palm, tobacco
Nigeria	Oil palm, rubber, cocoa, rice
Philippines	Coconuts
Sri Lanka	Coconuts, rubber, pineapples
Thailand	Rubber, cotton, maize
Trinidad	Coconuts, sugar cane

In the sugarcane fields extending along the KwaZulu-Natal coast, triffid weed infestations harbour wild pigs which results in crop losses (Erasmus, 1988).

Many commercial fields and housing developments have been destroyed by bush fires which have been facilitated by dry and dense *C. odorata* thickets (Hoevers & M'Boob, 1994). In South Africa, the replacement of previously fire-excluding fringes of riverine vegetation with *Chromolaena* has made indigenous forests susceptible to fire (MacDonald, 1984). The occurrence of this invader in timber plantations and undeveloped land in urban municipalities can fuel fires (Liggit, 1983).

The invasion of open fields and pastures by *C. odorata* frequently forms dense stands that, through competition, exclude almost all forage species (Liggit, 1983). *Chromolaena* has a rapid growth rate and can rapidly spread (Ambika, 1998). After clearing of forests, the weed may impede the process of succession by overgrowing and shading equally heliophilic forest trees (Jandova *et al*., 2014). Due to its rapid vegetative growth, it may smother the development of young trees, thus contributing to forest degeneration (de Foresta, 1994). The competitive ability and possibly its production of phytotoxins lead to reduced crop cultivation. The leaves possess a large amount of allelochemicals (Ambika & Jayachandra, 1990) which impede the growth of commercial and domestic crops (Gill *et al*., 1994; Abdul-Wahab & Rice, 1967; Neil & Rice, 1971; El-Kenany & El-Darier, 2013). A crude alcohol extract of *C. odorata* was shown to reduce seed germination and seedling growth of tomato (Tijani-Eniola & Fawusi, 1989). Farmers in the forest region of Chailla in the Congo avoid growing cassava in areas where *C. odorata* is abundant because the roots of the crop are more susceptible to rotting (Gill *et al*., 1994).

Chromolaena odorata has significant beneficial effects in some agro-ecosystems due to its rapid growth and high biomass potential. The weed may add to the organic matter in the soil through copious amounts of leaf litter (M'Boob, 1991). *Chromolaena odorata* seems to play an important role in the development of traditional bush fallow systems in West and Central Africa (Remington & Sahrawat, 1992; Tondoh *et al*., 2013). In Ghana, litter fall of the weed helps to improve soil conditions, especially in cassava and maize cultivation (Timbilla & Braimah, 1991). Rice farmers in Cote d' Ivoire have developed management techniques which allow them to derive benefits such as improved soil fertility and reduced weeding

frequency (Remington & Sahrawat, 1992). Mulching is prevalent in countries in Africa where resource poor farmers use the weed to ensure greater crop yields (Tondoh *et al*., 2013; El-Keblawy & Abdelfatah, 2014). In Nigeria, mulching with dry *C. odorata* seedlings decreases soil bulk density and increases soil moisture retention leading to a better yield of yams (Opara-Nadi & Lal, 1987). Furthermore, using *C. odorata* shoots as mulch stimulates the growth and yield of cucumber plants (Eussen & Slamet, 1973).

Chromolaena odorata may stimulate regeneration in heavily disturbed forests that are grass dominated (Hoevers & M'Boob, 1994). This is due to improvement in the organic matter composition of the soil. *Chromolaena odorata* may enhance secondary succession of disturbed sites when combined with other tree species (Hoevers & M'Boob, 1994). In Sierra Leone, the weed is used for soil erosion control on account of its vigour and rapid lateral spread (M'Boob, 1991).

1.9.2. *Effects on fauna*

Chromolaena is not eaten by livestock because the leaves are poisonous, possibly due to the high concentration of nitrates (Sajise *et al*., 1974). The species is believed to cause diarrhea and death of livestock in the southern Philippines (Aterrado & Talatala-Sanico, 1988). Furthermore, it has been reported that families have died in Congo after eating the leaves of *Solanum aethiopicum* L. contaminated with *C. odorata* (Bani & Le Gall, 1994). Cattle avoid pastures invaded by *C. odorata* and this subsequently leads to overgrazing of non-infested lands. This may be inconvenient for nomadic and semi-nomadic livestock systems where free grazing is practiced around settlements during the rainy season (Hoevers & M'Boob, 1994).

The weed is reported to harbour insect pests e.g. *Zonocerus variegatus*, a grasshopper, which reproduces freely under the dense canopy where they are protected from predators (Bani, 1990). The grasshoppers oviposit in the shade of *C. odorata* thickets (Chapman *et al*., 1986; Chiffaud & Mistre, 1990). The weed is therefore directly responsible for grasshopper outbreaks (Chapman *et al*., 1986; Popov, 1988). In Ghana and Nigeria, grasshoppers feed in adjacent cassava farms, thereby reducing crop yield (M'Boob, 1991).

Chromolaena odorata prevents the nesting of crocodiles at traditional nesting sites in Lake St. Lucia, a world heritage site (Leslie, 2000). The shading effects of the weed reduce the

incubation temperature of eggs, thus altering the sex ratio of the developing hatchlings (Leslie, 2000). Since *C. odorata* is reported to be extending its climatic limits from South Africa into neighbouring countries, other regions with Nile crocodiles may experience similar problems.

1.9.3. *Medicinal properties*

Decoctions of leaves from *C. odorata* are used as a cure for various ailments (Inta *et al*., 2013). Leaf extracts are used to cure skin diseases and to treat indigestion (Thang *et al*., 2001; Asase & Kadera, 2013). In northern Africa, liquid extracts are used to clot blood, abort foetuses during the early stages of pregnancy and to treat abdominal pains (Timbilla & Braimah, 1994). Extracts can also be used as a disinfectant for old wounds and boils (Iwu *et al*., 1999) and to treat patients with malaria and jaundice (Vital & Rivera, 2009). In rural African villages, extracts from the weed have been used to embalm bodies (Timbilla & Braimah, 1994). In Vietnam, weed extracts are used as teeth cleaning agents and as a cure for certain eye problems (Thang *et al*., 2001). In India, the weed is used for the treatment of diabetes (Onkaramurthy *et al*., 2013). Studies have also shown that fatty acids isolated from the weed possess anti-inflammation properties (Hahn *et al*., 2011). The leaves also have insect repelling properties (Hoevers & M'Boob, 1994; Nong *et al*., 2013a; Nong *et al*., 2013b). *Chromolaena odorata* can reduce the incidence of nematodes when used as a fallow crop (M'Boob, 1991).

1.10. Physiological studies

Physiological studies on *C. odorata* are limited and very little information is available on future spread and the environmental limits that control such encroachment. Gas exchange measurements under natural and simulated conditions may provide increased understanding of distributional patterns and explain weed invasion success. Although limited success has been achieved using chemical, mechanical and biological control, the development of an effective weed strategy may benefit from physiological studies. Furthermore, understanding the status and management of this noxious weed may help to identify and control further species that may pose a similar risk. Research that focuses on gas exchange studies is essential to understand the physiological mechanisms that promotes invasion by *C. odorata*.

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2. Gas exchange characteristics

2.1. Abstract

Gas exchange and chlorophyll *a* fluorescence characteristics were investigated in the introduced weed *Chromolaena odorata* under field conditions. Leaf measurements were taken from 06h00 to 16h00 at hourly intervals on the youngest fully expanded leaves from five plants over four days in November (summer). Trends in CO_2 uptake paralleled incident photosynthetic photon flux density (PPFD), the relationship being almost linear. Maximum CO_2 uptake (16-18 µmol $m^{-2}s^{-1}$) occurred at a PPFD of 1800- 2100 µmol $m^{-2}s^{-1}$. Trends in leaf conductance and transpiration were similar to those for CO_2 assimilation. Leaf water potential (Ψ) decreased from -0.35 MPa at dawn to a minimum value of -1.0 MPa at midday and recovered to -0.6 MPa at 16h00. Trends in water use efficiency (WUE) were similar to those of leaf Ψ. Quantum yield of PSII decreased from 0.74 at dawn to a minimum of 0.45 at midday followed by a recovery to 0.7 at 16h00. Maximal ETR through PSII (> 200 µmol m^{-2} s^{-1}) occurred at PPFD >2000 µmol $m^{-2}s^{-1}$. ETR followed a trend similar to those for incident PPFD, CO_2 uptake, leaf conductance and transpiration. Intrinsic photochemical efficiency of PSII (F_v/F_m) values were between 0.82-0.83 at dawn, then decreased to 0.77 at midday, but recovered to their predawn values at 16h00. Trends in quantum yield and ETR through PSII, CO_2 uptake, leaf conductance and transpiration were tightly coupled to those of incident PPFD. The ability of *C. odorata* to acclimatise to high incident PPFD may be an adaptive strategy to maximise carbon gain and minimise water loss.

2.2. Introduction

Despite increased interest in the spread and control of *C. odorata*, little is known of its photosynthetic characteristics under field conditions. Since its introduction to South Africa in the 1940's, the weed has spread rapidly along the entire KwaZulu-Natal coast and became a troublesome alien invader. Alien invader species are widely acknowledged by the International Union for the Conservation of Nature (IUCN) to be among the biggest threats to the loss of biological diversity. The invader is capable of producing vast quantities of seed which germinate readily. The weed reduces crop yields substantially and threatens

biodiversity in sensitive ecosystems. *Chromolaena* poses a serious threat to conservation, forestation, agricultural crops and other land uses. Although current practice emphasises specific procedures for controlling the weed, less information is available on effective strategies for curtailing its spread throughout the country.

Studies on leaf gas exchange are important as they provide an instantaneous measure of the net photosynthetic rates and water status of leaves (Blaikie & Chacko, 1998). Furthermore, chlorophyll *a* fluorescence provides information of photosynthetic electron transport processes that are not revealed by the more traditional methods of measuring gas exchange. Moreover, it is a sensitive indicator of damage to photosystem reaction centres caused by environmental stressors (Schreiber *et al*., 1995). Dynamic models that describe plant growth based on evidence obtained from ecophysiological studies can predict plant responses to a changing environment. This is important because plant productivity studies form the basis for understanding the function of ecosystems and formulating appropriate management plans (Dias-Filho *et al*., 1995).

Studies have shown that invaders often differ from indigenous species in ways that potentially contribute to their success within introduced habitats (Yamashita *et al*., 2000; Horton *et al*., 2001). Assimilating and utilising resources, i.e. plant efficiency, has implications for growth, survival and reproductive capacity (Lambers & Poorter, 1992). Plants with high net carbon assimilation have the potential to grow faster than species with low rates (Ewe & Sternberg, 2003). Leaf gas exchange of exotic invaders is often higher than those of native species (Pattison *et al*., 1998; Barusch & Goldstein, 1999; Durand & Goldstein, 2001). Although *Chromolaena* exhibits traits such as high growth rates and large seed outputs (Goodall & Erasmus, 1996), it may also be possible that the ecophysiology, in particular, its photosynthetic characteristics, contribute to its present distribution. Examining the eco-physiological characteristics of *Chromolaena* may be used to model future trends in distribution and abundance of this species.

The aim of this study was to determine photosynthetic performance of *C. odorata* by monitoring diurnal changes in gas exchange, chlorophyll *a* fluorescence and leaf water relations under field conditions.

2.3. Materials and methods

2.3.1. *Study Area*

The study was undertaken on the University of KwaZulu-Natal, Westville campus (29°49'S 30°56'E). Uniform, healthy, herbaceous shrubs of *C. odorata* of approximately 1 m height, occurring in a dense stand, were selected for the study. *In situ* measurements of gas exchange, chlorophyll fluorescence and water potential were taken in the field under naturally varying microclimatic conditions during November 2002 (summer).

2.3.2. *Gas exchange*

Five healthy plants were selected from the stand. All plants were fully exposed to sunlight. Gas exchange measurements were taken at hourly intervals on the abaxial surface of the most recently expanded mature leaves (n=5). Measurements commenced at 06h00 and terminated at 16h00. Leaves of five separate plants were monitored on each day and measurements were taken with a portable infrared gas analyser (IRGA) (Li-6400, Li-Cor, Nebraska, USA). Individual leaves were supported and held in position in the assimilation chamber with the aid of an extendable ultra fluid action tripod (Maxtec- model 1493). Photosynthetic photon flux density and leaf temperature were measured with a silicon photodiode and thermocouple respectively, both housed within the 0.25 L cuvette of the IRGA. Gas exchange measurements were taken under natural conditions of air temperature, incident PPFD, CO_2 concentration and vapour pressure deficit (VPD). Under natural conditions, air temperature and VPD values within the cuvette were maintained close to those of the ambient air. The leaf chamber was held at right angles to incident radiation to prevent shading inside the curvette.

2.3.3. *Chlorophyll fluorescence*

Chlorophyll fluorescence measurements were taken with a portable pulse amplitude modulation fluorometer (PAM- 2000, Walz, Effeltrich, Germany) following the procedure outlined by Rossa *et al*. (1998). Measuring and saturating light pulses were applied through fibre optics aligned at an angle of 60° to the leaf. Operation of the fluorometer and data capture was conducted with a palmtop computer (HP 200 LX). Fluorescence measurements

were made simultaneously with those of gas exchange on a part of the same leaf adjacent to the assimilation chamber. Potential quantum yield of PSII was measured after 15 min dark adaptation using a dark leaf clip with a shutter (H. Walz GmbH, Effeltrich, Germany). This period ensured relaxation of all fast components of non-photochemical quenching, q_n (Keiller *et al*., 1994). Photosystem II quantum yield ($F_m - F/F_m$) of energy conversion was calculated according to Genty *et al*. (1989) where F is the light adapted fluorescence and F_m the maximum light adapted fluorescence when a saturating light pulse of 7500 µmol $m^{-2}s^{-1}$ PPFD for 700 ms duration is superimposed on the prevailing environmental irradiance level (Schreiber & Bilger, 1993). Electron transport rates through PSII were determined according to Krause and Winter (1996). Since the emitter detector unit of the PAM 2000 shows temperature dependence (Herppich *et al*., 1994), all fluorescence parameters were corrected for this variable.

2.3.4. *Water use efficiency*

Water use efficiency was calculated by the ratio of CO_2 uptake (*A*) and transpiration (*E*) (WUE =*A*/*E*).

2.3.5. *Leaf water potential*

Leaf water potential (Ψ) was measured in duplicate at hourly intervals from 06h00 to 16h00 with a Scholander pressure chamber (Soil Moisture Equipment Corp., Santa Barbara, USA). Measurements were made on plants adjacent to those used for gas exchange. Selected twigs for measurements were approximately of the same diameter and fitted snugly into the rubber stopper. Water loss of twigs after excision was kept to a minimum by covering the healthy twigs with aluminium foil. In addition, water loss in the pressure chamber itself was reduced by lining the chamber with moistened filter paper. Each measurement represented the average data of two separate plants.

2.3.6. *Statistics*

Pearson's correlation coefficient (Microsoft Excel 2010) was used to analyse the relationship between variables. Quantitative values are presented as mean ± standard error of the mean (SE). Graphs were drawn with lines of best fit using quadratic equations and regression coefficients indicated by R^2 values. Statistical significance was at $P \leq 0.05$.

2.4. Results

Figures 1 and 2 illustrate diurnal trends in PPFD, CO_2 uptake, leaf conductance, transpiration and ambient temperature on the four measurement days. Maximum PPFD ranged from 1500 to 2000 µmol $m^{-2}s^{-1}$ between 10h00 and 12h00. Measurements were terminated on each day, at 16h00, due to low PPFD. Trends in CO_2 uptake were similar to those for incident PPFD. Maximum CO_2 uptake values ranged from 12 -18 µmol $m^{-2}s^{-1}$ (Fig. 1B) over all days. Leaf conductance (Fig. 1C) and transpiration (Fig. 2A) followed trends similar to CO_2 uptake. Maximum leaf conductance and transpiration values ranged from 0.15 to 0.2 mol $m^{-2}s^{-1}$ and from 4.2 to 5.2 mmol $m^{-2}s^{-1}$, respectively, between 10h00 and 11h00 (Fig. 1C & 2A).

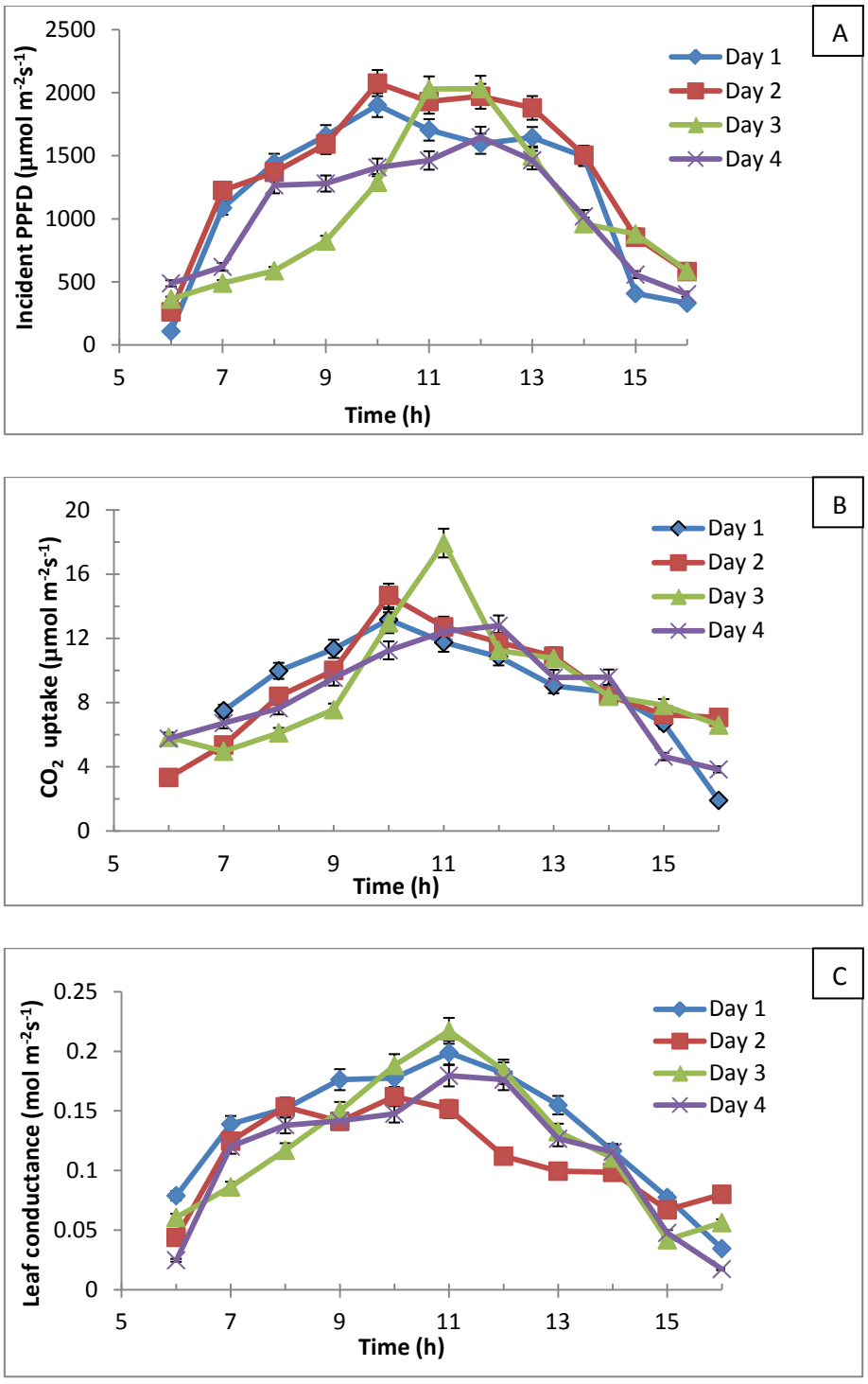

Fig. 1. Diurnal changes of incident photosynthetic photon flux density (A), CO_2 uptake (B) and leaf conductance (C) in healthy single stem of *Chromolaena odorata* plants on four randomly selected days. Each point is the mean of five independent readings. Vertical bars represent standard error. Bars not visible indicate standard error smaller than the symbol.

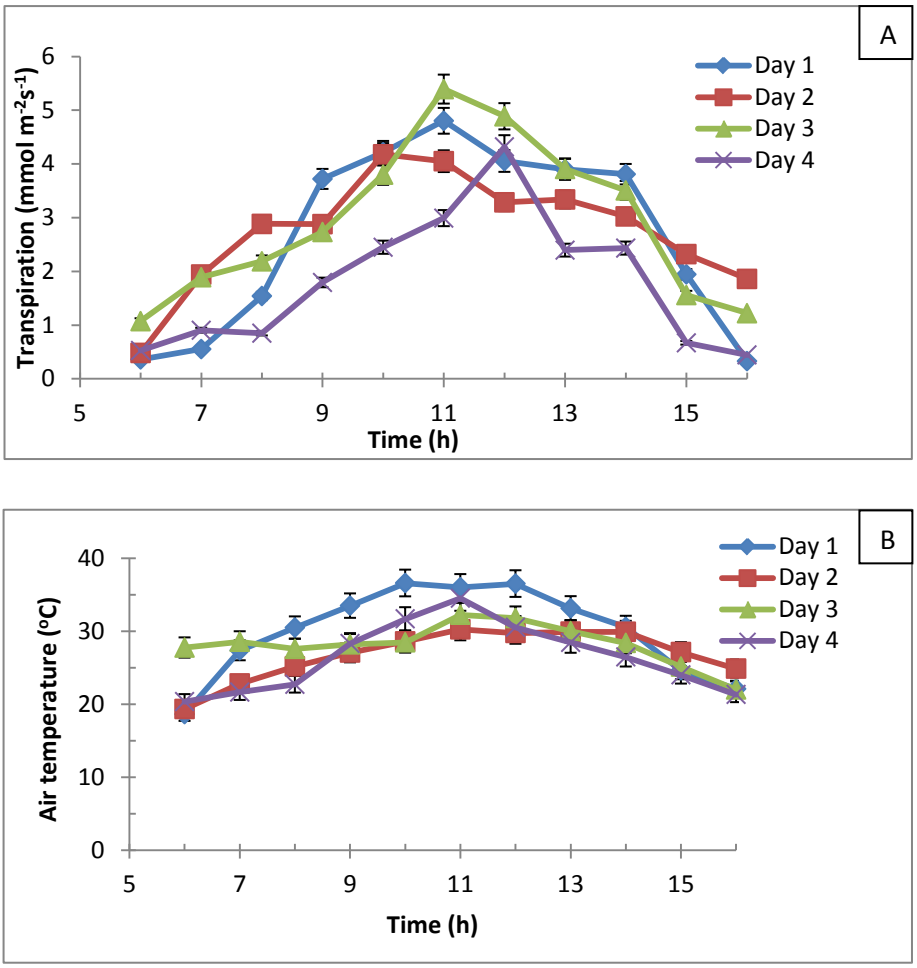

Fig. 2. Diurnal changes in transpiration (A) and ambient temperature (B) in healthy single stem of *Chromolaena odorata* plants on four randomly selected days. Each point is the mean of five independent readings. Vertical bars represent standard error. Bars not visible indicate standard error smaller than the symbol.

The relationship between CO_2 uptake and incident PPFD was almost linear with maximum CO_2 uptake values (12-14 µmol $m^{-2}s^{-1}$) occurring above 2000 µmol $m^{-2}s^{-1}$ PPFD (Fig. 3A). There was no saturation of CO_2 uptake above 2000 µmol $m^{-2}s^{-1}$ PPFD. Maximum CO_2 assimilation occurred at leaf conductance and transpiration values above 0.2 mol $m^{-2}s^{-1}$ (Fig. 3B) and 5 mmol $m^{-2}s^{-1}$ (Fig. 3C) respectively.

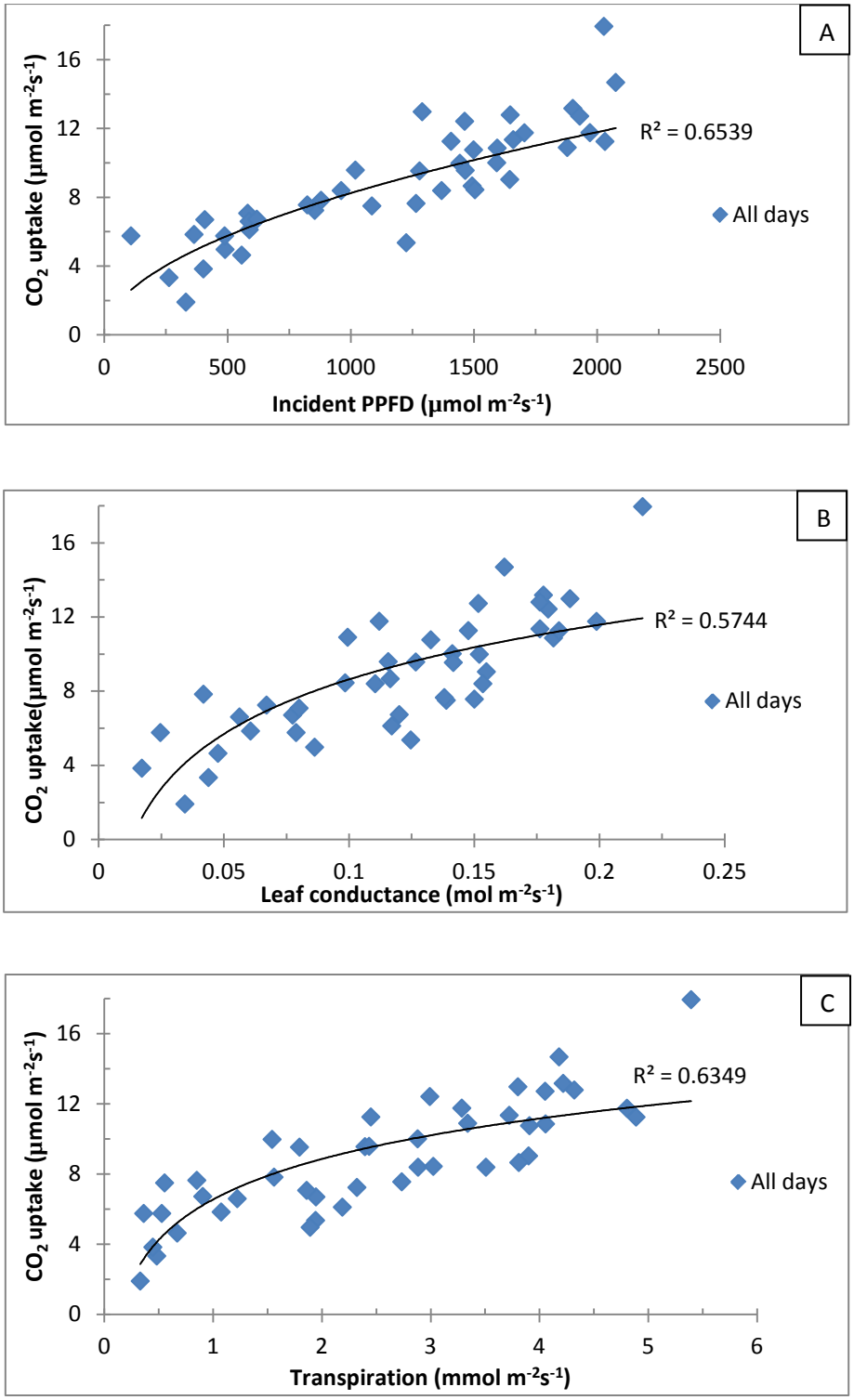

Fig. 3. Relationship between CO_2 uptake and incident photosynthetic photon flux density (A), CO_2 uptake and leaf conductance (B) and CO_2 uptake and transpiration (C) in healthy single stem of *Chromolaena odorata* plants. Each point is the mean of five independent readings. The regression lines are indicated by R^2 values.

Leaf water potential decreased from -0.35 MPa at 06h00 to -1.0 MPa between 12h00 and 13h00 but recovered to between -0.5 to -0.8 MPa at 16h00 (Fig. 4). The decrease in water potential from 06h00 to 12h00 coincided with the increase in incident PPFD.

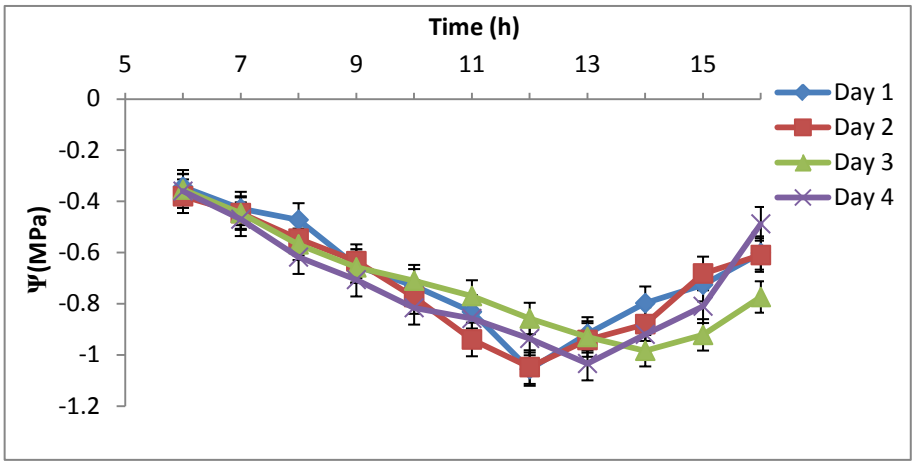

Fig. 4. Diurnal trends of leaf water potential in healthy single stem of *Chromolaena odorata* plants on four randomly selected days. Each point is the mean of two independent readings. The vertical bars represent standard error. Bars not visible indicate standard error smaller than the symbol.

WUE values decreased from 06h00 to midday followed by a gradual recovery to 16h00 (Fig. 5). The recovery in WUE, after 12h00, coincided with an increase in leaf water potential.

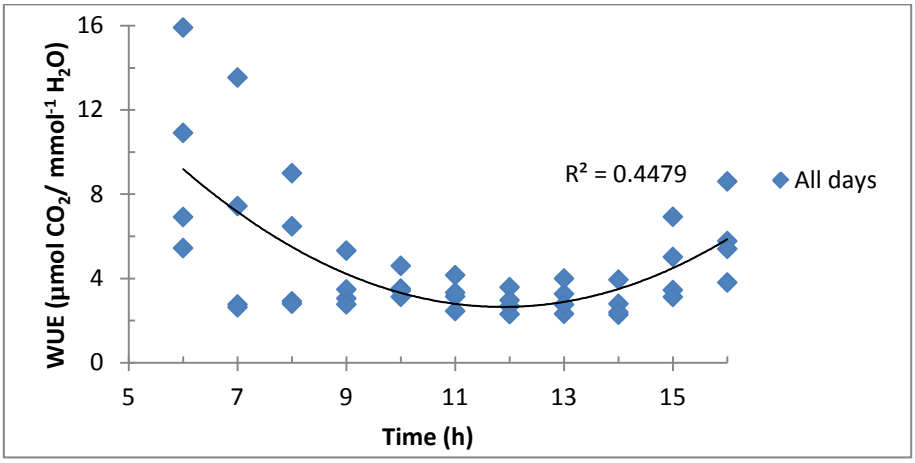

Fig. 5. Diurnal trend of water use efficiency in healthy single stem of *Chromolaena odorata* plants. Each point is the mean of five independent readings. The regression line is indicated by R^2 value.

The quantum yield of PSII generally decreased from 0.72 at 06h00 to a minimum (0.45 -0.55) at 12h00 followed by an increase (0.5-0.65) at 16h00 (Fig. 6A). Decreases in quantum yield and F_v/F_m from dawn to midday coincided with the increase in incident PPFD (Fig. 7A, C). Trends in quantum yield and ETR through PSII, CO_2 uptake, leaf conductance and transpiration were tightly coupled to those of incident PPFD. Intrinsic photochemical efficiency of PSII values decreased from 0.82 at 06h00 to approximately 0.77 at midday (Fig. 6C) followed by a recovery to their predawn values at 16h00. The decrease in Ψ (below -0.82 MPa) was associated with a decrease in leaf conductance (Fig. 8B).

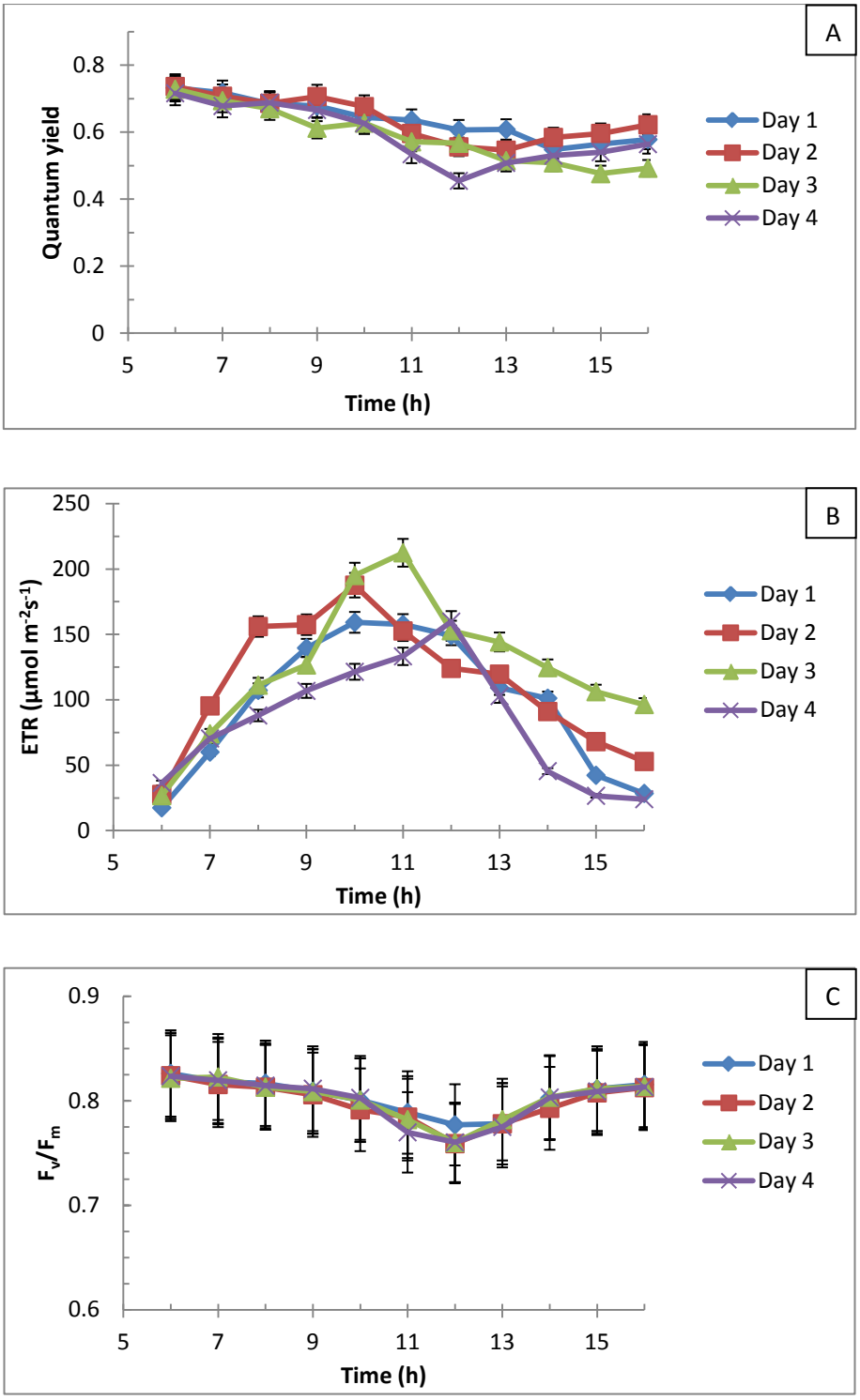

Fig. 6. Diurnal changes of quantum yield (A), electron transport rate through PSII (B) and the potential quantum yield of PSII photochemistry in the dark adapted state (F_v/F_m) (C) in healthy single stem of *Chromolaena odorata* plants. Each point is the mean of five independent readings. Vertical bars represent standard error. Bars not visible indicate standard error smaller than the symbol.

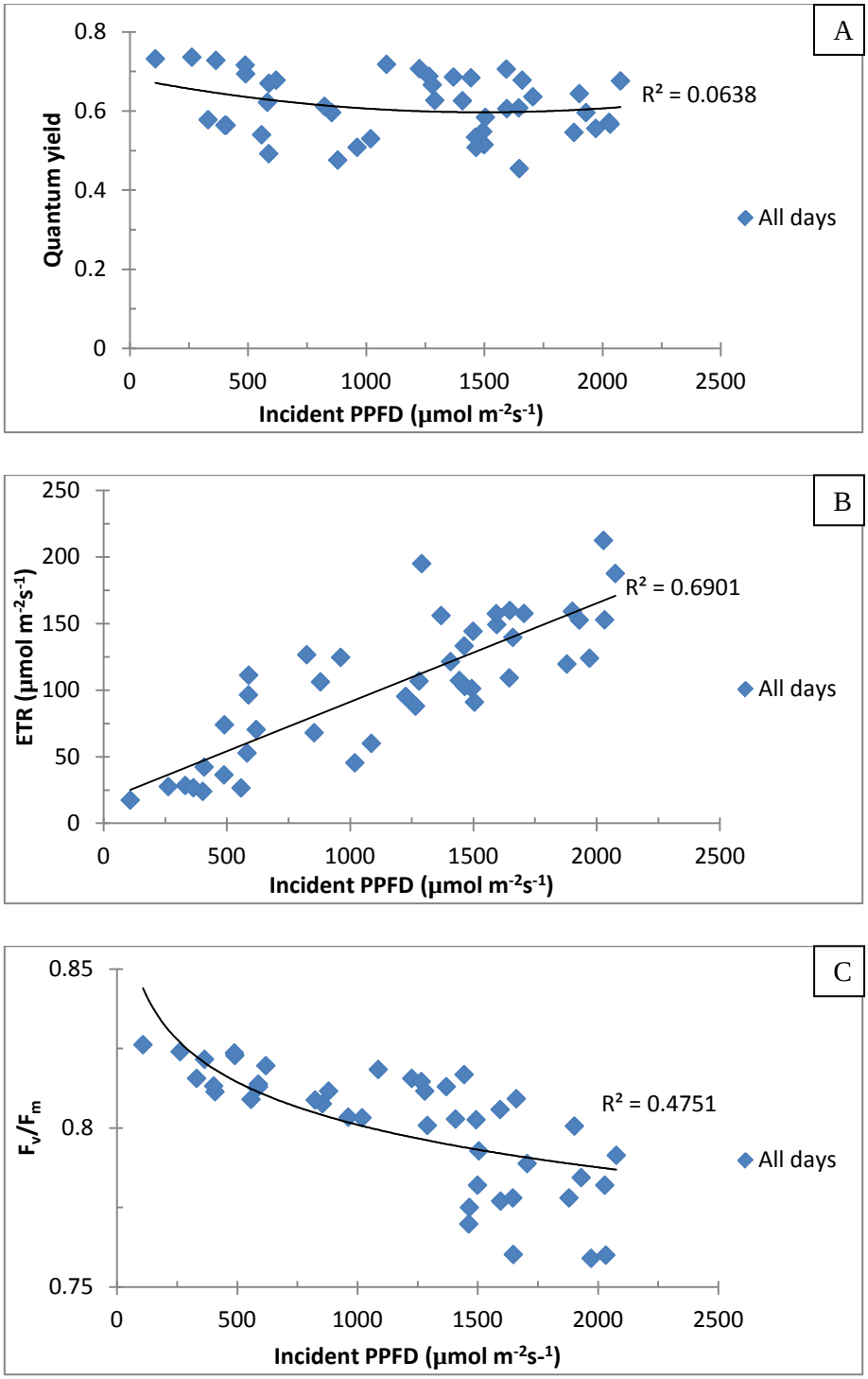

Fig. 7. Relationship between quantum yield and incident photosynthetic photon flux density (A), electron transport rate and incident photosynthetic photon flux density (B) and the potential quantum yield of PSII photochemistry in the dark adapted state (F_v/F_m) and incident photosynthetic photon flux density (C) in healthy single stem of *Chromolaena odorata* plants. Each point is the mean of five independent readings. The regression lines are indicated by R^2 values.

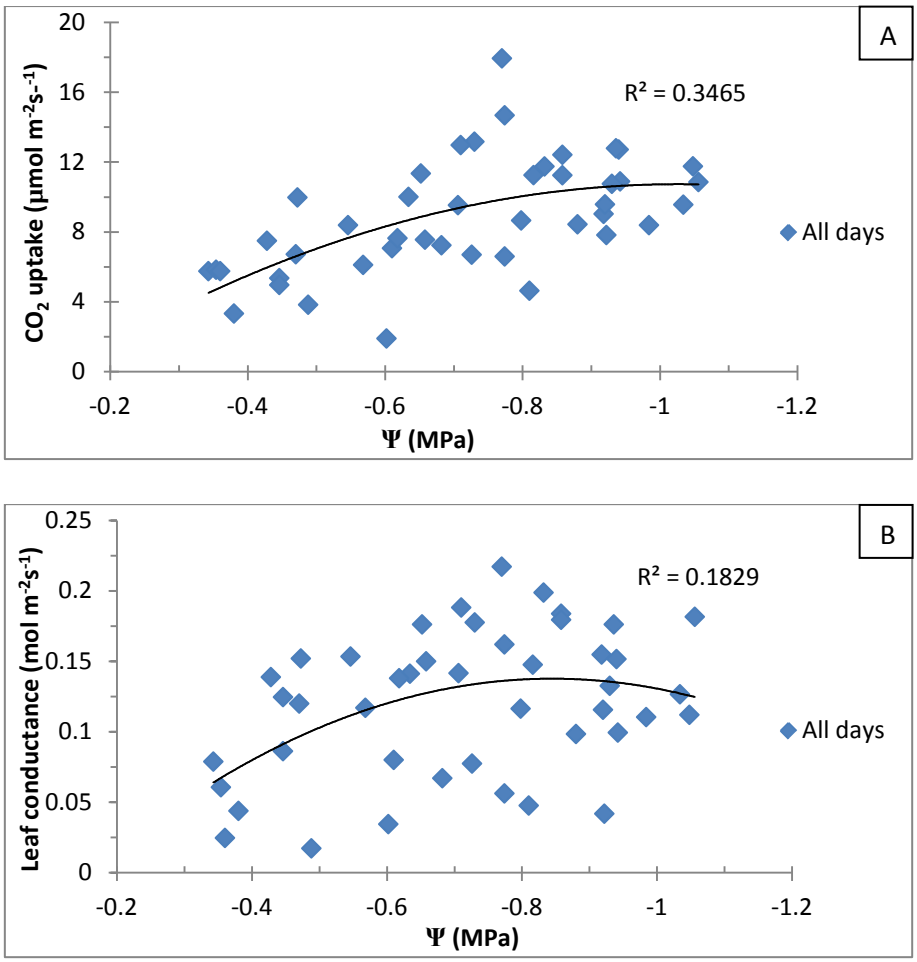

Fig. 8. Relationship between CO_2 uptake and leaf water potential (A) and leaf conductance and leaf water potential (B) in healthy single stem of *Chromolaena odorata* plants. Each point is the mean of five (CO_2 uptake and leaf conductance) and two (leaf water potential) independent readings. The regression lines are indicated by R^2 values.

Pearson's coefficient of determination for various variables are presented in Table 1 for all days. Values that show significant correlation are indicated in bold. Significant correlations existed between ETR and CO_2 uptake, leaf conductance and transpiration, transpiration and ETR and F_v/F_m and Ψ.

Table 1. Pearson's correlation coefficients for various variables (values in bold are significant at $P \leq 0.05$).

	A	**PPFD**	***g***	***E***	***Ci***	**Temp.**	**Yield**	**ETR**	**F_v/F_m**	**Ψ**
A	1									
PPFD	**0.682**	1								
g	0.494	0.409	1							
E	0.618	0.576	**0.808**	1						
Ci	-0.15	0.041	0.375	0.174	1					
Temp.	0.551	0.598	0.581	0.685	0.233	1				
Yield	-0.07	-0.123	-0.026	-0.070	0.003	-0.135	1			
ETR	**0.724**	0.584	0.673	0.727	-0.012	0.500	-0.013	1		
F_v/F_m	0.512	-0.595	-0.223	-0.448	0.149	-0.644	0.165	-0.371	1	
Ψ	0.400	-0.527	-0.108	-0.349	0.121	-0.578	0.214	-0.338	**0.789**	1

Abbreviations { *A*=CO_2 uptake, PPFD=photosynthetic photon flux density, *g*=leaf conductance, *E*=transpiration, *Ci*=internal CO_2 concentration Temp.=ambient temperature, ETR=electron transport rate, F_v/F_m=ratio of variable to maximal fluorescence, Ψ=leaf water potential}

2.5. Discussion

Gas exchange data indicated that *C. odorata* has a relatively high CO_2 assimilation rate compared to other C_3 invaders. Maximum CO_2 uptake (*A*) ranged between 16 – 18 µmol $m^{-2}s^{-1}$ (Fig. 1B) compared to values of 12.93 and 14.6 µmol $m^{-2}s^{-1}$ for C_3 invaders such as *Rubus discolour* Weihe & Nees and *Tridax procumbens* L. (McDowell, 2002). Photosynthetic rates of *C. odorata* are comparable to several tropical dicotyledonous C_3 weeds from India (Rajendrudu *et al*., 1987) and *Ipomoea asarifolia* (Desr.) Roem & Schult. and *Stachytarpheta cayennensis* (Rich.) Vahl., two Amazonian weeds (Dias-Filho *et al*., 1995), where measurements between 17-23 µmol $m^{-2}s^{-1}$ were observed. Invasive species exhibit high plasticity (Niinemets *et al*., 2003) by possessing thinner leaves with a higher specific area to maximise light capture (Feng *et al*., 2007) and lower carbon cost per unit photosynthetic area than indigenous species (Nagel & Griffen, 2001; Niinemets *et al*., 2003). However, other researchers have indicated that invasive species exhibit similar or even lower light saturated photosynthetic rates since a higher root mass fraction decreases leaf mass allocation and therefore, carbon gain (Smith & Knapp, 2001; Ewe & Sternberg, 2003). In *C. odorata*, there was no saturation of photosynthetic CO_2 uptake or ETR through PSII with increasing PPFD (Figs. 3A & 7B). In many species, daily carbon gain is linearly related to PPFD and typically does not reach light saturation because of leaf movements and shading effects (Rossa *et al*., 1998). However, in this study, leaves were held horizontally in the microcuvette and exposed to direct PPFD of up to 2200 µmol $m^{-2}s^{-1}$ with no shading. Under such conditions, photosynthetic CO_2 uptake in *C. odorata* appeared to be primarily determined by PPFD. The ability to acclimatise to high incident PPFD may therefore be an adaptive strategy to maximise carbon gain and ensure rapid growth. The high CO_2 uptake rate of *C. odorata* in high light environments may explain the success of the species in colonising open habitats.

Increase in leaf conductance (*g*) with increases in PPFD allowed *C. odorata* to assimilate carbon more efficiently. Increased transpiration (*E*) after midday probably resulted in a water deficit resulting in reduced leaf conductance and CO_2 exchange. This conclusion

was supported by a significant correlation between leaf conductance and transpiration (Table 1).

The low leaf water potential at midday resulted in reduced *g* and *A* (Zhang *et al*., 1997; Hirasawa & Hsiao, 1999). Water stress affects the potential for photosynthetic acclimation to irradiance (Craven *et al*., 2010). High air temperature and PPFD result in high evaporative demand (Tenhunen *et al*., 1987), thereby causing a reduction in *g*, *A* and *E*. Thus, stomatal closure at high PPFD regulated gas exchange, prevented further increase in water loss and consequently reduced *A*. Similar stomatal responses were reported in temperate (Gealy, 1989; Gerber & Dawson, 1990; Gealy *et al*., 1991) and tropical weeds (Rajendrudu *et al*., 1987; Feng *et al*., 2007). The limiting effects of stomatal closure at high PPFD on carbon assimilation have been reported previously in indigenous flora (Gonzalez-Rodriguez *et al*., 2002; Shirke & Pathre, 2003; Elfadl & Luukkanen, 2006; Yue *et al*., 2006).

The quantum yield of PSII photochemistry is directly related to the rate at which CO_2 is assimilated in the leaf (Baker & Rosenqvist, 2004). In this study, photosynthetic quantum yield decreased from an early morning maximum to noon, by an average of 26%, but showed a sustained increase thereafter, till 16h00 (Fig. 6A). The reduction in the photosynthetic quantum yield may be a response to protect the photochemical apparatus from transient photooxidation (Kalina *et al*., 2000; Osorio *et al*., 2006). If damage to the photosystems did occur, it was reversible, as there was complete recovery in quantum yield and F_v/F_m, after midday. Controlled dissipation of excess energy within the photochemical systems prevents irreversible damage to the photosynthetic apparatus when stomatal closure limits CO_2 uptake (Bjorkman & Demmig, 1987).Thus, while sustained decreases in maximal PSII efficiency and photosynthetic capacity are key characteristics of photoinhibition, they are also the features that provide powerful photoprotection against the formation of harmful reactive oxygen species at high PPFD (Demmig-Adams *et al*., 2006).

Decreases in F_v/F_m with increase in PPFD have been attributed to slowly relaxing quenching processes and photo damage to PSII reaction centres (Baker & Rosenqvist, 2004). Non-photochemical quenching (NPQ) processes help to regulate and protect photosynthesis in environments in which light energy absorption exceeds the capacity for light utilisation. A value of 0.83 has been reported as an average reference for the optimal quantum efficiency of unstressed leaves of higher plants (Bjorkman & Demmig, 1987). A 5% midday decrease in F_v/F_m indicated that leaves were probably water stressed, suggested by a significant correlation between F_v/F_m and leaf water potential (Table 1). Water stress results in greater sensitivity of gas exchange to temperature (Tenhunen *et al*., 1987) causing stomatal closure and decreased photosynthesis by reducing electron transport and photophosphorylation. Water stress also reduces chlorophyll synthesis and carboxylating enzymes (Egea *et al.*, 2012).

2.6. Conclusion

The results indicated that *C. odorata* exhibits photosynthetic uptake rates higher than most C_3 plants, comparable to reported weeds from India and South America. High CO_2 uptake rates in *C. odorata* are attributed to a lack of light saturation of photosynthetic CO_2 or ETR through PSII with increasing PPFD. High photosynthetic uptake rates, probably allow for greater carbon gain in high light environments contributing to increased growth rates compared to indigenous plants. In addition, the ability to recover rapidly from the effects of water stress and transient photooxidation may confer on *C. odorata* greater vigour enabling the weed to acclimatise to a broader range of environmental stressors. High WUE may be a mechanism to increase the efficiency of resource utilisation ensuring that more carbon is available for growth and reproduction. This trait may be important to the success and spread of *C. odorata* in KwaZulu-Natal compared to indigenous species.

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3. Photosynthetic characteristics of sun and shade leaves

3.1. Abstract

In situ measurements of gas exchange, chlorophyll fluorescence and leaf water potential were determined in completely shaded and fully exposed adjacent stands of *Chromolaena odorata* growing in the field under naturally varying microclimatic conditions. Physiological studies of sun and shade plants may provide information into the efficiency of leaf adaptation to incident photosynthetic photon flux density (PPFD). The objective of the study was to review the mechanisms underlying the differences in the photosynthetic characteristics of *C. odorata* growing in exposed and shaded environments. The responses of *C. odorata* to exposed and shaded conditions will affect overall productivity. This study will provide information on the degree of versatility of the weed to invade new and existing sunny and shaded habitats and may be useful in management plans to control its spread. Leaf measurements were taken from 08h00 to 16h00, at hourly intervals, on the youngest fully expanded leaves from five plants over three days in summer. Maximum incident PPFD was 1300 µmol $m^{-2}s^{-1}$ and 250 µmol $m^{-2}s^{-1}$ for the exposed and shaded sites respectively. Maximal CO_2 uptake was 12 µmol $m^{-2}s^{-1}$ and 1.8 µmol $m^{-2}s^{-1}$ for sun and shade plants respectively. CO_2 uptake increased from dawn to a maximum at 11h00 and thereafter, decreased. Trends in incident PPFD, electron transport rate (ETR), leaf conductance and transpiration were similar to those for CO_2 exchange. In sun and shade plants, trends in leaf water potential followed closely those for CO_2 uptake and leaf conductance. In both treatments, the decrease in quantum yield of PSII from dawn to midday coincided with an increase in incident PPFD. The midday depression of photosynthetic quantum yield in sun and shade was associated with a decrease in the photochemical efficiency of PSII (F_v/F_m). Sun plants had lower specific leaf areas than shade plants. Concentrations of total chlorophyll, total carotenoids and chlorophylls *a* and *b* were significantly lower by 59, 25, 5 and 197% respectively in sun compared to shade leaves. Proline concentrations were considerably lower in shade than

sun leaves. The data suggest that *C. odorata* is able to acclimatise to low incident PPFD, thereby outcompeting less tolerant species.

3.2. Introduction

Photosynthetic responses of herbaceous species to different photon flux densities have been well documented (Bjorkman, 1981) and the ability of sun and shade plants to capture and utilise light is an important characteristic of growth potential (Feng *et al*., 2002). Competition for light is the primary factor influencing weed establishment (D' Antonio *et al*., 2001; Standish *et al*., 2001). Successful weed species may be more invasive than indigenous species by having morphological and physiological traits which increase the efficiency of light capture and use (Pattison *et al*., 1998).

Chromolaena odorata is a problematic weed in KwaZulu-Natal and occurs mostly in disturbed sites as dense, tangled, freely occurring bushes or alternatively forms extensive undergrowth. Light availability may lead to changes in photosynthetic acclimation, plant morphology and growth (Chazdon, 1988; Pan *et al*., 2013). As a result, weeds in exposed habitats may exhibit photosynthetic characteristics different from those in the shade (Bjorkman, 1981; Marchiori *et al*., 2014). Morphological and physiological adjustments to irradiance intensity may delay acclimation and growth response that are critical for seedling survival (Tucker *et al*., 1987). Sims & Pearcy (1991, 1992) reported that fully developed leaves of *Alocasia macrorrhiza* (L.) G. Don, an understorey herb, exhibited no ability for photosynthetic acclimation to changes in photon flux densities. However, significant differences between growth light environment and water stress were observed by Valladares and Pearcy (1997) in *Heteromeles arbutifolia* (Lindl.) M. Roemer. They reported that drought increased photoinhibition of leaves exposed to full sunlight.

High photon flux densities, which cause photoinhibition, lead to a reduction in quantum yield of photosynthesis (Powles, 1984; Kyle, 1987). Moreover, high light can reduce photochemical activity of photosystems and limit carbon gain (Valladares & Pearcy, 1997). When more light is absorbed than can be utilised by the photosystems, plants

dissipate excess excitation energy in a controlled manner (Thiele *et al*., 1998; Krause & Weiss, 1991).

A positive link between the accumulation of proline, an organic, compatible, osmoregulatory solute in plants and high PPFD, has been documented (Ferdausi *et al*., 2009; Kamran *et al*., 2009). It has been shown that high solar radiation will induce proline accumulation in sun leaves to protect enzymes against peroxidative processes (Saradhi *et al*., 1995). High light intensity and temperature enhance the accumulation of proline in plants (Shiraishi, 1996). However, the role of proline remains controversial (Wadhwa *et al*., 2010) although it has been proposed to contribute to osmotic adjustment (Ferdausi *et al*., 2009), stabilise subcellular structures (Samuel *et al*., 2000), act as a scavenger of free radicals (Chen & Dickman, 2005) and is a major constituent of cell wall structural proteins in plant morphogenesis (Chen *et al*., 2006). In addition, proline may protect enzymes from dehydration injury (Wadhwa *et al*., 2010).

In shade plants, a large leaf area improves the ability to capture light (Niinemets *et al*., 1998) and increases longevity (Reich *et al*., 1992). Sun leaves, on the other hand, are comparatively thicker than those in the shade, with a higher leaf dry mass per fresh leaf area. In addition, sun leaves have a higher photosynthetic capacity per unit leaf area than shade leaves due to more efficient quantum utilisation (Niinemets & Tenhunen, 1997). Sun leaves can reduce water loss through a decrease in size and conductance and an increase in thickness while those in the shade develop low solute osmotic potentials to maintain turgor (Rhizopoulou *et al*., 1991).

Plants can quickly induce carbon assimilation during sun flecks (Singsaas *et al*., 2000). Some herbs in shaded environments display rapid leaf movements which minimise the photoinhibitory effects of high irradiance levels during sun flecks (Powles & Bjorkman, 1981). If the sun fleck light intensity is greater than what the photosystems can utilise, the quantum yield of photosynthesis will be reduced by non-photochemical quenching (Watling *et al*., 1997).

Comparative studies on physiological characteristics of sun and shade plants may provide information on the efficiency of leaf acclimation to radiant energy use (Givnish, 1988). According to Boardman (1977), plants that occupy low light habitats perform efficiently in those environments but are capable of higher rates of photosynthesis at greater light intensities. On the other hand, plants that grow in exposed habitats have a higher capacity for photosynthesis but are less efficient than shade plants at lower light intensities. This type of differentiation originates at the level of the photosynthetic machinery (Malkin & Fork, 1981). One of the possible factors which contributes to the difference between sun and shade plants is the ratio of antennae chlorophyll to the reaction centre (Boardman, 1977). Photosynthetic capacity is defined in terms of the higher level of electron transport enzymes and activity associated with CO_2 fixation per chlorophyll molecule.

The objective of the study was to review the mechanisms underlying the differences in photosynthetic characteristics of *C. odorata* growing in sun and shaded environments. The responses of *C. odorata* to sun and shaded conditions will affect overall productivity. This part of the study will provide information on the degree of versatility of the weed to invade new and existing sunny and shaded habitats and may be useful in management plans to control its spread.

3.3. Materials and methods

3.3.1. *Study area*

The study area was previously described in chapter 2. Two uniform stands of *C. odorata*, one forming a dense stand fully exposed to sunlight (sun plants) and the other completely shaded by indigenous *Acacia* trees (shade plants) were chosen for the study. The plots were selected on the basis of being of similar site quality such as slope and vegetation characteristics. The stands consisted of plants that were single stem, approximately 1.5 m in height and disease free. *In situ* measurements of gas exchange and chlorophyll fluorescence were made under naturally varying microclimatic conditions on three successive hot and cloudless days in November 2003 (summer).

3.3.2. *Gas exchange*

Gas exchange measurements were taken as outlined in chapter 2.

3.3.3. *Chlorophyll fluorescence*

Chlorophyll fluorescence measurements were taken as outlined in chapter 2.

3.3.4. *Leaf water potential*

Leaf water potential measurements were taken as outlined in chapter 2.

3.3.5. *Proline*

Proline was extracted and analysed according to the method of Bates *et al*. (1973). Fifty milligrams of fresh leaf material from randomly selected adjacent plants were sampled every hour, frozen in liquid nitrogen immediately after collection, and ground in a pestle and mortar. The homogenate was mixed with 1 mL aqueous sulfosalicyclic acid (3% w/v) and filtered through filter paper (Whatman #1). The extracted solution was reacted with an equal volume of glacial acetic acid and ninhydrin reagent (1.25 mg ninhydrin in 30 mL of glacial acetic acid, 20 mL 6 M H_3PO_4) and incubated at 95^oC for 1 h. The reaction was terminated by placing the test tubes in an ice bath. The reaction mixture was vigorously mixed with 2 mL toluene. After warming to 25^oC, the absorbance of the chromophore was determined with a spectrophotometer (Beckman DU-600) at 520 nm using L-proline as a standard.

3.3.6. *Leaf area*

Leaves from randomly selected sun and shaded plants were collected early in the morning, placed in an insulated cooler-box and immediately transported to the laboratory. Leaf area was determined using a Licor Model 3100 leaf area meter (Licor, Nebraska, USA).

3.3.7. *Chlorophyll and total carotenoids*

Fresh leaf samples were weighed (0.1 g) and extracted separately in 100% acetone. A pinch of sand (laboratory reagent-purified by acid) was added to samples and the contents homogenised in a pestle and mortar after the addition of 1 mL acetone. An additional 4 mL of acetone were added to the ground mixture. The mixture was then poured into test tubes and centrifuged for 5 min at 2500 rpm (Hettich Universal Centrifuge-Tiiv Bayer, Germany). Absorbance of the supernatant was read at 470, 645 and 662 nm respectively using a spectrophotometer (Cary 50 UV-Visible spectrophotometer, Varian, Australia). There were 18 replicates for each treatment. The amount of chlorophylls *a*, *b* and total carotenoids was determined using the following formulae (Dere *et al*., 1998).

$C_a = 11.75\ A_{662} - 2.350\ A_{645}$

$C_b = 18.61\ A_{645} - 3.960\ A_{662}$

$C_{x+c} = 1000\ A_{470} - 2.270\ C_a - 81.4\ C_b/227$

Total chlorophyll was calculated by adding chlorophylls *a* and *b*.

3.3.8. *Statistics*

Statistical analyses were similar to those outlined in Chapter 2. Significance values were assessed by Student's t-tests.

3.4. Results

Figures 1 (A-C) and 2 (A-B) illustrate diurnal trends in PPFD, CO_2 uptake, leaf conductance, transpiration and ambient temperature of sun and shade leaves. Maximum incident PPFD for sun and shade plants was 1300 µmol $m^{-2}s^{-1}$ and 200 µmol $m^{-2}s^{-1}$ respectively (Fig. 1A). Maximum CO_2 uptake was 1.8 µmol $m^{-2}s^{-1}$ for shade and 12 µmol $m^{-2}s^{-1}$ for sun plants (Fig. 1B). CO_2 uptake increased from dawn to a maximum at 11h00,

and thereafter, decreased. Trends in incident PPFD, ETR, leaf conductance and transpiration were similar to those for CO_2 exchange.

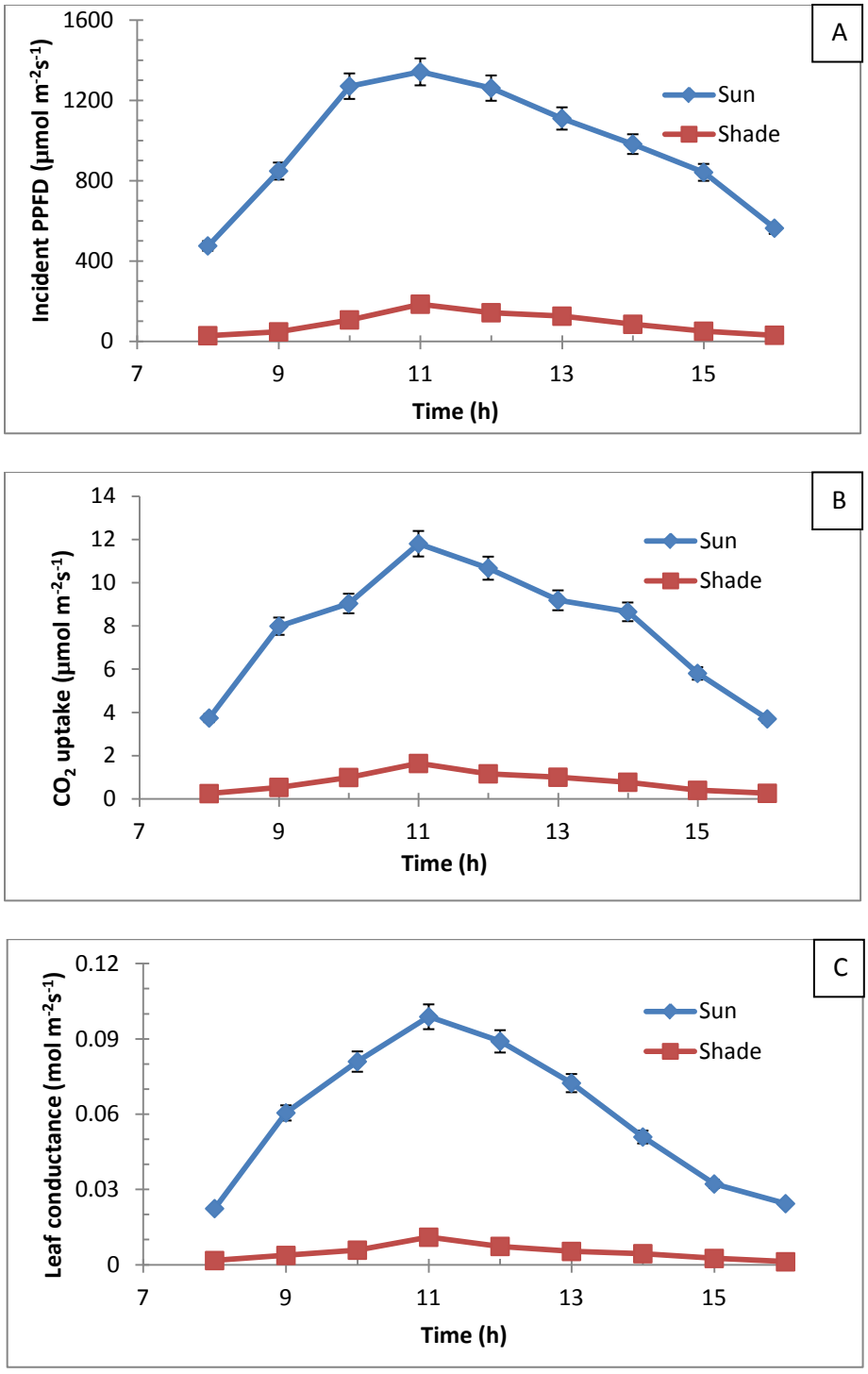

Fig. 1. Diurnal changes in incident photosynthetic photon flux density (A), CO_2 uptake (B) and leaf conductance (C) in healthy single stem of *Chromolaena odorata* plants growing in sun and shade. Each point is the average of 15 individual readings (n=15) taken on three randomly selected days. Vertical bars represent standard error. Bars not visible indicate standard error smaller than the symbol.

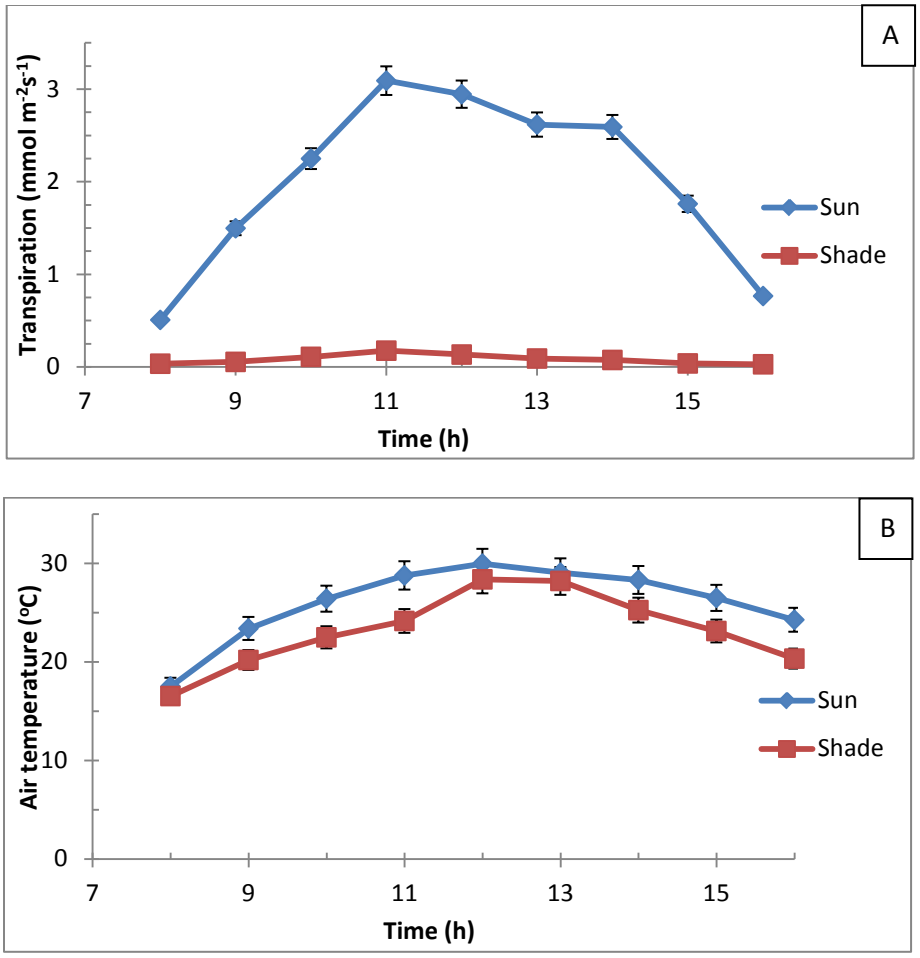

Fig. 2. Diurnal changes in transpiration (A) and ambient temperature (B) in healthy single stem of *Chromolaena odorata* plants growing in sun and shade. Each point is the average of 15 individual readings (n=15) taken on three randomly selected days. Vertical bars represent standard error. Bars not visible indicate standard error smaller than the symbol.

CO_2 uptake and incident PPFD in sun and shade plants were highly correlated (R^2 = 0.90 in sun and 0.86 in shade) suggesting that gas exchange was primarily driven by PPFD (Fig. 3A & D). There was no saturation of CO_2 uptake at high PPFD in sun plants. Lower CO_2 uptake rates in shade plants were associated with lower PPFD (Fig. 3D). In sun plants CO_2 uptake increased from 3 to 11 µmol $m^{-2}s^{-1}$ with increases in incident PPFD from 460 to 1300 µmol $m^{-2}s^{-1}$ (Fig. 3A). High leaf conductance in sun plants resulted in greater transpiration (Fig. 3C) compared to those in the shade treatment (Fig. 3F).

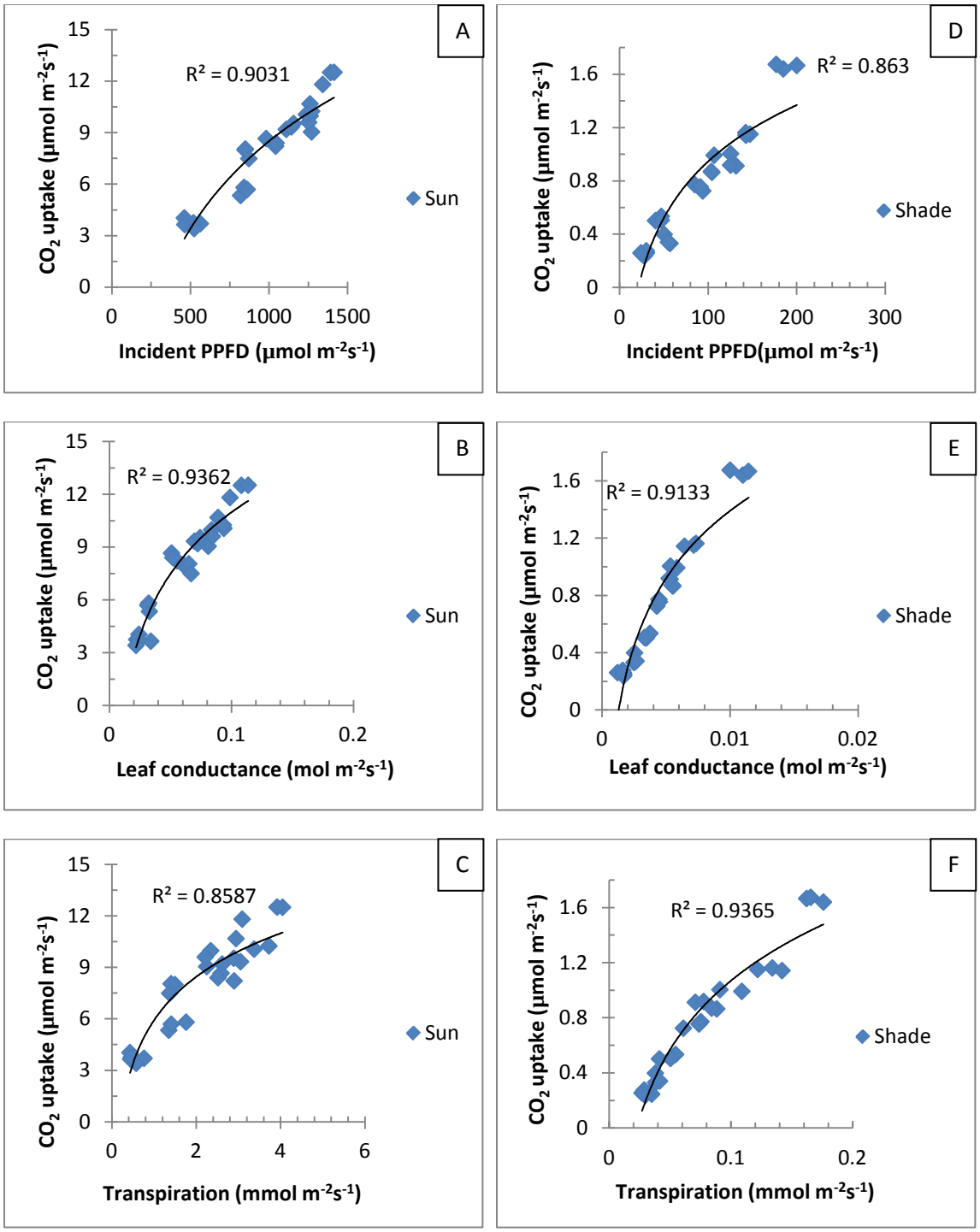

Fig. 3. Relationship between CO_2 uptake and incident photosynthetic photon flux density (A, D), CO_2 uptake and leaf conductance (B, E) and CO_2 uptake and transpiration (C, F) in healthy single stem of *Chromolaena odorata* plants growing in sun and shade respectively. Individual readings (n=15) were averaged and taken on three randomly selected days. The regression lines are indicated by R^2 values.

Quantum yield of PSII in shade plants decreased from 0.78 at dawn to 0.5 at midday and recovered to 0.75 at dusk. In sun plants, quantum yield of PSII followed similar trends but with lower values (Fig. 4A). Intrinsic photochemical efficiency of PSII (F_v/F_m) in sun and shade plants was above 0.82 at dawn, decreased at midday to 0.76-0.81 but recovered to dawn values at dusk (Fig. 4C). Trends in F_v/F_m in shade plants were similar to those for photosynthetic quantum yield.

In sun plants, quantum yield of PSII and F_v/F_m decreased with increases in incident PPFD from dawn to dusk (Fig. 7A, C). In shade plants quantum yield of PSII and F_v/F_m decreased from maximal (0.78 and 0.83) to minimal values (0.52 and 0.81). However, they recovered at higher PPFD (Fig. 7D, F). The ETR was considerably higher in sun than shade plants (Fig. 7B, E).

There was no correlation between leaf water potential and CO_2 uptake and leaf conductance and transpiration between sun and shade plants (Fig. 8).

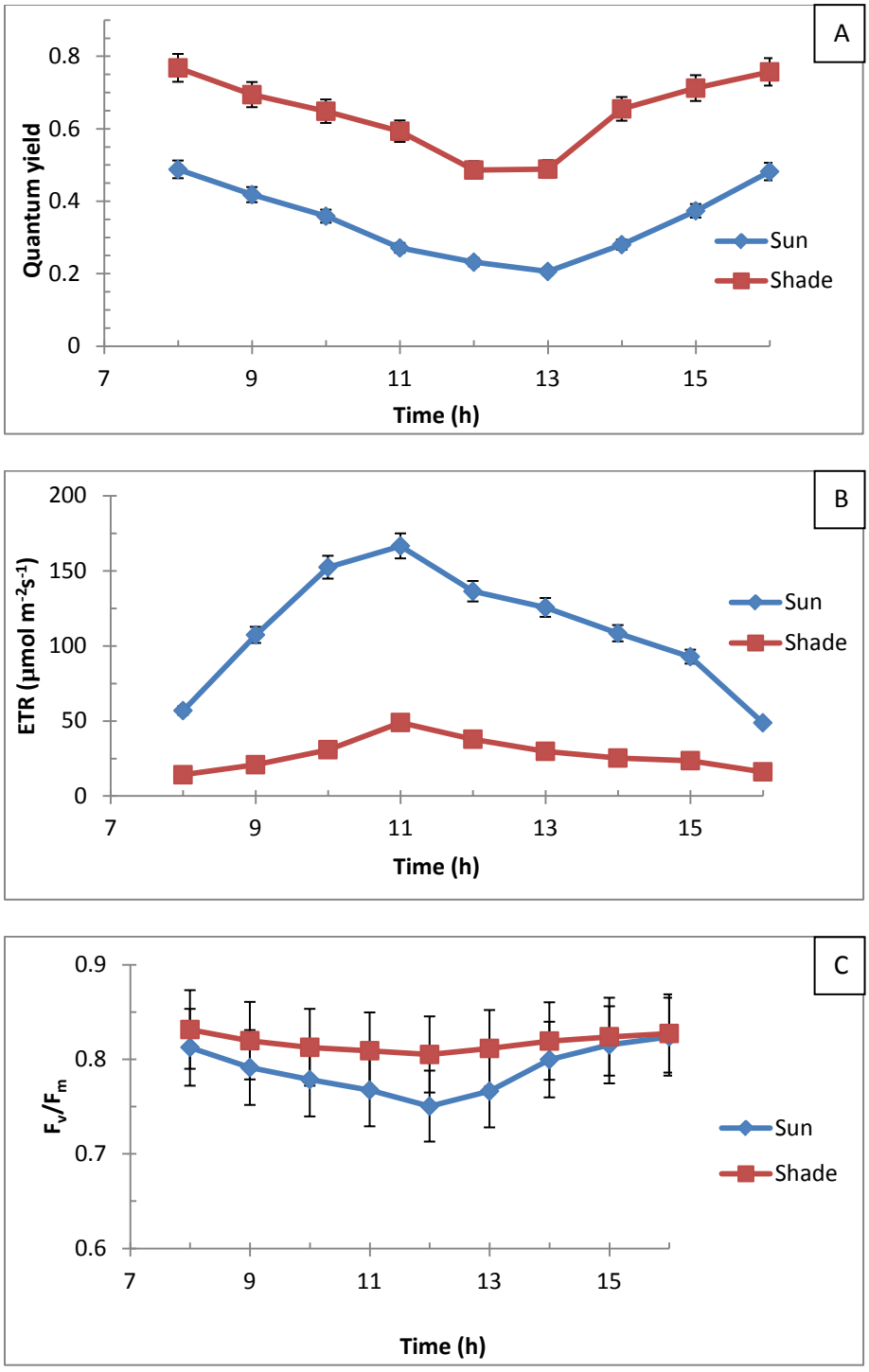

Fig. 4. Diurnal changes in photosynthetic quantum yield (A), electron transport rate through PSII (B) and the potential quantum yield of PSII photochemistry in the dark adapted state (F_v/F_m) (C) in healthy single stem of *Chromolaena odorata* plants growing in sun and shade. Each point is the average of fifteen individual readings (n=15) taken on three randomly selected days. Vertical bars represent standard error. Bars not visible indicate standard error smaller than the symbol.

Trends in leaf water potential were similar to those for photosynthetic quantum yield in both treatments (Fig. 5). Proline concentrations in sun plants increased from dawn to midday and thereafter decreased up to dusk. In shade plants, trends in proline concentrations were similar to those for sun plants but concentrations were much lower (Fig. 6).

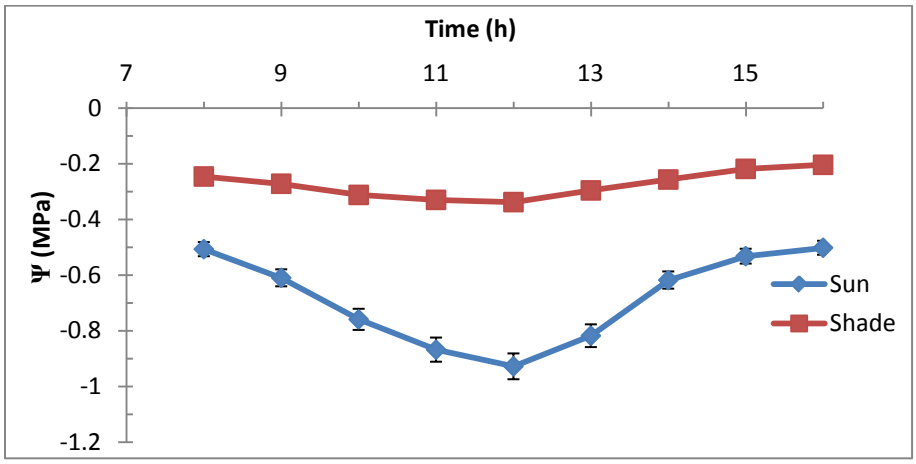

Fig. 5. Diurnal changes in leaf water potential in healthy single stem of *Chromolaena odorata* plants growing in sun and shade. Each point is the average of two individual readings (n=2) taken on three randomly selected days. Vertical bars represent standard error. Bars not visible indicate standard error smaller than the symbol.

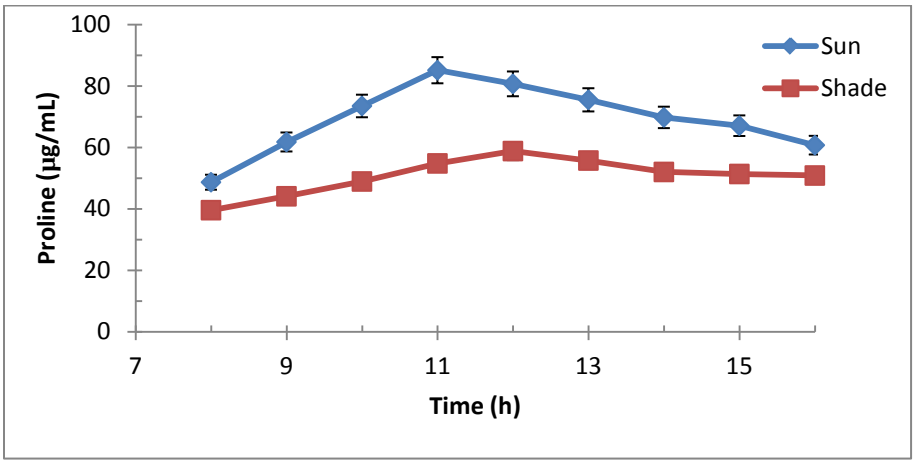

Fig. 6. Diurnal changes in proline concentration in healthy single stem of *Chromolaena odorata* plants growing in sun and shade. Each point is the average of 15 individual readings (n=15) taken on three randomly selected days. Vertical bars represent standard error. Bars not visible indicate standard error smaller than the symbol.

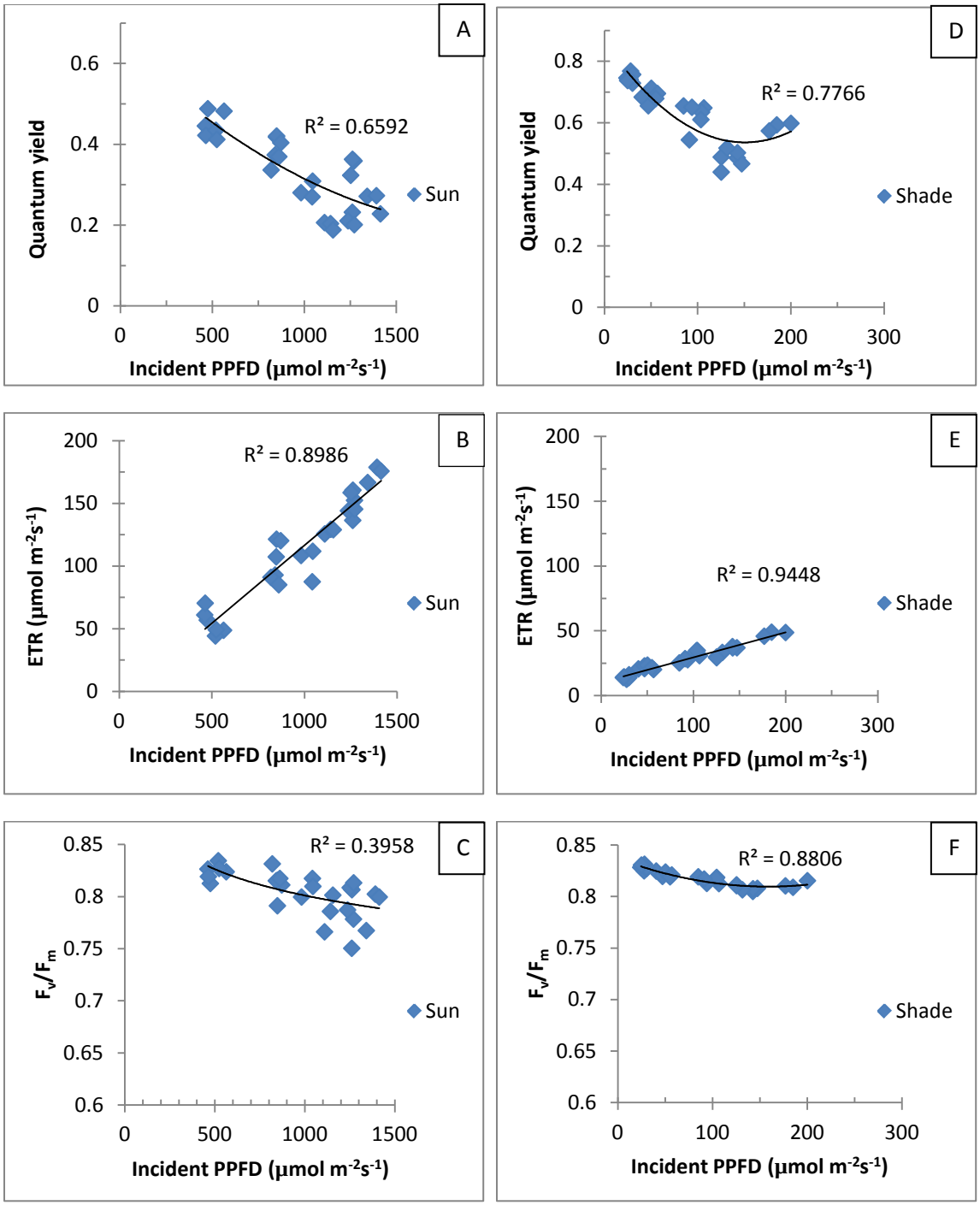

Fig. 7. Relationship between incident phoosynthetic photon flux density and quantum yield (A, D), incident photosynthetic photon flux density and electron transport rate (B, E) and incident photosynthetic photon flux density and the potential quantum yield of PSII photochemistry in the dark adapted state (C, F) in *Chromolaena odorata* plants growing in sun and shade respectively. Individual readings (n=15) were averaged and taken on three randomly selected days. The regression lines are indicated by R^2 values.

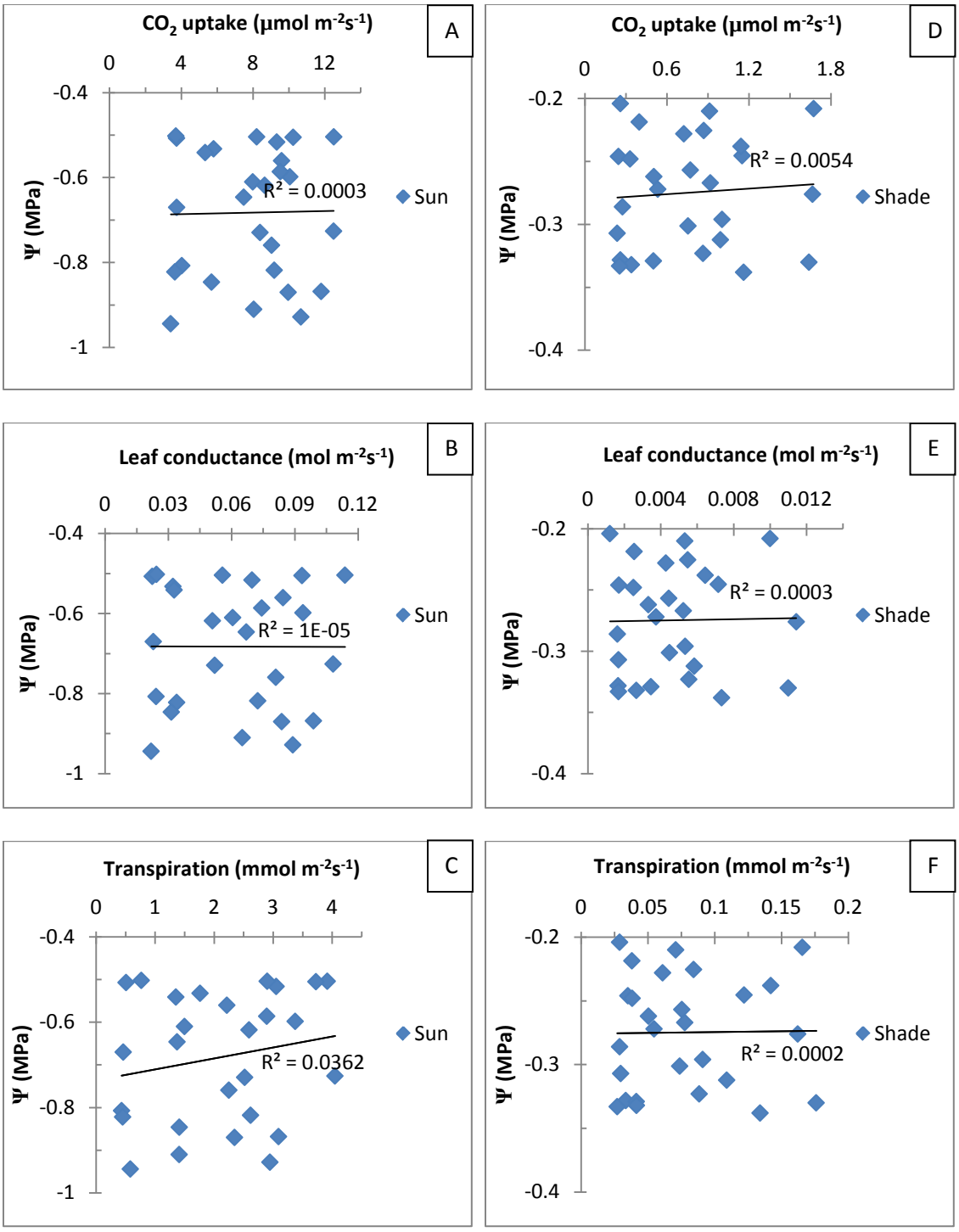

Fig. 8. Relationship between leaf water potential and CO_2 uptake (A, D), leaf water potential and leaf conductance (B, E) and leaf water potential and transpiration (C, F) in *Chromolaena odorata* plants growing in sun and shade respectively. Individual readings were averaged (CO_2 uptake, leaf conductance, transpiration- n=15) and (Ψ- n=2) and taken on three randomly selected days. The regression lines are indicated by R^2 values.

Proline concentrations were significantly higher in sun than shade plants (Fig. 9). Proline concentrations in sun and shade plants were significantly correlated with leaf conductance and ambient temperature respectively (Table 3).

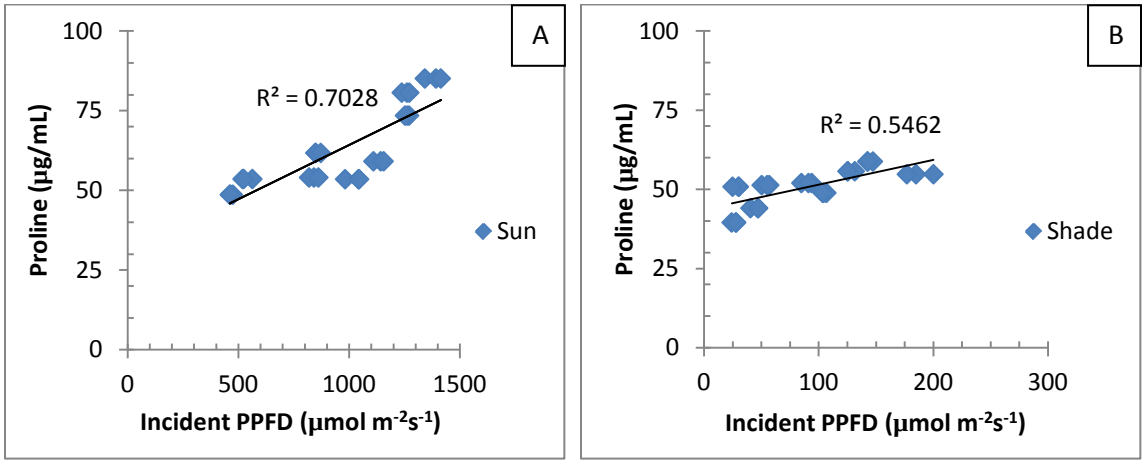

Fig. 9. Relationship between incident photosynthetic photon flux density and proline concentration in healthy, single stem of *Chromolaena odorata* plants growing in sun (A) and shade (B). Individual readings (n=15) were averaged and taken on three randomly selected days. The regression lines are indicated by R^2 values.

Sun leaves had lower specific areas than shade leaves. The concentrations of total chlorophyll, total carotenoids and chlorophylls *a* and *b* were significantly lower in sun compared to shade leaves by 59, 25, 5 and 197% respectively (Table 1).

Table 1. Differences in chloroplastic pigments, proline and leaf area between sun and shade leaves of *Chromolaena odorata* (n = 15).

PARAMETER	SUN	SHADE
Total chlorophyll (µg g^{-1})	332.88 ± 9.39	528.31 ± 7.50*
Chlorophyll *a* (µg g^{-1})	239.59 ± 4.28	251 ± 0.94*
Chlorophyll *b* (µg g^{-1})	93.30 ± 5.19	276.79 ± 8.21*
Chlorophyll *a*/*b* ratio	2.67 ± 0.11	0.92 ± 0.03*
Total carotenoids (µg g^{-1})	21511.64 ± 471.78	26962.71 ± 70.04*
Proline (µg mL^{-1})[a]	80.70±1.00	56.58±0.98*
Specific leaf area (cm^2 g^{-1})	24.31 ± 1.50	42.92 ± 0.98*

Means ± SE are indicated. Statistical significance of unpaired "t" tests is indicated at $P \leq 0.05$ (*). [a]Proline concentration at 12h00.

Photosynthetic characteristics and Ψ of sun leaves were significantly greater than those in the shade (Table 2).

Table 2. Ecophysiological differences (maximal values) between sun and shade leaves.

PARAMETER	SUN	SHADE
A (µmol $m^{-2}s^{-1}$)	12	1.8*
PPFD(µmol $m^{-2}s^{-1}$)	1300	250*
g (mol $m^{-2}s^{-1}$)	0.1	0.007*
E (mmol $m^{-2}s^{-1}$)	3.0	0.15*
WUE (µmol CO_2/ $mmol^{-1}$ H_2O)	4.0	12*
Temp. (^oC)	28	27
Yield	0.78	0.49*
ETR (µmol $m^{-2}s^{-1}$)	165	48*
F_v/F_m	0.82	0.83
Ψ (MPa)	-0.48	-0.25*

Statistical significance of unpaired "t" tests is indicated at $P \leq 0.05$ (*).

Abbreviations {*A*=CO_2 uptake, PPFD=photosynthetic photon flux density, *g*=leaf conductance, *E*=transpiration, WUE=water use efficiency, Temp. =ambient temperature, ETR=electron transport rate, F_v/F_m=ratio of variable to maximal fluorescence, Ψ=leaf water potential}

Leaf conductance was significantly correlated with CO_2 uptake and PPFD in both sun and shade plants (Table 3). Significant correlations also existed between ETR and CO_2 uptake and leaf conductance and ETR (Table 3).

Table 3. Pearson's correlation coefficients for various variables between sun and shade plants (values in bold are significant at $P \leq 0.05$).

	A- sun	*A*- shade	PPFD- sun	PPFD- shade	*g*- sun	*g*- shade	*E*- sun	*E*- shade	Temp.- sun	Temp.- shade	Yield- sun	Yield- shade	ETR- sun	ETR- shade	F_v/F_m sun	F_v/F_m shade	Ψ- sun	Ψ- shade	Proline sun	Proline shade
A- sun	1																			
A-Shade	**0.917**	1																		
PPFD-Sun	**0.953**	**0.898**	1																	
PPFD-shade	**0.909**	**0.962**	**0.908**	1																
g-Sun	**0.932**	**0.917**	**0.906**	**0.878**	1															
g-shade	**0.902**	**0.962**	**0.866**	**0.940**	**0.899**	1														
E-sun	**0.911**	**0.890**	**0.914**	**0.921**	**0.847**	**0.850**	1													
E-shade	**0.863**	**0.949**	**0.846**	**0.919**	**0.880**	**0.925**	**0.832**	1												
Temp.-sun	0.731	0.697	**0.796**	**0.772**	0.634	0.652	**0.813**	0.647	1											
Temp.-shade	0.684	0.659	0.734	0.721	0.583	0.606	0.755	0.614	**0.936**	1										
Yield-sun	-0.759	-0.746	-0.786	-0.825	-0.691	-0.679	-0.859	-0.675	-0.815	-0.817	1									
Yield-shade	-0.747	-0.703	-0.767	-0.772	-0.707	-0.631	-0.791	-0.650	-0.750	-0.759	**0.878**	1								
ETR-sun	**0.916**	**0.855**	**0.913**	**0.835**	**0.914**	**0.850**	**0.812**	**0.807**	0.624	0.553	-0.637	-0.654	1							
ETR-shade	**0.912**	**0.938**	**0.910**	**0.931**	**0.886**	**0.928**	**0.874**	**0.898**	0.723	0.655	-0.712	-0.674	**0.863**	1						
F_v/F_m-sun	-0.639	-0.616	-0.621	-0.622	-0.607	-0.603	-0.557	-0.581	-0.592	-0.600	0.540	0.584	-0.590	-0.576	1					
F_v/F_m-shade	-0.745	-0.716	-0.777	-0.762	-0.711	-0.672	-0.770	-0.675	-0.746	-0.709	**0.806**	**0.774**	-0.649	-0.694	0.634	1				
Ψ -sun	-0.885	-0.892	-0.883	-0.910	-0.909	-0.849	-0.857	-0.863	-0.749	-0.711	**0.813**	**0.842**	-0.866	-0.855	0.681	**0.781**	1			
Ψ -shade	-0.869	-0.852	-0.840	-0.834	-0.916	-0.831	-0.780	-0.840	-0.563	-0.519	0.666	**0.771**	-0.866	-0.809	0.644	0.686	**0.918**	1		
Proline -sun	**0.807**	**0.842**	**0.800**	**0.804**	**0.828**	**0.829**	0.739	0.846	0.680	0.660	-0.580	-0.558	**0.794**	**0.819**	-0.595	-0.581	-0.815	-0.768	1	
Proline- shade	0.576	0.621	0.655	0.703	0.519	0.565	0.716	0.594	**0.861**	**0.851**	-0.783	-0.725	0.443	0.627	-0.402	-0.647	-0.664	-0.429	0.574	1

Abbreviations {*A*=CO_2 uptake, PPFD=photosynthetic photon flux density, *g*=leaf conductance, *E*=transpiration, Temp. =ambient temperature, ETR=electron transport rate, F_v/F_m=ratio of variable to maximal fluorescence, Ψ= leaf water potential}

3.5. Discussion

Incident PPFD fluctuations are the main causes of variation in leaf photosynthetic rates in the natural environment (Vadell *et al*., 1993). In this study, sun plants were exposed to 5 times greater incident PPFD than shade plants (Table 2). In addition, sun plants were able to exhibit higher conductance, higher transpiration and lower leaf water potential compared to shade plants. Increases in leaf conductance with PPFD have been documented in most species (Abrams & Mostoller, 1995; Muraoka *et al*., 1997; Niinemets *et al*., 1998; Horton *et al*., 2010). In direct sunlight, greater leaf conductance caused sun plants to take up approximately ten times greater CO_2 than shade plants. There was no saturation of CO_2 uptake at high incident PPFD in sun plants. Rapid CO_2 fixation is particularly important in dominant communities (Feng *et al*., 2002). Sun plants were more efficient at assimilating CO_2 compared to shade plants due to significantly higher photosynthetic electron transport (Horton *et al*., 2010). In sun plants, high CO_2 uptake may improve carbon gain and offset any reduction in assimilation due to self shading. This may explain why *C. odorata* dominates open spaces particularly well. These results support those of Marek *et al*. (1989) who showed that assimilation activity of exposed spruce foliage was higher compared to those in a shaded habitat.

In sun plants, higher incident PPFD, coupled with higher temperature at midday resulted in decreased leaf conductance, CO_2 uptake and transpiration. The decrease in leaf water potential in sun plants in the morning was probably due to high evapotranspiration. Leaf conductance in sun plants was probably efficiently regulated to maximise CO_2 uptake and minimise water loss. A similar study with *Myrtus communis* L. showed that stomatal closure of sun leaves was efficiently regulated to control water loss during water deficiency (Mendes *et al*., 2001). Lower chlorophyll *b* in sun than shade plants could be due to exposure to high incident PPFD. Lower chlorophyll content in sun compared to shade plants may result in less light being absorbed and more transmitted. In addition, lower chlorophyll content of sun leaves may be due to reduced specific leaf area compared to shade leaves (Table 1). Excess incident PPFD, that is not absorbed, can be dissipated as heat, thus protecting the photosystems from photoinhibition (Allred *et al*., 2010). Photoinhibition is described as any decrease in the quantum yield of photosynthesis during exposure to high or moderate light (Genty *et al*., 1989; Tyystjarvi *et al*., 1994; Valladares & Pearcy, 1997). Photoinhibition may lead to damage of chlorophyll molecules (Souza *et al*., 2004) and may inactivate PSII

enzymes resulting in decreased carbon assimilation as demonstrated previously (Colom & Vazzana, 2003). Lower leaf surface area in sun compared to shade leaves may possibly assist in increasing water use efficiency by conserving water lost through transpiration (Table 2).

Shade plants were able to fix CO_2 although light was limiting within the canopy. The species is tolerant to low light conditions despite reduced CO_2 uptake. Water stress in shade plants was lower than those in the sun, probably because of lower ambient temperature and incident PPFD. A study on *Gethyllis multifolia* L. Bolus *and Gethyllis villosa* Thunb. showed that shade can lessen the effects of drought and high incident PPFD (Daniels *et al*., 2013). Shade plants had significantly higher specific leaf areas than sun plants, possibly, to increase leaf area for light capture. In addition, concentrations of total chlorophyll, total carotenoids and chlorophylls *a* and *b* were significantly higher in shade than sun plants probably to intercept light more efficiently (Table 1). Compared to sun plants, shade plants typically have more light harvesting complexes per unit area in order to capture as much light as possible (Wadhwa *et al*., 2010). Species with lower stomatal conductance and more negative water potential have higher chlorophyll contents than species with higher stomatal conductance and less negative water potential (Wadhwa *et al*., 2010).

Photosynthetic quantum yield, in both sun and shade plants, decreased from morning to midday but recovered thereafter (Fig. 4A). Diurnal changes in quantum yield is a measure of the proportion of photons absorbed by chlorophylls associated with PSII and indicates overall photosynthetic efficiency (Genty *et al*., 1989). The recovery in photosynthetic quantum yield after midday suggests that if damage did occur to PSII reaction centres, it was fully reversible. Midday depression in quantum yield in *Oryza sativa* L. was attributed to stomatal closure in response to high PPFD (Panda, 2011). The decrease in quantum yield from dawn to midday in sun plants and then subsequent increase to dusk may therefore be a response to increased water loss. In both treatments, reduced leaf conductance limited transpiration and caused an increase in quantum yield. The ability to rapidly recover from low quantum yield values at midday may be a mechanism to recover from water stress. This advantage may enable the weed to be more successful in colonising disturbed habitats compared to other indigenous species. Muraoka and co-workers (1997) found that quantum yield in sun leaves of *Arisaema heterophyllum* Blume remained unchanged throughout the day and carbon assimilation did not correlate with electron transport rate. However, these authors found that

quantum yield and carbon assimilation markedly decreased in shade leaves at midday and depended on the electron transport rate through PSII.

The photochemical efficiency of PSII ($F_v/F_{m)}$ ratio can be used for assessment of photoinhibition in leaves (Ball & Farquhar, 1984; Tyystjarvi *et al*., 1994; Kalina *et al*., 2000). In sun and shade plants, F_v/F_m values were between 0.82-0.83 at dawn and at dusk although a 6% decrease was observed at midday (Fig. 4C). Although stomatal closure may limit the rate of CO_2 assimilation, controlled dissipation of excess energy within the photochemical system can prevent irreversible damage to the photosynthetic apparatus (Demmig-Adams *et al*., 1989). Simultaneous depression of photosynthetic CO_2 uptake and F_v/F_m under natural high light has been reported for several species (Demmig-Adam *et al*., 1989; Epron *et al*., 1992; Valladares & Pearcy, 1997). The recovery in F_v/F_m after midday, supports trends in photosynthetic quantum yield, indicating that there was no permanent damage to PSII in *C. odorata* under sun or shade.

A significant linear relationship between incident PPFD and proline in sun plants was observed. Proline concentration in sun plants at midday was significantly higher than those in the shade (Table 1). Accumulation of free proline occurs under water stress conditions (Rhizopoulou *et al*., 1991; Zhu, 2002). Proline accumulates as a reserve osmolyte for the synthesis of chlorophyll upon relief of stress (William & Sharon, 1981). In shade plants incident PPFD, temperature and water potential were lower than in sun plants. Furthermore, the lower leaf conductance in shade plants contributed to reduced water loss. A similar study confirmed that midday proline concentrations in *Chenopodium murale* L. and *Ocimum basilicum* L. were higher in sun compared to shade plants (Batanouny *et al*., 1984). Proline may serve an important role in enabling greater carbon gain at high incident PPFD by protecting carboxylation enzymes from dehydration or light injury (Wadhwa *et al*., 2010).

3.6. Conclusion

This study showed that the light environment significantly influenced gas exchange parameters in *C. odorata.* High incident PPFD may allow greater carbon gain, thereby contributing to increased growth and spread of the species. Shade plants have significantly larger leaf surface areas and greater concentrations of total chlorophyll, total carotenoids and chlorophylls *a* and *b* than sun plants, which probably maximises CO_2 uptake. *Chromolaena*

odorata can successfully acclimatise to low PPFD thus outcompeting less tolerant species under low light conditions. Although shade plants exhibit lower photosynthetic rates compared to those in the sun, they may be capable of rapid growth and establishment in direct sunlight. The high plasticity of *C. odorata* to grow under reduced light could pose a serious threat in coastal forest ecosystems where high fuel loads can possibly alter fire regimes. Leaf conductance was efficiently regulated, especially in sun plants to enable water conservation and maximise water use efficiency. The significantly higher concentration of proline in sun plants may play a key function in protecting the photosynthetic apparatus of PSII from water stress during periods of high light intensity.

3.7. References

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4. Seasonal variation in photosynthetic characteristics

4.1. Abstract

The aim of this study was to characterise seasonal gas exchange characteristics in *Chromolaena odorata*. *In situ* measurements of gas exchange and chlorophyll fluorescence were taken in the field under naturally varying microclimatic conditions on five successive days in summer, as well as in winter. In summer, maximum CO_2 uptake (16 µmol $m^{-2}s^{-1}$) occurred at a photosynthetic photon flux density (PPFD) of 2000 µmol $m^{-2}s^{-1}$. In winter, maximum CO_2 uptake (13 µmol $m^{-2}s^{-1}$) occurred at a PPFD of 1400 µmol $m^{-2}s^{-1}$. CO_2 uptake increased with increasing incident PPFD from dawn to a maximum between 10h00 and 11h00. Thereafter, CO_2 uptake decreased with incident PPFD. In both seasons, trends in incident PPFD, leaf conductance, transpiration and electron transport rate (ETR) followed closely those of CO_2 uptake. There was no saturation of CO_2 exchange at high PPFD in summer or in winter. Maximum leaf conductance was 0.18 mol $m^{-2}s^{-1}$ in summer and 0.11 mol $m^{-2}s^{-1}$ in winter. Maximum transpiration ranged from 4.3 to 4.5mmol $m^{-2}s^{-1}$ over both seasons. Photosynthetic quantum yield of PSII in summer and winter decreased as incident PPFD increased. Maximum ETR through PSII in summer and winter was between 175-180 µmol $m^{-2}s^{-1}$. Leaf water potential was 17% higher in summer than winter, possibly due to increased soil moisture in the former season. The midday depression in the potential quantum yield of PSII in the dark adapted state (F_v/F_m), in both seasons, was possibly due to reversible photoinhibition, suggested by recovery in F_v/F_m to predawn values at 16h00.

4.2. Introduction

The impact of invasive weeds on indigenous species, communities and ecosystems has been widely discussed (Simberloff, 1997; Vitousek *et al*., 1997; Mack *et al*., 2000). However, the mechanisms by which invasive plants spread rapidly are lacking. An understanding of these mechanisms is essential to predict distribution patterns and may be useful for control efforts (Baruch and Goldstein, 1999; Mack *et al*., 2000; Kolar and Lodge, 2001). Indigenous vegetation in KwaZulu-Natal is being strangled by a proliferation of invasive alien plants, one of which is *C. odorata*. The weed is highly adaptable and currently invades a wide range

of ecological niches, especially along the eastern coast of KwaZulu-Natal. During summer, both drought and excess light can affect plant carbon assimilation (Gulias *et al*., 2009; Lu *et al*., 2012). Light is the main meteorological driver of photosynthesis and, at higher latitudes, the marked variability in photoperiod leads to high seasonal variation in diurnal course of CO_2 uptake (Poyatos *et al*., 2012). The degree of precipitation normally regulates photosynthesis in summer while low temperature normally reduces carbon assimilation in winter (Flexas *et al*., 2001). Marked changes in seasonal patterns of gas exchange are mostly observed in deciduous plants where photosynthetic parameters dynamically acclimate to temperature (Makela, 2008). Ball *et al*. (1994) showed that cold induced changes in net carbon assimilation rate may be correlated with growth. They showed that in some woody species, a reduction in PSII photochemical efficiency, is quantitatively reflected in seasonal variations of both carbon assimilation and growth.

In temperate regions, a reduction in winter temperature can be more limiting than summer drought (Corcuera *et al*., 2005; Methy, 2000). DeMatta *et al*. (1997) showed that F_v/F_m and CO_2 uptake decreased markedly in winter. Furthermore, other parameters that affect CO_2 uptake include variations in the leaf nitrogen content, drought (Wilson *et al*., 2000) and the duration of the growing season (Karlsson *et al*., 2003). Barros *et al*. (1997) correlated leaf conductance and shoot growth in coffee during the initial stage of the declining growth period. They hypothesised that photosynthesis might modulate growth, depending on the time of the year.

The aim of this study was to characterise seasonal gas exchange characteristics in *C. odorata* to determine summer and winter patterns. Although a number of research projects on subtropical weeds have addressed how light availability affects rates of gas exchange, there is comparatively little information on how these rates are affected by seasonal changes. Physiological changes to environmental parameters may enable future prediction in species distribution patterns and ecosystem functioning. This information forms the basis for the development of strategies to monitor and document the spread of this noxious weed.

4.3. Materials and methods

4.3.1. *Study area*

The study area was the same as the one previously described in chapter 2. *In situ* measurements of gas exchange and chlorophyll fluorescence were taken under naturally varying microclimatic conditions on five successive days in July (winter) and in December 2004 (summer).

4.3.2. *Gas exchange*

Gas exchange measurements of *C. odorata* were taken as outlined in chapter 2.

4.3.3. *Chlorophyll fluorescence*

Chlorophyll fluorescence measurements of *C. odorata* were taken as outlined in chapter 2.

4.3.4. *Leaf water potential*

Leaf water potentials were measured as outlined in chapter 2.

4.3.5. *Statistics*

Analysis was similar to the procedure outlined in Chapter 2 and 3.

4.4. Results

Maximum PPFD was 2000 µmol $m^{-2}s^{-1}$ in summer and 1400 µmol $m^{-2}s^{-}1$ in winter (Fig. 1A & D). CO_2 uptake increased with increasing incident PPFD from dawn to a maximum between 10h00 and 11h00. Thereafter, CO_2 uptake decreased with incident PPFD up to 16h00. In both seasons, trends in incident PPFD, leaf conductance, transpiration and ETR followed closely those of CO_2 uptake. CO_2 uptake at maximum PPFD was 16 µmol $m^{-2}s^{-1}$ in summer (Fig. 1B) and 13 µmol $m^{-2}s^{-1}$ in winter (Fig. 1E). In summer, maximum leaf conductance and transpiration were 0.18 mol $m^{-2}s^{-1}$ and 4.5 mmol $m^{-2}s^{-1}$ respectively at 2000

μmol $m^{-2}s^{-1}$ PPFD (Figs. 1C & 2A). In winter, maximum leaf conductance and transpiration were 0.11 mol $m^{-2}s^{-1}$ and 4.25 mmol $m^{-2}s^{-1}$ respectively at 1400 μmol $m^{-2}s^{-1}$ PPFD (Figs. 1F & 2C). Maximum ambient temperature was between 35-37^{o}C in both seasons (Figs. 2B & D).

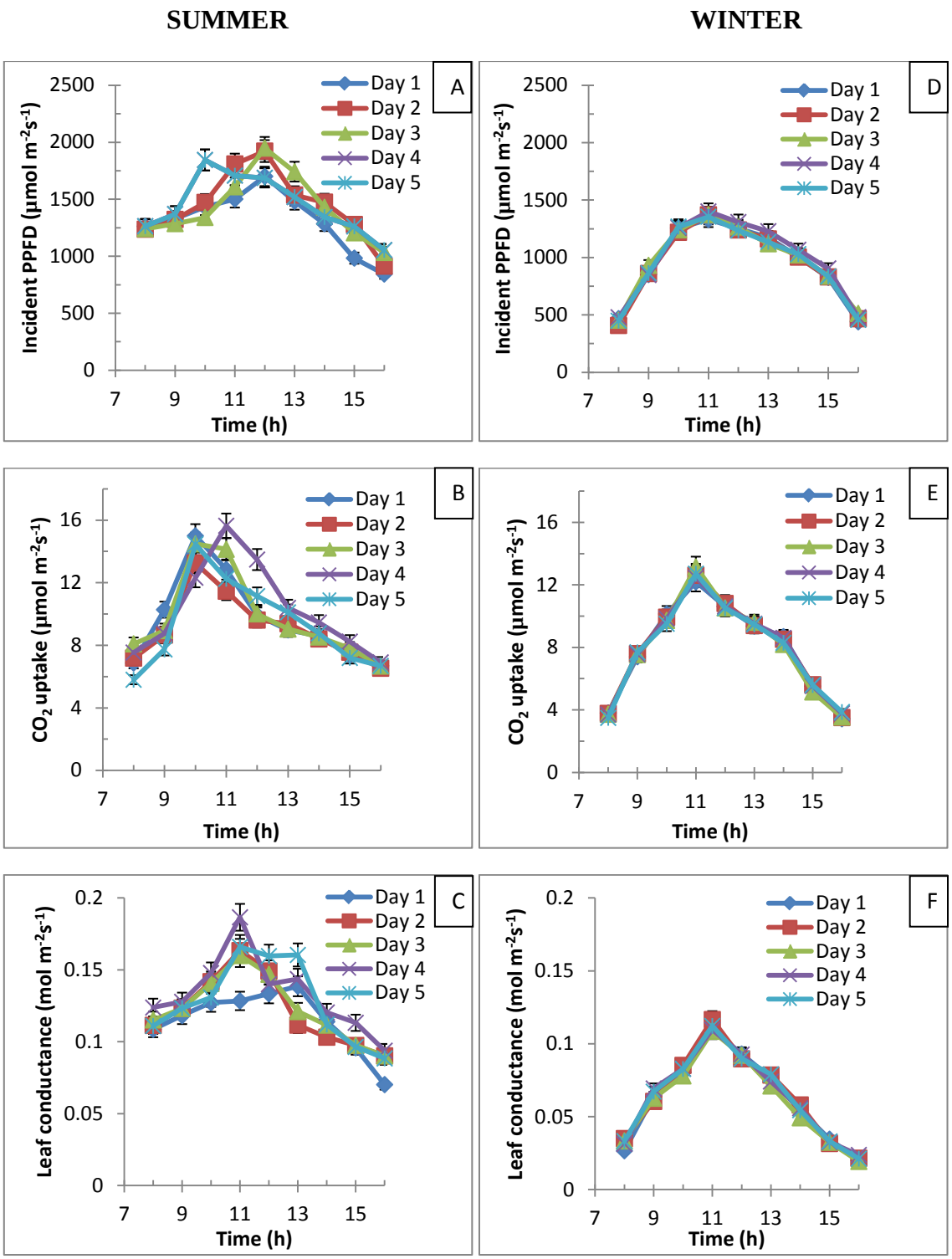

Fig. 1. Diurnal changes in incident photosynthetic photon flux density (A, D), CO_2 uptake (B, E) and leaf conductance (C, F), in summer and winter respectively. Healthy single stem of *Chromolaena odorata* plants were measured on five consecutive days in each season. Each point is the mean value of five independent readings. Vertical bars represent standard error. Bars not visible indicate standard error smaller than the symbol.

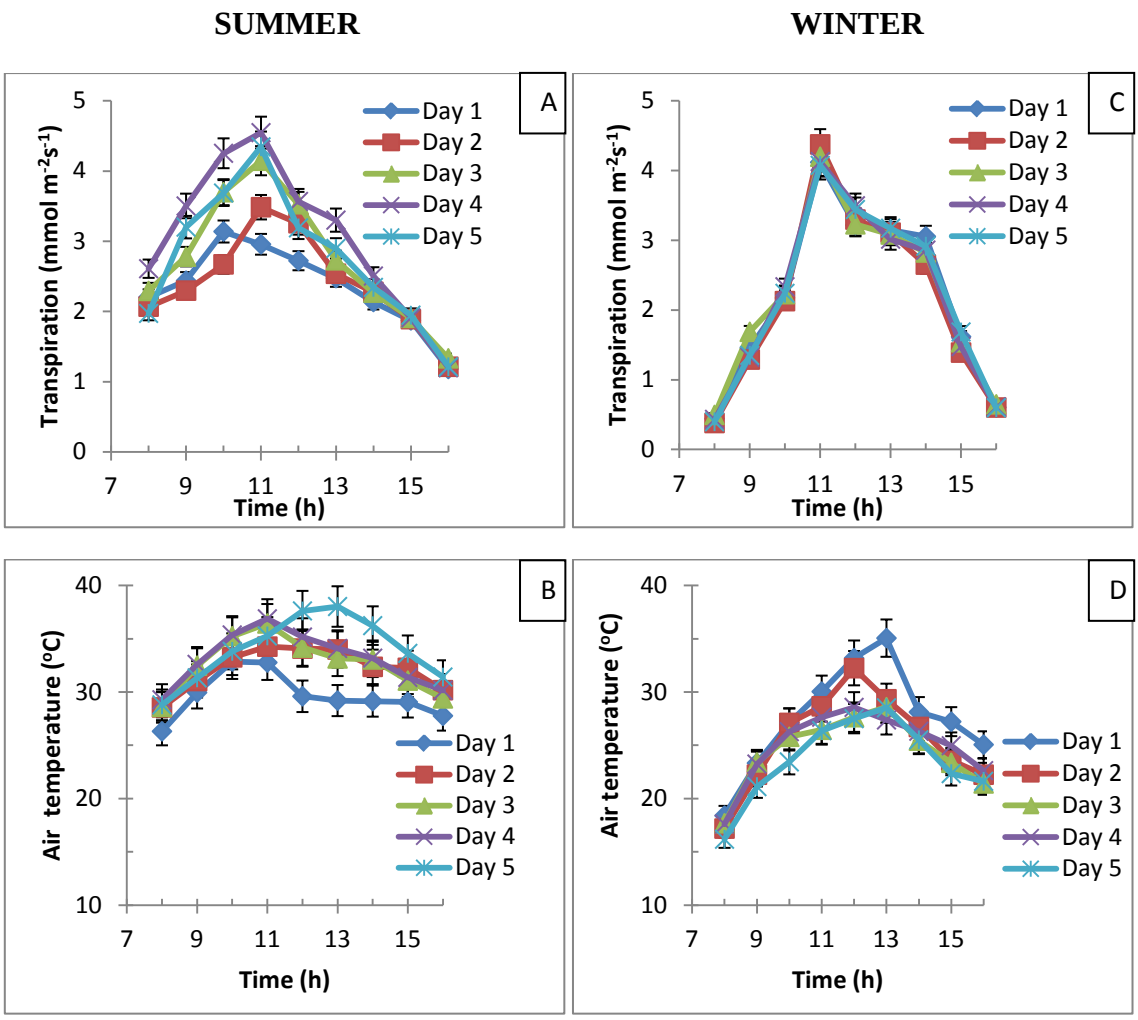

Fig. 2. Diurnal changes in transpiration (A, C) and ambient temperature (B, D) in summer and winter respectively. Healthy single stem of *Chromolaena odorata* plants were measured on five consecutive days in each season. Each point is the mean value of five independent readings. Vertical bars represent standard error. Bars not visible indicate standard error smaller than the symbol.

There was no saturation of CO_2 exchange at high PPFD in summer or in winter. In summer, mean CO_2 uptake increased from 5 to 13 µmol $m^{-2}s^{-1}$ with increases in incident PPFD from 850 to 1900 µmol $m^{-2}s^{-1}$ (Fig. 3A). In winter, mean CO_2 uptake increased from 3 to 12 µmol $m^{-2}s^{-1}$ with increases in incident PPFD from 400 to 1400 µmol $m^{-2}s^{-1}$ (Fig. 3D). CO_2 uptake increased with increases in leaf conductance in summer (Fig. 3B) and winter (Fig. 3E). Higher leaf conductance in summer contributed to greater transpiration compared to the winter.

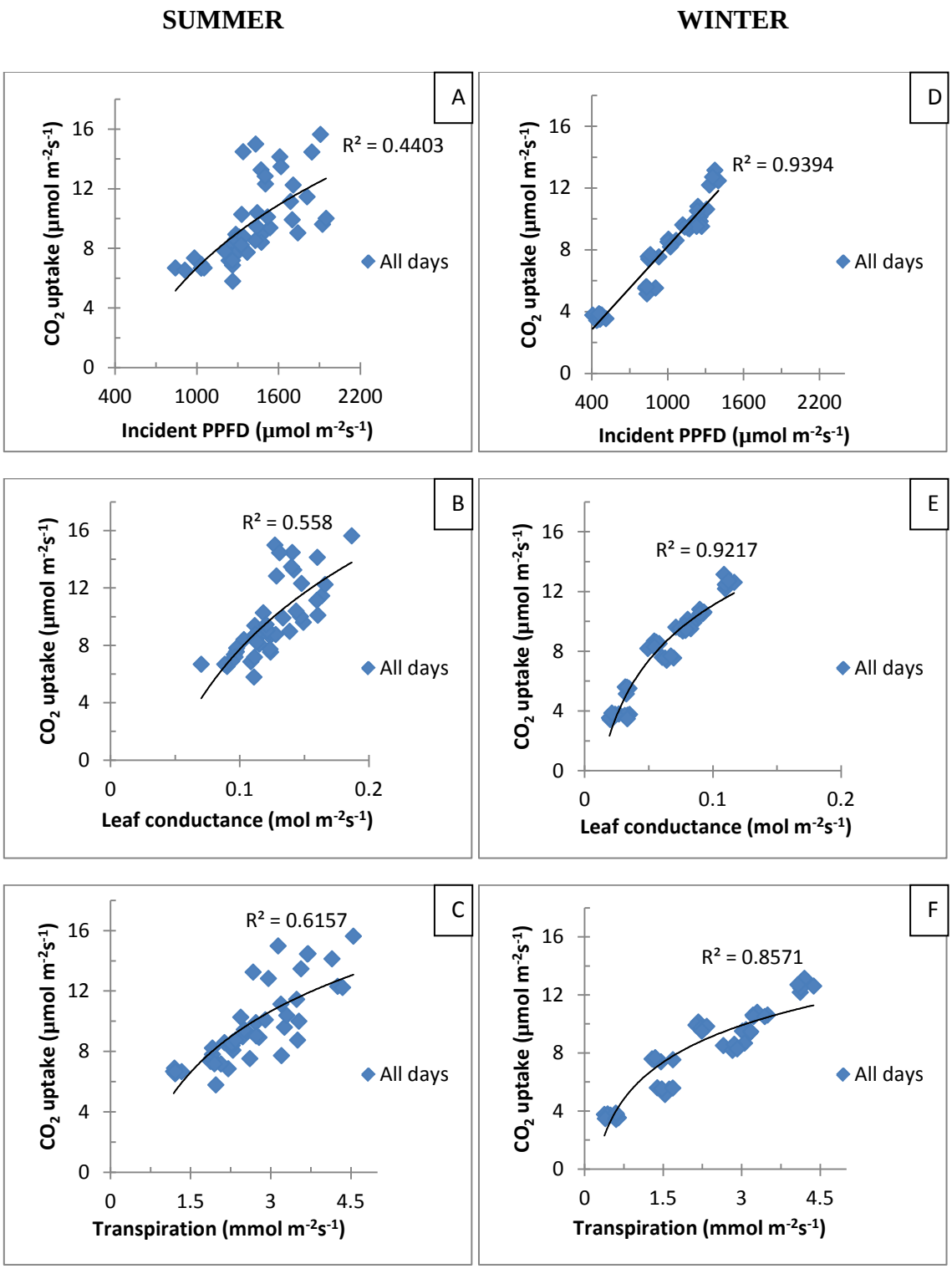

Fig. 3. Relationship between CO_2 uptake and incident photosynthetic photon flux density (A, D), CO_2 uptake and leaf conductance (B, E) and CO_2 uptake and transpiration (C, F) in summer and winter respectively. Healthy single stem of *Chromolaena odorata* plants were measured on five consecutive days in each season. Each point is the mean value of five independent readings. The regression lines are indicated by R^2 values.

In both seasons, photosynthetic quantum yield of PSII decreased from the predawn maximum to a minimum value at midday, followed by a recovery at dusk. (Figs. 4A, D). Quantum yield through PSII was considerably lower in winter than in summer possibly due to reduced soil moisture. In both seasons, the midday depression of photosynthetic quantum yield was associated with a decrease in the photochemical efficiency of PSII (F_v/F_m). Maximum ETR through PSII in both seasons was between 175-180 µmol $m^{-2}s^{-1}$ (Figs. 4B, E). Minimum F_v/F_m values ranged from 0.75 to 0.79 at midday for both seasons (Figs. 4C, F).

In both seasons, decreases in quantum yield of PSII and F_v/F_m, from dawn to midday, coincided with increases in incident PPFD (Figs. 5A, D, C, F). The relationship between ETR through PSII and PPFD was linear in winter and curvilinear in summer (Figs. 5B, E).

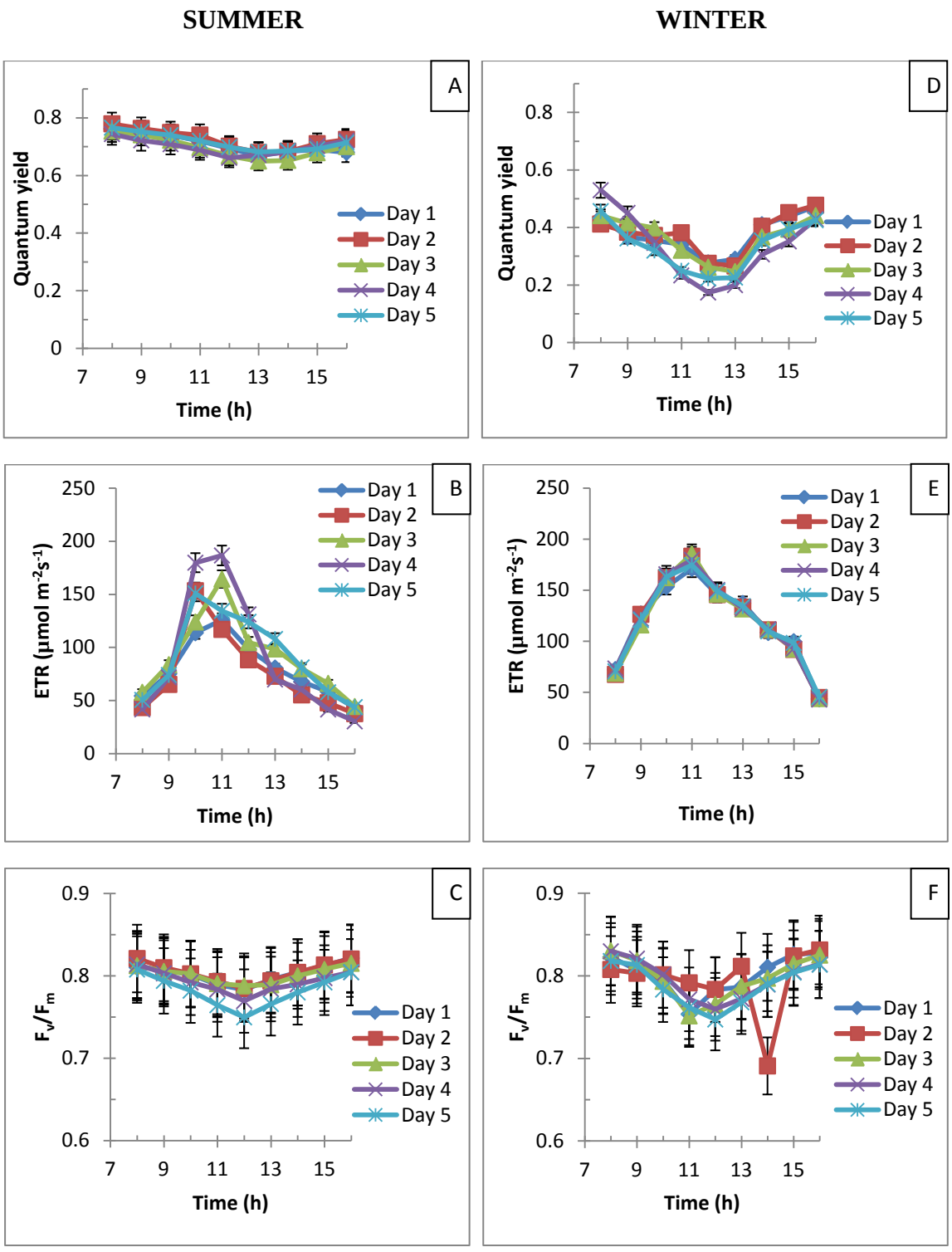

Fig. 4. Diurnal changes in photosynthetic quantum yield (A, D), electron transport rate through PSII (B, E) and the potential quantum yield of PSII photochemistry in the dark adapted state (F_v/F_m) (C, F) in summer and winter respectively. Healthy single stem of *Chromolaena odorata* plants were measured on five consecutive days in each season. Each point is the mean value of five independent readings. Vertical bars represent standard error. Bars not visible indicate standard error smaller than the symbol.

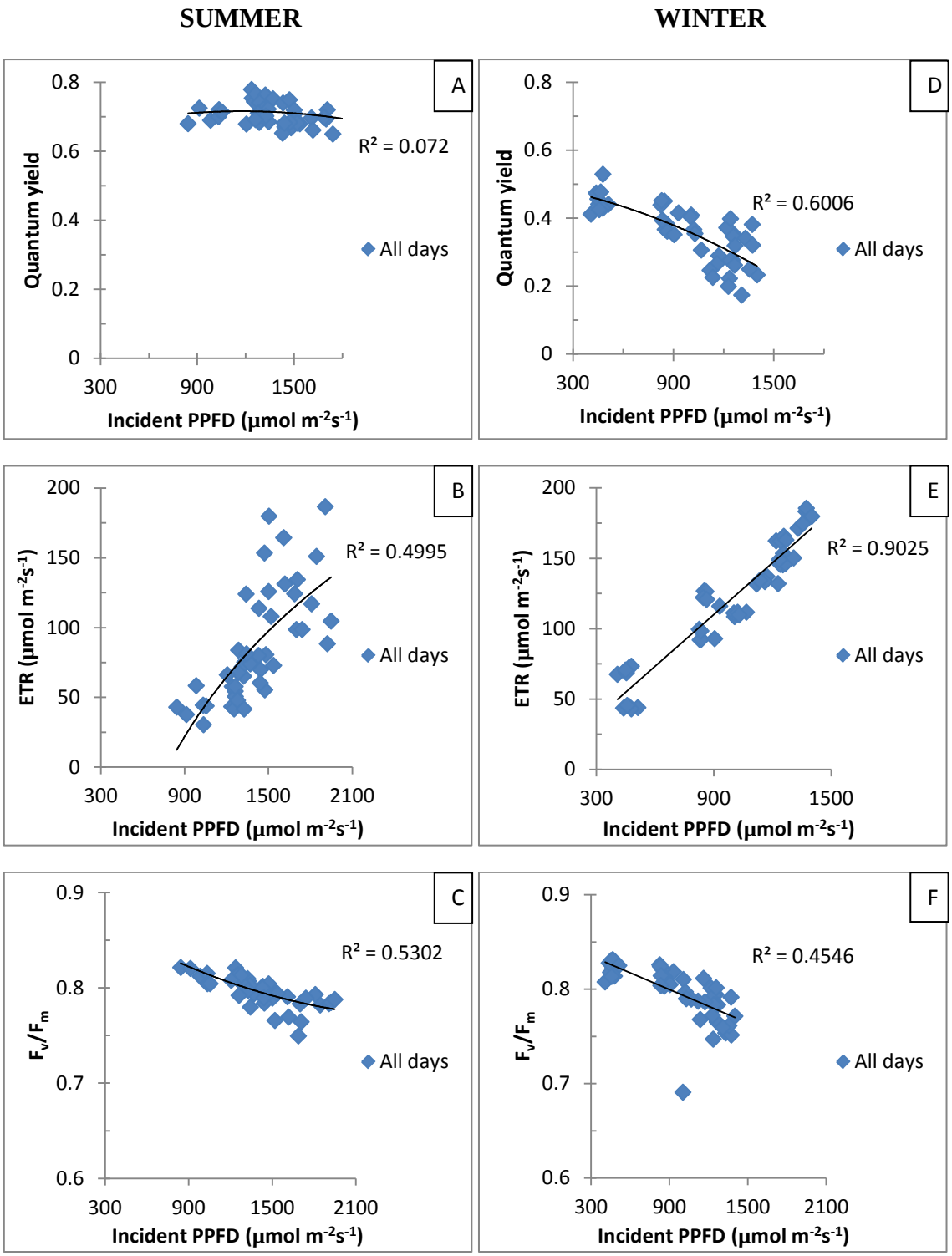

Fig. 5. Relationship between photosynthetic quantum yield and incident photosynthetic photon flux density (A, D), electron transport rate through PSII and incident photosynthetic photon flux density (B, E) and the potential quantum yield of PSII photochemistry in the dark adapted state (F_v/F_m) and incident photosynthetic photon flux density (C, F) in summer and winter respectively. Healthy single stem of *Chromolaena odorata* plants were measured on five consecutive days in each season. Each point is the mean value of five independent readings. The regression lines are indicated by R^2 values.

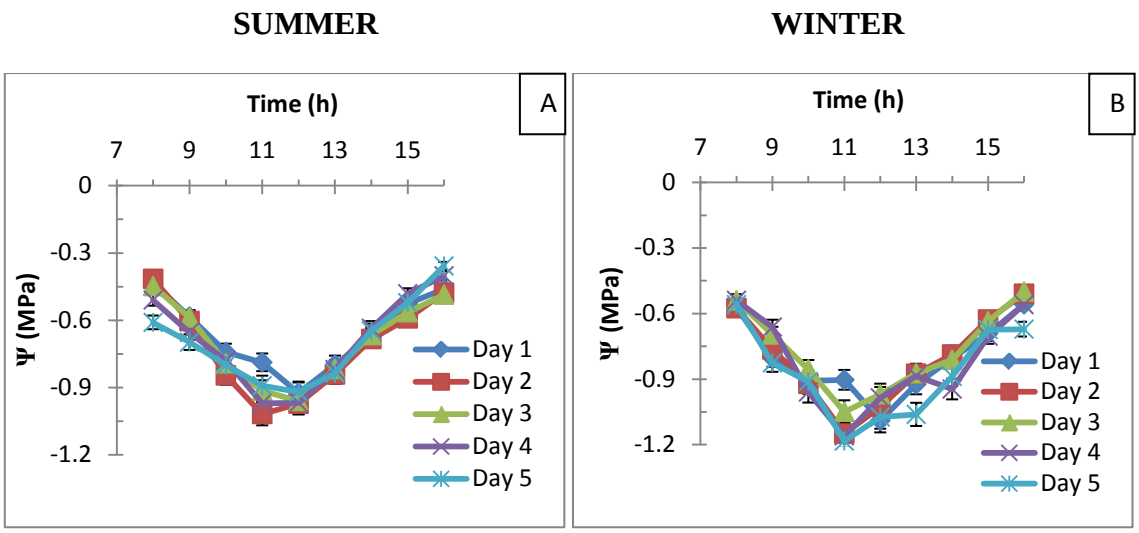

Fig. 6. Diurnal changes in leaf water potential (Ψ) in *Chromolaena odorata* in summer (A) and winter (B). Healthy single stem of *Chromolaena odorata* plants were measured on five consecutive days in each season. Each point is the mean value of two independent readings. Vertical bars represent standard error. Bars not visible indicate standard error smaller than the symbol.

Leaf water potential was significantly higher in summer compared to winter (Figs. 6A, B). Higher leaf water potentials in summer and winter were associated with increases in leaf conductance and CO_2 uptake (Figs. 7A-D).

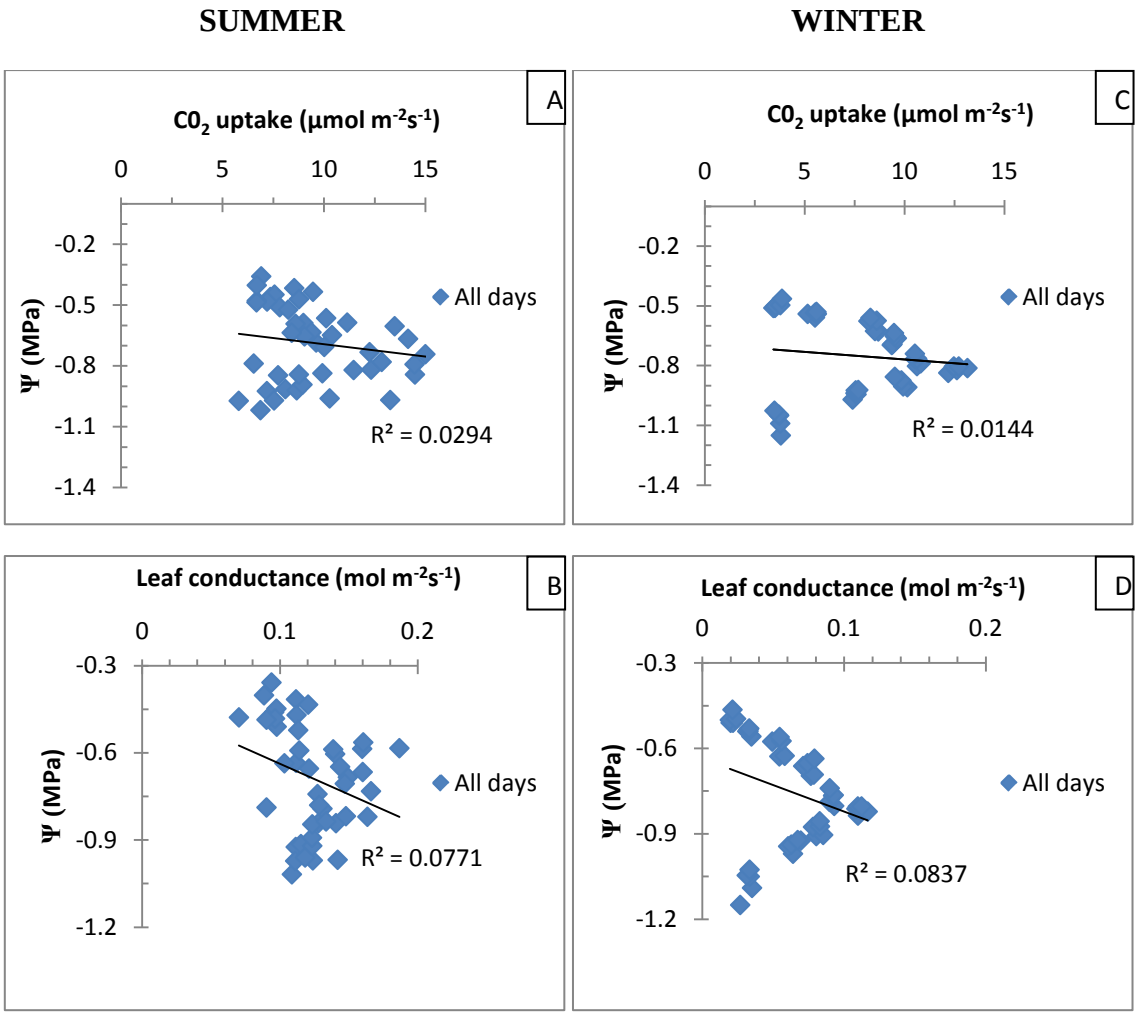

Fig. 7. Relationship between CO_2 uptake and leaf water potential (A, C) and leaf conductance and leaf water potential (B, D) in summer and winter. Healthy single stem of *Chromolaena odorata* plants were measured on five consecutive days in each season. Each point is the mean value of five (CO_2 uptake and leaf conductance) and two (leaf water potential) independent readings. The regression lines are indicated by R^2 values.

Photosynthetic quantum yield and leaf water potential were significantly higher in summer than in winter (Table 1). Leaf conductance was significantly correlated with PPFD and transpiration in winter (Table 2). In addition, ETR was significantly correlated with transpiration in both seasons (Table 2).

Table 1. Ecophysiological differences (maximal values) between summer and winter measurements.

PARAMETER	SUMMER	WINTER
A (µmol $m^{-2}s^{-1}$)	16	13
PPFD (µmol $m^{-2}s^{-1}$)	2000	1400
g (mol $m^{-2}s^{-1}$)	0.18	0.11
E (mmol $m^{-2}s^{-1}$)	4.5	4.3
WUE (µmol CO_2/ $mmol^{-1}$ H_2O)	3.55	3.02
Temp. (^oC)	37	36
Yield	0.78	0.53*
ETR (µmol $m^{-2}s^{-1}$)	182	180
F_v/F_m	0.82	0.83
Ψ (MPa)	-0.40	-0.47*

Statistical significance of unpaired "t" tests is indicated at $P \leq 0.05$(*).

Abbreviations { *A*=CO_2 uptake, PPFD=photosynthetic photon flux density, *g*=leaf conductance, *E*=transpiration, WUE=water use efficiency, Temp. =ambient temperature, ETR=electron transport rate, F_v/F_m=ratio of variable to maximal fluorescence, Ψ=leaf water potential}

Table 2. Pearson's correlation coefficients for summer and winter measurements (values in bold are significant at $P \leq 0.05$).

	A **(W)**	***A*** **(S)**	**PPFD (W)**	**PPFD (S)**	***g*** **(W)**	***g*** **(S)**	***E*** **(W)**	***E*** **(S)**	**Temp. (W)**	**Temp. (S)**	**ETR (W)**	**ETR (S)**	***Yield*** **(W)**	***Yield*** **(S)**	F_v/F_m **(W)**	F_v/F_m **(S)**	**Ψ (W)**	**Ψ (S)**
A**(W)**	1																	
A**(S)**	-0.048	1																
PPFD (W)	**0.963**	-0.058	1															
PPFD (S)	**0.824**	0.006	**0.803**	1														
g**(W)**	**0.952**	-0.029	**0.897**	**0.829**	1													
g**(S)**	0.724	0.024	0.671	0.699	0.743	1												
E**(W)**	**0.935**	-0.059	**0.915**	0.776	**0.862**	0.641	1											
E**(S)**	**0.785**	0.043	0.760	0.755	**0.813**	0.754	0.653	1										
Temp. (W)	0.746	-0.099	**0.783**	0.546	0.651	0.397	**0.803**	0.360	1									
Temp. (S)	0.734	-0.037	0.755	0.625	0.674	0.595	0.715	0.655	0.452	1								
ETR (W)	**0.947**	-0.029	**0.940**	**0.805**	**0.933**	0.731	**0.834**	**0.841**	0.633	0.698	1							
ETR(S)	**0.780**	-0.016	0.764	0.670	**0.793**	0.650	0.664	**0.777**	0.446	0.665	**0.830**	1						
Yield (W)	-0.712	0.177	-0.732	0.701	-0.674	-0.578	-0.737	-0.539	-0.638	-0.636	-0.635	-0.530	1					
Yield (S)	-0.093	-0.008	-0.100	0.043	-0.052	-0.081	-0.099	-0.126	-0.137	-0.091	-0.071	-0.075	0.035	1				
$\mathbf{F_v/F_m}$ **(W)**	-0.408	0.032	-0.394	0.373	-0.374	-0.305	-0.428	-0.296	-0.308	-0.354	-0.350	-0.273	0.336	0.020	1			
$\mathbf{F_v/F_m}$**(S)**	-0.701	0.038	-0.709	0.698	-0.677	-0.585	-0.731	-0.601	-0.534	-0.757	-0.632	-0.571	**0.788**	0.081	0.388	1		
Ψ (W)	-0.823	-0.651	-0.804	0.732	-0.795	-0.642	-0.802	-0.661	-0.650	-0.652	-0.777	-0.664	0.696	0.286	0.358	0.716	1	
Ψ(S)	-0.854	-0.660	-0.843	**0.813**	-0.854	-0.677	-0.821	-0.727	-0.674	-0.651	-0.822	-0.698	0.676	0.313	0.340	0.669	0.735	1

Abbreviations {W=winter, S=summer, *A*=CO_2 uptake, PPFD=photosynthetic photon flux density, *g*=leaf conductance, *E*=transpiration, Temp.=ambient temperature, ETR=electron transport rate, F_v/F_m=ratio of variable to maximal fluorescence, Ψ=leaf water potential}

4.5. Discussion

Significant correlation between incident PPFD and leaf conduction, transpiration and ETR through PSII was evident in winter, indicating the importance of light on photosynthetic parameters. The study showed that leaf conductance tightly controlled CO_2 uptake and transpiration in winter. A 13% reduction in CO_2 uptake, possibly due to stomatal limitations imposed by lower PPFD, occurred in winter. Seasonal reductions in leaf conductance due to lower PPFD were responsible for lower photosynthetic rates of *Citrus sinensis* (L.) Osbeck (Ribeiro *et al*., 2009). Early morning incident PPFD measurements were considerably higher in summer (1200 µmol $m^{-2}s^{-1}$) than winter (500 µmol $m^{-2}s^{-1}$) resulting in higher CO_2 uptake rates (Figs. 1A & D). There was no saturation of CO_2 uptake at high incident PPFD (Figs. 3A, D). The relationship between CO_2 uptake and incident PPFD was almost linear in both summer and winter. However, the relationship between CO_2 uptake and leaf conductance and transpiration was non linear. This suggests that incident PPFD drives leaf conductance and subsequently CO_2 uptake and transpiration in both seasons.

Air temperature affects seasonal variation in photosynthetic capacity in winter (Harris *et al*., 2006; Hughes & Smith, 2007; Bartak *et al*., 2012). Diurnal time courses in summer and winter ambient temperatures (Fig. 2B, D) were similar in our study (Table 1). Early summer mornings were characterised by heavy cloud cover which dissipated during mid morning, whereas winter was characterised by clear blue skies. In summer, fluctuations in incident PPFD due to cloud presence accounted for the variation in gas exchange parameters. Higher CO_2 uptake rates, lower evapotranspiration (Rocha *et al*., 2004; Weng *et al*., 2005; Riberio *et al*., 2009) and altered stomatal dynamics on cloudy compared to clear days were reported previously (Flexas *et al*., 2001). In our study CO_2 uptake (Figs. 1B, E) and transpiration (Figs. 2A, C) were higher in summer compared to winter, probably due to higher incident PPFD.

Stomatal control is the major physiological factor that optimises water use under high temperature (Guerfel *et al*., 2009). Plants can reduce excessive water loss by closing stomata at high temperatures (Fernandez *et al*., 1997; Moriana *et al*., 2002). Higher leaf conductance in summer, compared to winter, was probably due to greater soil moisture availability in the former season (Figs. 1C, F). Plants had higher water use efficiency in summer compared to winter, probably to maximise CO_2 uptake and minimise water loss (Table 1).

The summer months (December - February) are characterised by higher air temperatures and solar radiation, longer photoperiods and more abundant rainfall than winter (June-August). Seasonal variation between net photosynthesis and leaf conductance was due to rainfall in other studies (Flexas *et al*., 2001; Ribeiro *et al*., 2009). Rainfall in KwaZulu-Natal summers generally occurs as late afternoon thunderstorms.

Higher PPFD in summer may possibly affect photosynthetic enzymes and cause a reduction in ETR and F_v/F_m (dos Santos *et al*., 2013; Guerfel *et al*., 2009). The F_v/F_m ranged from 0.82 to 0.75 in summer and from 0.81 to 0.75 in winter. Generally, F_v/F_m values below 0.75 are indicative of a stressful condition in plants (Bolhar-Nordenkampf *et al*., 1989). Reduced F_v/F_m at midday in both seasons, indicated that dynamic photoinhibition probably occurred. Dynamic photoinhibition occurs at midday (dos Santos *et al*., 2013) with excessive PPFD stress (Maxwell & Johnson, 2000; Casaroli *et al*., 2007) resulting in decreased quantum efficiency. In summer, photoinhibition at high incident PPFD caused greater stomatal closure and reduced water loss through transpiration (Guerfel *et al*., 2009). In *Quercus ilex L.*, F_v/F_m varied with seasonal changes in temperature and soil water availability (Ogaya & Penuelas, 2003). Decreased F_v/F_m at midday in both seasons was reversible with values at dawn closely approximating those at dusk suggesting no damage to the photosynthetic apparatus (Bolhar-Nordenkampf *et al*., 1989).

Lower ETR values at midday, in summer and winter, were possibly due to stomatal limitations that reduced excessive water loss. Similarly, other researchers have shown that a reduction in CO_2 uptake and ETR at midday was caused by stomatal closure in response to water loss (Ogaya & Penuelas, 2003; Llusia & Penuelas, 2000). In this study, high ETR values coincided with high incident PPFD. The relationship between incident PPFD and ETR was more noticeable in winter, despite incident PPFD values being lower. Midday photosynthetic quantum yield decreased by 20% in summer and 50% in winter. Plants were more stressed in winter than summer, evident by significantly lower leaf water potential and photosynthetic quantum yield values. Recovery in leaf water potential by late afternoon, in both seasons, suggested that plants recovered from the effects of transient water stress.

4.6. Conclusion

Leaf conductance, CO_2 uptake, transpiration and ETR in winter were more tightly coupled than in summer. It is likely that the differences observed in gas exchange and chlorophyll fluorescence parameters, between seasons, were due to fluctuations in incident PPFD caused by increased cloud cover in summer. In summer, cloud cover can possibly reduce the likelihood of water stress in subtropical climates. Incident PPFD drives leaf conductance and subsequently transpiration in both seasons. Reductions in photosynthetic quantum yield in summer were probably caused by photoinhibition and in winter, by water stress.

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5. Effects of water stress on photosynthetic performance

5.1. Abstract

The effects of water stress on leaf gas exchange and chlorophyll fluorescence characteristics of *Chromolaena odorata* were investigated under controlled conditions. Thirty healthy seedlings of *C. odorata* of uniform size (approximately 45 cm height) were selected from the field and transplanted into 10 litre plastic pots filled with a soil mixture consisting of equal quantities of sand, loam and compost. Plants were randomly assigned into two groups (well watered = WW and water stressed = WS). Plants were allowed to acclimate for three months and were watered daily to the point of saturation. After acclimation, WW plants were watered daily until water drained freely from the pots. In the WS treatment, water was withheld for the duration of the experiment. Gas exchange measurements were taken every two days, starting from the day after water was withheld (day 2), to the end (day 10), when further measurements were not possible due to severe wilting. Maximum CO_2 uptake in the WW treatment ranged from 10.5 to 12.8 µmol $m^{-2}s^{-1}$ over ten days. In the WS treatment, maximum CO_2 uptake decreased progressively from 5.8 µmol $m^{-2}s^{-1}$ on day 2 to 1.0 µmol $m^{-2}s^{-1}$ on day 10. Trends in leaf conductance, transpiration and electron transport rate (ETR) in both treatments followed a pattern similar to CO_2 uptake. There was no saturation of CO_2 uptake at high photosynthetic photon flux density (PPFD) in the WW treatment. In the WS treatment, CO_2 uptake at saturating PPFD was 4.7 µmol $m^{-2}s^{-1}$ over 10 days. In the WW treatment, quantum yield of PSII decreased from a maximal value at dawn to midday and thereafter recovered to early morning values at dusk on all days. In the WS treatment, quantum yield decreased progressively from day 2 to day 10. Trends in the potential quantum yield of PSII in the dark adapted state (F_v/F_m) followed a similar pattern to photosynthetic quantum yield in both treatments. Leaf water potential values ranged from -0.25 to -0.3 MPa for the WW treatment over 10 days. In the WS treatment, leaf water potential was -0.25 MPa at the commencement of treatment and decreased to -1.9 MPa on day 10. Proline concentrations on all days showed little deviation from initial values in WW plants. Concentrations of proline increased with the duration of stress in WS plants and followed a trend similar to leaf water potential. This study showed that leaf wilting in *C. odorata* is an

adaptive response to prevent excessive loss of water which may be strategically important in water stressed environments.

5.2. Introduction

There are numerous studies on plant response to soil water status (Abril & Hanano, 1998; Arora *et al*., 2001; Chaitanya *et al*., 2003; Tsuji *et al*., 2003; Gindaba *et al*., 2004; Yang *et al*., 2006; Efeoglu *et al*., 2009). Drought is the primary environmental condition that has a great impact on physiological process and growth in plants (Borchert, 1991; Kramer, 1986). Plant responses to water stress may involve adaptive changes and/or deleterious effects (Chaves *et al*., 2002). Under field conditions, drought response can cause changes in biomass accumulation, growth rate and many other physiological or structural traits (Arora *et al*., 2001; Chaitanya *et al*., 2003). Plant strategies to cope with drought normally involve stress avoidance and "tolerance" strategies that vary with genotype (Chaves *et al*., 2002). Early responses to water stress aid survival, whereas acclimation, using new metabolic and structural capabilities, help to improve plant functioning (Bohnert & Shaveleva, 1998).

Nutrient uptake by plants is decreased under drought conditions due to reduced transpiration, impaired active transport and membrane permeability, resulting in reduced root absorbing power (Tanguilig *et al*., 1987). Most of the damaging effects of drought are associated with the photosynthetic process of the plant (Efeoglu *et al*., 2009). Photosynthesis is known to be very sensitive to environmental stress. When soil water availability is limited, photosynthetic rate often limits plant growth (Huang & Fu, 2000). The decrease in photosynthetic activity under drought stress can be attributed to both stomatal and non-stomatal limitations (Shangguan *et al*., 1999; Yordanov *et al*., 2003; Zlatev & Yordanov, 2004). Non-stomatal limitations to photosynthesis have been attributed to reduced carboxylation efficiency (Jia & Gray, 2004), reduced ribulose-1,5-bisphosphate (RuBP) regeneration (Lauer & Boyer, 1992), reduced amount of functional ribulose-1,5-bisphosphate carboxylase (Kanechi *et al*., 1995) or to the inhibited functional activity of PSII. Stomatal closure is generally accepted to be the main determinant for decreased photosynthesis under mild to moderate stress (Sharkey, 1990; Chaves, 1991; Ort *et al*., 1994; Nayyer & Gupta, 2006; Yang *et al*., 2006). Stomatal closure may limit CO_2 uptake and promote an imbalance between photochemical activity of PSII and the electron requirement of the Calvin-Benson cycle. This may lead to an excess of absorbed excitation energy and subsequent photoinhibitory damage to PSII reaction centres (Foyer &

Noctor, 2000). Many plants exposed to high solar radiation and low water availability have evolved mechanisms to protect against photoinhibition. Inhibition or damage to the primary photochemical and biochemical processes may occur simultaneously (Lawlor, 2002). Photo-protective mechanisms may involve physiological adjustments in leaf morphology, anatomy, leaf biochemistry and photochemistry (Lovelock & Clough, 1992; Havaux & Niyogi, 1999).

The present study aims to determine the effects of drought stress on leaf gas exchange and chlorophyll fluorescence in *C. odorata*. Monitoring changes in stomatal conductance, CO_2 exchange and PSII electron transport efficiency, may allow for an evaluation of the responses of *C. odorata* to water stress. Information on the the degree of drought tolerance may be useful in explaining present distribution trends and predicting future encroachment patterns for *C. odorata*.

5.3. Materials & methods

5.3.1. *Plant material and experimental design*

The study was undertaken in June 2005 within the University of KwaZulu-Natal (Westville campus) conservancy (29°49'S 30°56'E), Durban, South Africa. Thirty healthy seedlings of *C. odorata* of uniform size (about 45 cm height) were selected from the field and transplanted in 10 L plastic pots filled with a soil mixture consisting of equal quantities of sand, loam and compost. The pots were kept out in the open under naturally varying microclimatic conditions. Plants were left to acclimate for three months and were watered daily to the point of saturation. At the end of three months, plants were randomly assigned into two groups, well watered (WW) and water stressed (WS). In the WW group, plants continued to be watered every day after sunset until water drained freely from the pots. Soil surfaces were covered with plastic film and aluminium foil to reduce evaporation, to minimise temperature fluctuation and to prevent rain from entering the pots. The WS plants were not watered for the duration of the study. Measurements were taken every two days starting from the day after water was withheld (day 2) to the end (day 10), when further measurements were not possible due to severe wilting.

5.3.2. *Gas exchange*

Gas exchange characteristics of *C. odorata* were measured (n=15) as outlined in chapter 1.

5.3.3. *Chlorophyll fluorescence*

Fluorescence characteristics of *C. odorata* were measured (n=15) as outlined in chapter 1.

5.3.4. *Leaf water potential*

Leaf water potential was measured (n=2) at 07h00, at two day intervals, with a Scholander pressure chamber (Soil Moisture Equipment Corp., Santa Barbara, USA). Measurements were made following the procedure outlined in chapter 1.

5.3.5. *Proline*

Proline was analysed (n=15) following the procedure outlined in chapter 2.

5.3.6. *Statistics*

Analysis was similar to the procedure outlined in Chapters 2 and 3.

5.4. Results

Diurnal time courses of leaf conductance, CO_2 uptake and transpiration for WW and WS plants are illustrated in Fig. 1 (A-F). Maximum CO_2 uptake in the WW treatment ranged from 10.5 to 12.8 µmol $m^{-2}s^{-1}$ (Fig. 1A) over all days. In the WS treatment, maximum CO_2 uptake decreased progressively from 5.8 µmol $m^{-2}s^{-1}$ at the commencement of the treatment to 1.0 µmol $m^{-2}s^{-1}$ at the end (Fig. 1D). Trends in leaf conductance, transpiration and ETR in both treatments followed a pattern similar to CO_2 uptake.

Over all days, quantum yield in WW plants decreased from a maximal value at dawn, to a minimum at midday and then recovered to dawn values at dusk (Fig. 2A). In the WS treatment, quantum yield of PSII decreased progressively from dawn to dusk with gradual

recovery (Fig. 2 D). Trends in the potential quantum yield of PSII in the dark adapted state followed a trend similar to photosynthetic quantum yield, in both treatments (Figs. 2C, F).

The CO_2 uptake increased with increases in incident PPFD in the WW treatment (Fig. 3A) with no light saturation. In the WS treatment, CO_2 uptake was saturated at a PPFD of 1300 µmol $m^{-2}s^{-1}$ (Fig. 3D). Lower leaf conductance in the WS treatment resulted in decreases in CO_2 uptake (Fig. 3E) and transpiration (Fig. 3F), unlike the WW treatment.

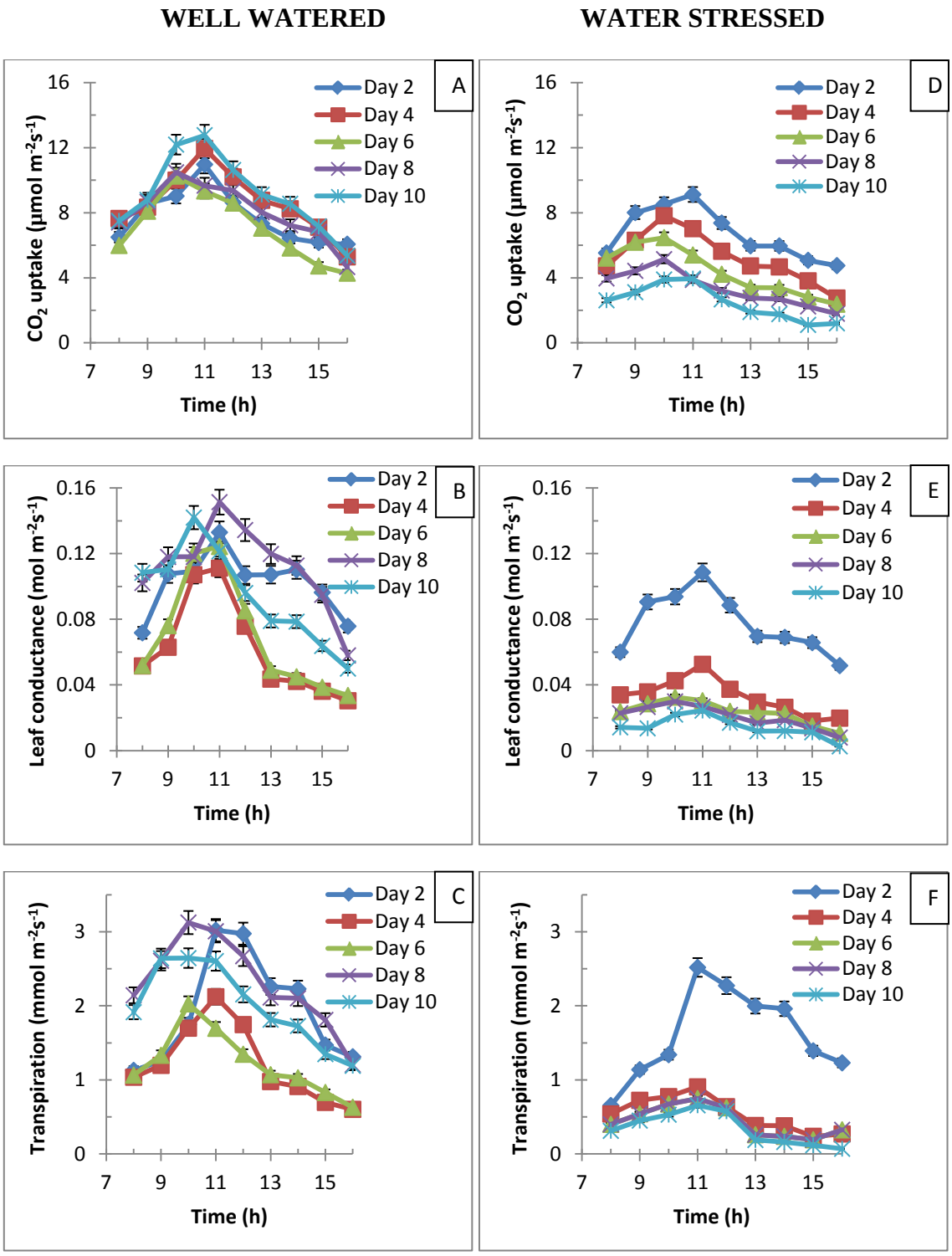

Fig. 1. Diurnal changes in CO_2 uptake (A, D), leaf conductance (B, E), and transpiration (C, F) in well watered and water stressed *Chromolaena odorata* plants. Measurements were taken on healthy single stem of plants at two day intervals for 10 days. Each point is the mean value of five independent readings. Vertical bars represent standard error. Bars not visible indicate standard error smaller than the symbol.

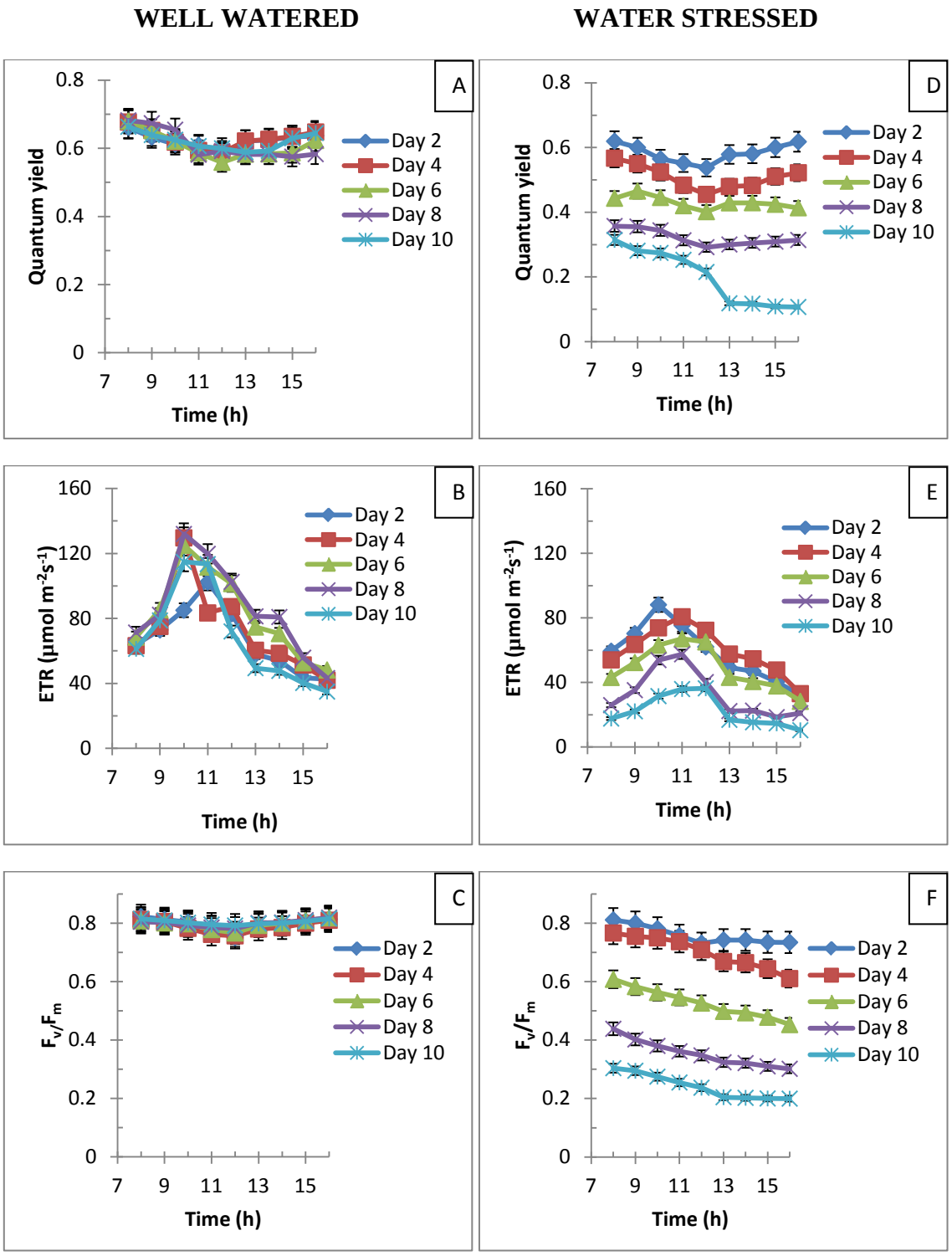

Fig. 2. Diurnal changes in photosynthetic quantum yield (A, D), electron transport rate through PSII (B, E), and the potential quantum yield of PSII photochemistry in the dark adapted state (F_v/F_m) (C, F) in well watered and water stressed *Chromolaena odorata* plants. Measurements were taken on healthy single stem of plants at two day intervals for 10 days. Each point is the mean value of five independent readings. Vertical bars represent standard error. Bars not visible indicate standard error smaller than the symbol.

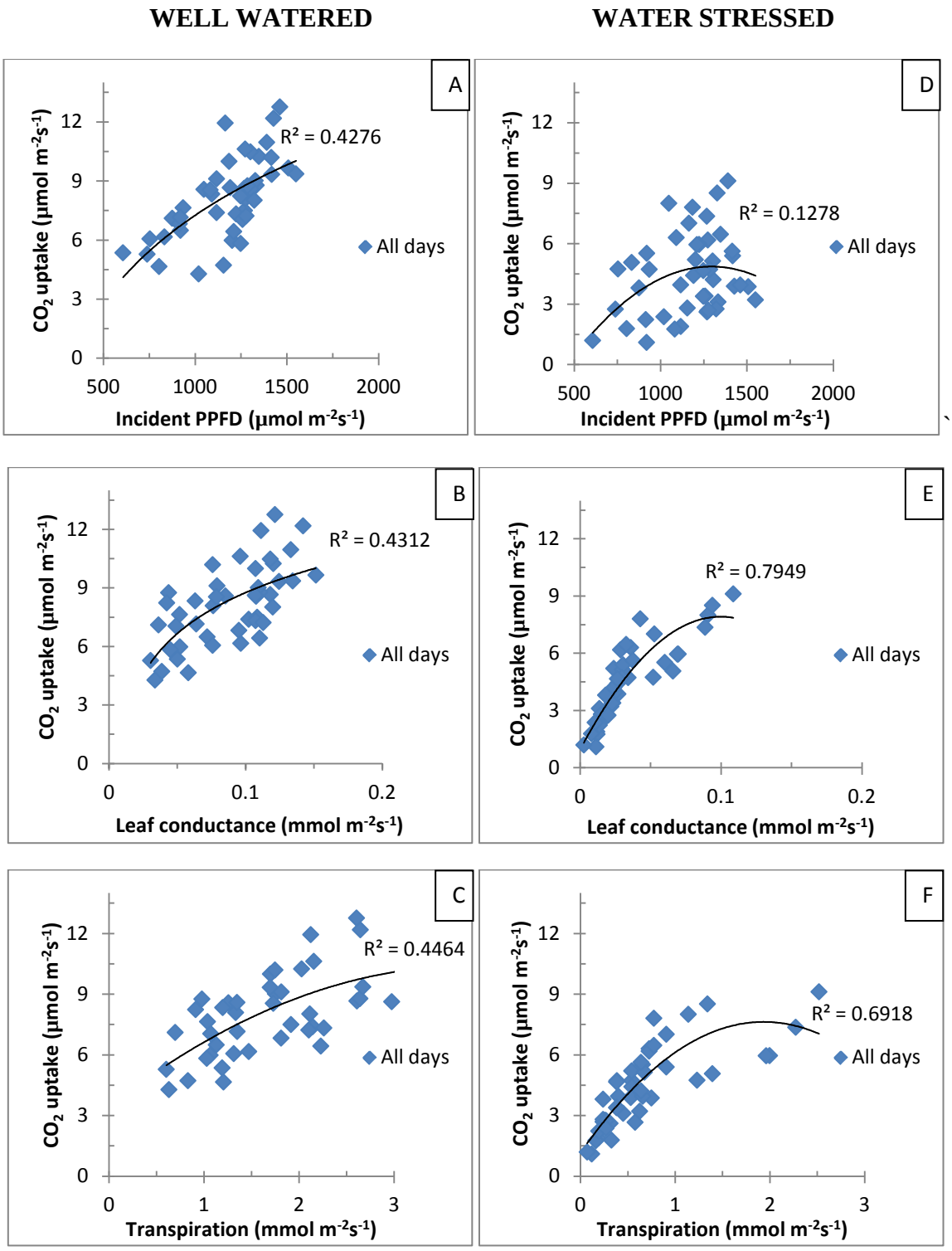

Fig. 3. Relationship between CO_2 uptake and incident photosynthetic photon flux density (A, D), CO_2 uptake and leaf conductance (B, E) and CO_2 uptake and transpiration (C, F) in well watered and water stressed *Chromolaena odorata* plants. Measurements were taken on healthy single stem of plants at two day intervals for 10 days. Each point is the mean value of five independent readings. The regression lines are indicated by R^2 values.

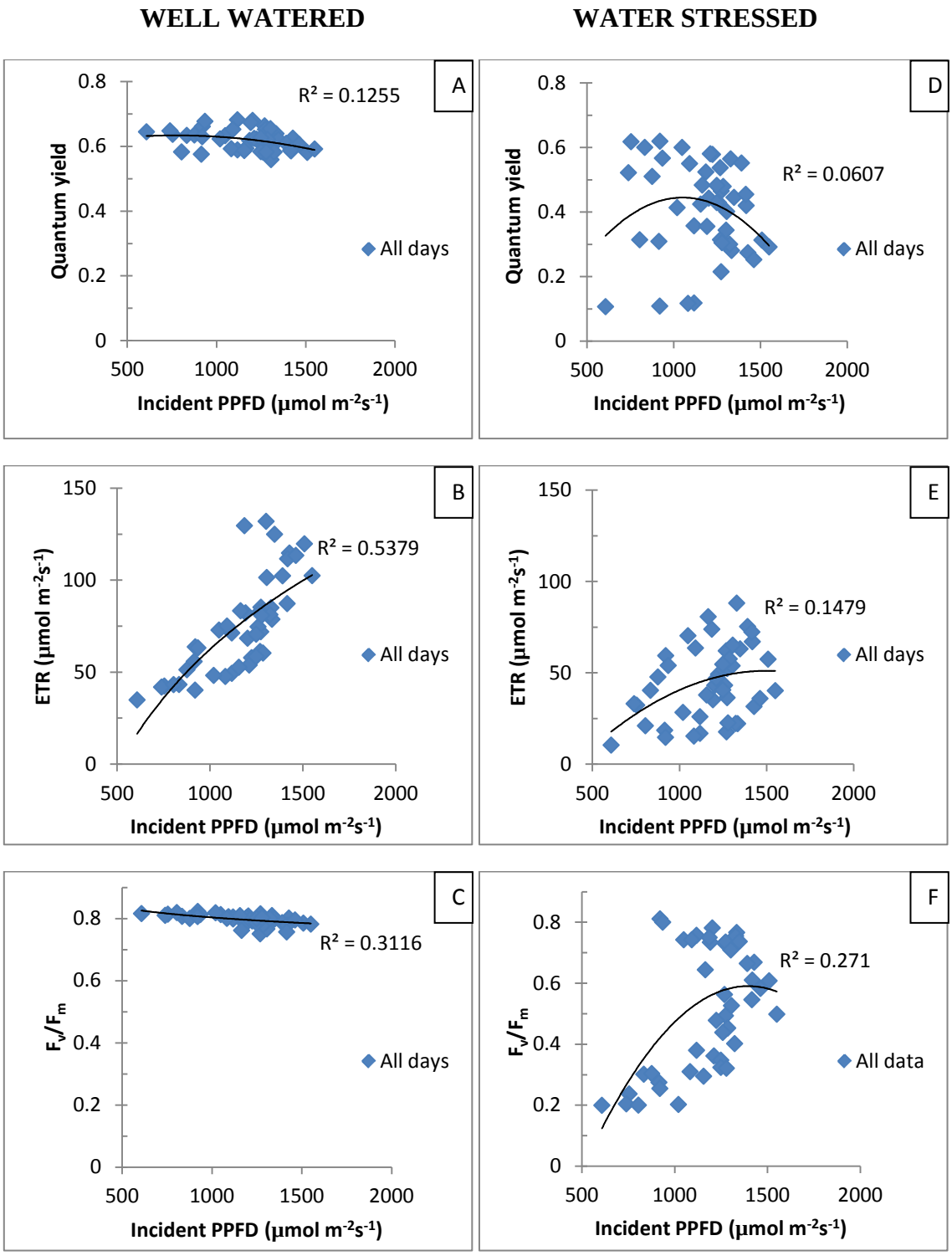

Fig. 4. Relationship between quantum yield and incident photosynthetic photon flux density (A, D), electron transport rate through PSII and incident photosynthetic photon flux density (B, E) and the potential quantum yield of PSII photochemistry in the dark adapted state (F_v/F_m) and incident photosynthetic photon flux density (C, F) in well watered and water stressed *Chromolaena odorata* plants. Measurements were taken on healthy single stem of plants at two day intervals for 10 days. Each point is the mean value of five independent readings. The regression lines are indicated by R^2 values.

In the WW treatment, photosynthetic quantum yield decreased as incident PPFD increased from 550 to 1500 µmol $m^{-2}s^{-1}$ (Fig. 4A). In the WS treatment, photosynthetic quantum yield increased as incident PPFD increased from 600 to 1100 µmol $m^{-2}s^{-1}$, and thereafter decreased (Fig. 4 D). Trends in the potential quantum yield of PSII in the dark adapted state followed a pattern similar to photosynthetic quantum yield, in both treatments.

Leaf water potential values ranged from -0.25 to -0.3 MPa for the WW treatment (Fig. 5) over 10 days. In the WS treatment, Ψ decreased from -0.25 MPa on day 0 to -1.9 MPa on day 10 (Fig. 5). The progressive decrease in Ψ with increases in water stress was associated with decreases in leaf conductance, CO_2 uptake and transpiration. Concentrations of proline increased progressively from day two to day 10 with increase in water stress in WS plants (Fig. 6) and were correlated with a decrease in Ψ. In WW plants, proline concentrations on all days showed little variation (Fig. 6).

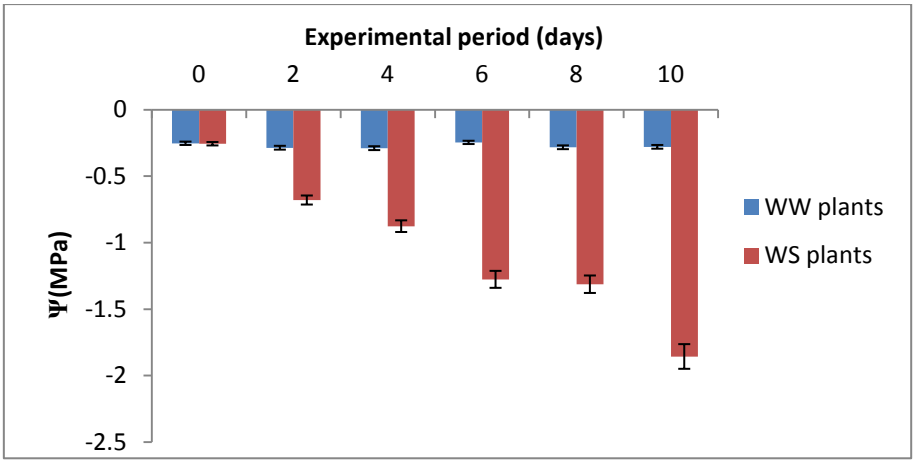

Fig. 5. Changes in leaf water potential in well watered and water stressed *Chromolaena odorata* plants over 10 days. Vertical bars represent the standard error.

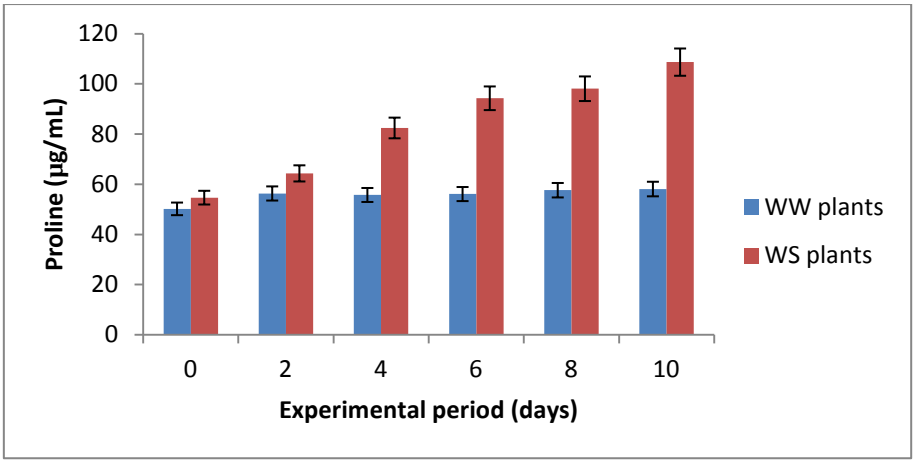

Fig. 6. Changes in proline concentration in well watered and water stressed *Chromolaena odorata* over 10 days. Vertical bars represent the standard error.

On day 10, maximum CO_2 uptake in the WW treatment was significantly greater than that in the WS treatment (Table 1). Leaf conductance was significantly correlated with CO_2 uptake and transpiration in the WS treatment (Table 2).

Table 1. Ecophysiological differences (maximal values) between well watered (WW) and water stressed (WS) plants on day 10.

PARAMETER	WW	WS
A (µmol $m^{-2}s^{-1}$)	12.8	4.0*
g (mol $m^{-2}s^{-1}$)	0.14	0.025*
E (mmol $m^{-2}s^{-1}$)	2.6	0.6*
WUE (µmol CO_2/ $mmol^{-1}$ H_2O)	4.92	6.67*
Yield	0.66	0.30*
ETR (µmol $m^{-2}s^{-1}$)	115	38*
F_v/F_m	0.82	0.30*
Ψ (MPa)	-0.3	-1.9*

Statistical significance of unpaired "t" tests is indicated at $P \leq 0.05$ (*).

Abbreviations {*A*=CO_2 uptake, *g*=leaf conductance, *E*=transpiration, WUE=water use efficiency, ETR=electron transport rate, F_v/F_m=ratio of variable to maximal fluorescence, Ψ=leaf water potential}

Table 2. Pearson's correlation coefficients between well watered (WW) and water stressed (WS) *C. odorata* plants (values in bold are significant at $P \leq 0.05$).

	A **(WW)**	***A*** **(WS)**	***g*** **(WW)**	***g*** **(WS)**	***E*** **(WW)**	***E*** **(WS)**	**ETR (WW)**	**ETR (WS)**	**Yield (WW)**	**Yield (WS)**	**F_v/F_m (WW)**	**F_v/F_m (WS)**	**Temp.**	**PPFD**
A **(WW)**	1													
A **(WS)**	0.370	1												
g**(WW)**	0.208	0.087	1											
g **(WS)**	0.193	**0.817**	0.010	1										
E **(WW)**	0.556	0.229	0.125	0.329	1									
E **(WS)**	0.163	0.694	0.035	**0.870**	0.500	1								
ETR (WW)	0.715	0.428	0.298	0.170	0.493	0.144	1							
ETR (WS)	0.394	**0.824**	0.034	0.632	0.049	0.466	0.483	1						
Yield (WW)	-0.134	0.126	0.033	0.030	-0.168	-0.022	-0.171	-0.102	1					
Yield (WS)	-0.128	0.730	0.002	0.688	-0.146	0.598	0.009	0.663	0.227	1				
F_v/F_m (WW)	-0.473	0.327	0.007	-0.264	-0.374	-0.324	-0.427	-0.502	0.483	-0.086	1			
F_v/F_m (WS)	-0.600	0.100	0.034	0.240	-0.397	0.111	-0.552	-0.130	0.519	0.448	0.642	1		
Temp.	0.322	0.325	0.008	-0.218	0.519	0.031	0.268	-0.242	-0.575	-0.521	-0.373	0.722	1	
PPFD	0.612	0.262	0.149	0.105	0.488	0.126	0.706	0.354	-0.369	-0.112	-0.554	0.695	0.491	1

Abbreviations {*A*=CO_2 uptake, *g*=leaf conductance, *E*=transpiration, ETR=electron transport rate, F_v/F_m=ratio of variable to maximal fluorescence, Temp.=ambient temperature, PPFD=photosynthetic photon flux density}

5.5. Discussion

There was a progressive decrease in leaf water potential with increase in water stress. Leaf water potential was measured at predawn to determine maximum values which can then be compared to midday minima (Jones, 1990). Generally, plants are considered stressed when the difference between treatments is more than 1.5 MPa (Flexas & Medrano, 2002). In our study the leaf water potential difference between the two treatments was 1.65 MPa (Fig. 5) suggesting that WS plants were severely stressed. Plants may lower water loss by reducing transpiration or by absorbing more water from the soil. A significant correlation between leaf conductance and transpiration suggests that partial stomatal closure in WS plants reduced water loss (Table 2).

In WS plants, decreases in leaf conductance ranged from 30% on day 2 to 87% on day 10. Leaf conductance was close to zero (0.001 mol $m^{-2}s^{-1}$) on day 10, suggesting almost complete stomatal closure (Fig. 1E). Stomatal closure was probably a regulatory response to limit water loss and maximise carbon gain. Water stressed leaves exhibited lower Ψ than WW plants (Fig. 5) and showed signs of severe wilting on day 10. This made further measurements of leaf water potential difficult. Leaf tips in WS plants were brown and leaves were in the process of abscising. Shedding of leaves commenced 15 days after the onset of stress and may represent an important strategy to conserve water and prevent mortality (Stagnari *et al*., 2014). In addition, the reallocation of nutrients stored in older to younger parts prior to abscission, serves to conserve plant resources (Chaves *et al*., 2003). The decrease in predawn Ψ in WS plants from -0.25 to -1.9 MPa over 10 days represented a reduction of 660% (Fig. 5). Partial closure of stomata in WS plants, probably due to lower Ψ, led to a 89% reduction in CO_2 uptake (Cornic, 2000; Molnar *et al*., 2004; Sun *et al*., 2013). The decrease in CO_2 uptake under water stress was probably due to an impairment of electron transport (Fig. 4E) (Flexas & Medrano, 2002). Decreased CO_2 uptake under low relative water content is caused by an impaired metabolism of photosynthetic enzymes, including ribulose bisphosphate carboxylase (Lawlor & Cornic, 2002; Campos *et al*., 2014). In sunflower, water stress inhibited regeneration of ribulose bisphosphate carboxylase (Tezara *et al*., 1999). Photosynthetic enzyme inhibition may cause a reduction in CO_2 uptake and increase WUE. Increase in WUE is a frequent water stress response in plants (Table 1) and is a mechanism of drought resistance (Starman & Lombardini, 2006). An increase in WUE in

the WS compared to the WW treatment decreased transpiration and is probably an adaptation to water stress.

Plants are regarded as being severely drought stressed when g > 0.05 mmol $m^{-2}s^{-1}$ (Flexas & Medrano, 2002). Water stressed plants were severely stressed from day six with maximum g values below 0.04 mol $m^{-2}s^{-1}$ (Fig. 1E). Severe water stressed conditions result in almost complete stomatal closure due to photoinhibition (Flexas & Medrano, 2002). Severe water stress (Epron *et al*., 1992; Flexas & Medrano, 2002; Souza *et al*., 2004) decreases F_v/F_m and is used as an indication of photoinhibition of PSII (Krause & Weiss, 1991). A reduction in photochemical efficiency of PSII and electron transport coincided with a decrease in leaf water content (Peeva & Cornic, 2009). This may indicate damage to PSII reaction centres caused by down regulation capacity of PSII electron transport (Osmond, 1994). However, the rapid decrease in F_v/F_m values suggested that chronic irreversible photoinhibition, rather than down regulation of photochemistry, occurred in WS plants.

The midday depression in F_v/F_m in WW plants (0.75- 0.77), due to photoinhibition, was probably caused by high PPFD. According to Mohammed *et al*. (1995), F_v/F_m values of 0.82-0.83 are typical of plants with a well functioning photosystem apparatus. Predawn values of the photochemical efficiency of PSII (0.81-0.83) in the WW treatment indicated that the photochemical apparatus of PSII was not affected by water stress (Fig. 4C). In WS plants, F_v/F_m values decreased progressively from 7% on day 2 to 76% on day 10 (Fig. 3F) suggesting a decrease in photochemical efficiency with increased stress.

Results from this study suggested that water stress reduced CO_2 exchange in *C. odorata.* Mechanisms to reduce photoinhibitory damage in plants include production of biochemical osmolytes such as glycine betaine, proline, ascorbate peroxidase and glutamine synthetase (Peeva & Cornic, 2009). Decrease in Ψ (Fig. 5) was accompanied by an increase in the concentration of proline in WS plants (Fig. 6) suggesting, that the metabolite was produced as a consequence of water stress. The synthesis of osmolytes such as proline has been suggested to stabilise membranes and maintain the conformation of proteins at low leaf water potentials (Efeoglu *et al*., 2009). Proline acts as an osmoprotector of cytosolic enzymes and organelles and enables plants to survive water stress (Csonka, 1989). Proline concentrations can increase up to 300 fold in plants exposed to water stress (Delauney & Verma, 1993). In our study, proline concentration in the WS treatment was 86% higher on day 10 compared to plants at

the onset of water stress. It is likely that proline, as well as other protective osmolytes not determined, may reduce photooxidative injury (Ashraf & Foolad, 2007) in stressed plants.

5.6. Conclusion

Chromolaena odorata responds to a reduction in soil water availability by reducing leaf conductance and Ψ. However, like most plant species, the weed is susceptible to prolonged water stress. The low tolerance to water stress may explain the limited abundance of the species in areas with low to medium rainfall. Increased proline concentration and leaf wilting probably increase WUE and may be an adaptive strategy to protect against dehydration injury, as in other mesic species. New leaves sprout readily when soil moisture increases suggesting an efficient strategy to recover after periods of prolonged water stress.

5.7. References

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6. Effects of glyphosate on plant mortality and photosynthetic characteristics

6.1. Abstract

The aim of this study was to determine the effectiveness of glyphosate, a common broad spectrum herbicide, on plant mortality in *Chromolaena odorata. Chromolaena odorata* plants of approximately 1.2 m in height were sprayed with 1% glyphosate. Gas exchange characteristics were monitored for five consecutive days after spraying. Plants were harvested daily 24 h after spraying and glyphosate concentrations were determined in leaves, stems and roots by high performance liquid chromatography (HPLC). After glyphosate application, CO_2 uptake decreased from 9.5 µmol $m^{-2}s^{-1}$ on day 1 to 0 µmol $m^{-2}s^{-1}$ on day five. Trends in leaf conductance, transpiration and electron transport rate (ETR) followed a pattern similar to CO_2 uptake. Saturation of CO_2 uptake occurred at a PPFD of 1750 µmol $m^{-2}s^{-1}$ on day one. Maximum CO_2 uptake occurred at a leaf conductance of 0.09 mol $m^{-2}s^{-1}$ on day one. There was a linear relationship between CO_2 uptake and transpiration over all days. Generally, trends in photosynthetic quantum yield and intrinsic photochemical efficiency of PSII (F_v/F_m) were similar to those of CO_2 uptake over all days. There were significant correlations between CO_2 uptake and transpiration, ETR and photosynthetic quantum yield and transpiration and F_v/F_m. Glyphosate concentration was high in leaves and stems after herbicide application (up to day three). After day three, most of the glyphosate was located in the roots suggesting that the herbicide accumulated below ground. The study showed that glyphosate rapidly decreases leaf conductance leading to a decrease in CO_2 uptake and transpiration. Glyphosate treatment of *C. odorata* resulted in 100% mortality within six days of spraying.

6.2. Introduction

Glyphosate and trichlopyr are two commonly used herbicides to treat *C. odorata* infestation (Langeland, 2012). Although trials have shown that trichlopyr is more effective than glyphosate (Goodall & Erasmus, 1996) its use is controversial because of associated links to cancer (Kegley *et al*., 2008) and higher toxicity rating in fish, amphibians, birds and aquatic

invertebrates (Rossi, 1998). Proper use of glyphosate to manage protected habitats from weeds is preferred, since it has fewer adverse environmental impacts than other commonly used herbicides (Langeland, 2012). Glyphosate is also readily biodegradable (Legrand, 2013) whereas trichlopyr residue has been found in the soil even after 477 days after application (Newton *et al*., 1990). Glyphosate was used in this study based on its low toxicity and environmental impacts.

Glyphosate has been used successfully to control a wide range of annual and perennial weeds in the last three decades (Franz *et al*., 1997). It inhibits the enzyme 5- enolpyruvylshikimate-3-phosphate (EPSP) synthase which catalyses the conversion of shikimate-3-phosphate and phosphoenolpyruvate (PEP) to yield EPSP and inorganic phosphate in the shikimate pathway (Geiger & Fuch, 2002). The inhibition of EPSP synthase results in shikimate accumulation and prevents the biosynthesis of the aromatic amino acids, phenylalanine, tyrosine and tryptophan (Huangfu *et al*., 2009).

Glyphosate is one of the best acidic herbicides demonstrating phloem mobility (Dewey & Appleby, 1983; Gougler & Geiger, 1981; Martin & Edgington, 1981) and easily passes through the cell membranes of parenchyma or phloem cells (de Ruiter & Meinen, 1996). Bromilow & Chamberlain (2000) tested more than fifty compounds, mostly acidic herbicides, and concluded that glyphosate was the most effective in the treatment of the invasive weed, *Ricinus communis* L. (castor bean). They demonstrated that high concentrations of glyphosate were found consistently in sink tissues such as the shoot and root apices. Another study, on the same species, showed that glyphosate was present in the phloem tissue of the shoot and root apices at levels of 30% and 20% respectively (Ferreira & Reddy, 2000). These studies confirmed the effectiveness of glyphosate in castor bean.

Glyphosate uptake by plant cells have been quantified in leaf discs (Ibaoui *et al*., 1986; Gougler & Geiger, 1981), cell suspensions (Mervosh & Balke, 1991; Richard & Slife, 1979; Royneberg *et al*., 1992), protoplasts (Denis & Delrot, 1993) and cell membrane vesicles (Reichers *et al*., 1994). Surfactants such as ammonium sulphate enhance foliar absorption of glyphosate (de Ruiter *et al*., 1988; de Ruiter *et al*., 1994; Gaskin & Holloway, 1992). Surfactant induced permeability of the cell membrane creates a steeper concentration gradient between the outside and the inside of the cuticle (de Ruiter & Meinen, 1996). However, other authors suggest that absorption and translocation of glyphosate can vary depending on the

chemical composition of the epicuticular wax of the species (Ferreira & Reddy, 2000). Furthermore, movement of glyphosate within a plant is related to the plant growth stage, sink-source relationships and the amount of herbicide absorbed (Duke, 1988; Franz *et al*., 1997; Sandberg *et al*., 1980).

Studies have also shown that glyphosate activity in water stressed plants is less effective than on non stressed ones due to reduced foliar absorption and translocation (de Ruiter & Meinen, 1998; Waldecker & Wyse, 1985; Klevorn & Wyse, 1984).

Glyphosate affects CO_2 exchange in plants (Madsen *et al*., 1995). Shaner and Lyon (1979) found a decrease in net photosynthesis and leaf conductance in bean plants 4 h after glyphosate application. According to Madsen *et al*. (1995) the diversion of carbon into the shikimate pathway causes a decrease in the amount of ribulose bisphosphate (RuBP) within 1 h after glyphosate application, followed by a decrease in net carbon exchange and a rise in leaf conductance after 1- 6 h. Measurements of chlorophyll fluorescence emission kinetics from leaves have been extensively employed in studies to determine the effectiveness of herbicides (Habash *et al*., 1985). Inhibition of photosynthetic electron transport by a herbicide results in modification of the kinetics of chlorophyll fluorescence induction due to the increased dissipation of absorbed radiant energy (Richard *et al*., 1983). This may result in irreversible damage to the thylakoid membrane (Singh *et al*., 1997) and cause reduced growth or total death of plants. Chlorophyll fluorescence kinetic studies on leaf tissues offer a potentially important research tool to assess the efficiency of herbicide penetration in weeds.

Chemicals are generally used to treat large weed infestations because of reduced costs. However, the use of chemicals for weed control in conservation areas has been largely avoided because of contamination and a risk to desirable species (Erasmus, 1985). The aim of this study was to determine the herbicide efficacy of glyphosate by monitoring photosynthetic performance as well as the concentration of the herbicide within the tissues.

6.3. Materials and methods

6.3.1. *Study area*

This study was undertaken in July 2007 within the University of KwaZulu-Natal Conservancy (29^{o}49'S 30^{o}56'E), Westville, KwaZulu-Natal. Healthy uniform shrubs of approximately 1.2 m in a dense stand were selected for the study. Springbok herbicide (360 g/L glyphosate-Shaik Agchem) was mixed with distilled water on site (1% solution – manufacturers recommended dosage). A surfactant/wetting agent (polyether-polymethyisiloxane-copolymer) was added to the glyphosate solution to improve coverage of spray droplets onto the leaves and to enhance absorption. Leaves were sprayed until completely wet using a conventional knapsack sprayer (Matabi K-12). *In situ* measurements of gas exchange were monitored for five consecutive days after spraying under naturally varying microclimatic conditions in June (winter) and concentrations of the herbicide monitored within the tissues.

6.3.2. *Gas exchange*

Gas exchange measurements were taken at hourly intervals on the abaxial surface of the most recently expanded mature leaves. Measurements were taken following the procedure outlined in chapter 2.

6.3.3. *Chlorophyll fluorescence*

Measurements were taken at hourly intervals following the procedure outlined in chapter 2.

6.3.4. *High performance liquid chromatography (HPLC)*

6.3.4.1. *Plant material*

Three plants were harvested daily, starting 24 h after spraying. Plants were dried at 60^{o}C in an oven (Labotec) for 48 h. The dried material was separated into leaves, stems and roots and milled into a fine powder. The powder was stored in 100 mL glass screw cap bottles, labelled and kept in an insulated box.

6.3.4.2. *Mobile phase*

A 4% solution of methanol was mixed with 0.816 g of potassium dihydrogen orthophosphate. The pH was adjusted to 1.9 using phosphoric acid.

6.3.4.3. *Analytical standard*

Two hundred milligrams of glyphosate acid (analytical standard) were weighed, placed into a 50 mL volumetric flask and diluted to volume using the mobile phase. The volumetric flask was placed in an ultrasonic water bath (Labotec) to mix the active ingredient and then cooled to ambient temperature. The standard was then allowed to settle for 1.5 h to dissolve in the mobile phase. The standard was filtered through a 0.45 µm cartridge filter (Whatman) prior to injection using a Millipore flask under vacuum.

6.3.4.4. *Sample preparation*

Half a gram of sample material (leaves, stems and roots) was weighed and placed in 50 mL volumetric flasks. The powder was diluted to volume with the mobile phase. The volumetric flasks were placed on a hot plate for 10 min to dissolve the glyphosate. The samples were cooled to ambient temperature and each filtered through a 0.45 µm cartridge filter using a Millipore flask under vacuum. Samples were filtered to prevent damage to the pistons and to prevent blockages in the system prior to analysis.

6.3.4.5. *Sample analysis*

Glyphosate concentration in tissues was determined by HPLC using a Hitachi chromatograph equipped with chemstation software, an autoinjector and a photodiode detector using a wavelength of 195 nm. The method used was a modification of the assay suggested by Smith (1990) and Zelaya *et al*. (2011). A Whatman Partsil 10 SAX column (250 mm x 4.6 mm, 5 µm particle size) was used with an injection volume of 50 µL. The mobile phase was set at 3 mL/min. Both sample and calibration standard were filtered through a 0.22 µm filter prior to injection. The approximate retention and total run time were 2.8 and 10 min respectively. The calibration standard was injected using a 100 µL syringe. The calibration standard was

re-injected till the amount/area ratio of active ingredient corresponded to within 1% of each other. The sample solution was then injected after ensuring that the liquid chromatograph had stabilized and that the pressure and baseline on the graphical display were constant. Glyphosate concentration in the sample was identified by comparing its retention time with that of the standard. The concentration in the sample was calculated was equal to the standard concentration x the peak area of the sample/peak area of the standard.

6.3.5. *Statistics*

Analysis was similar to the procedure outlined in Chapter 2 and 3.

6.4. Results

Figures 1 (A-C) and 2 (A-B) illustrate diurnal trends in PPFD, CO_2 uptake, leaf conductance, transpiration and ambient temperature on five consecutive measurement days after application of glyphosate. CO_2 uptake decreased from 9.5 µmol $m^{-2}s^{-1}$ on day one to 0 µmol $m^{-2}s^{-1}$ on day five (Fig. 1B). Trends in leaf conductance, transpiration and ETR followed trends similar to CO_2 uptake (Figs. 1C, 2A, 4B).

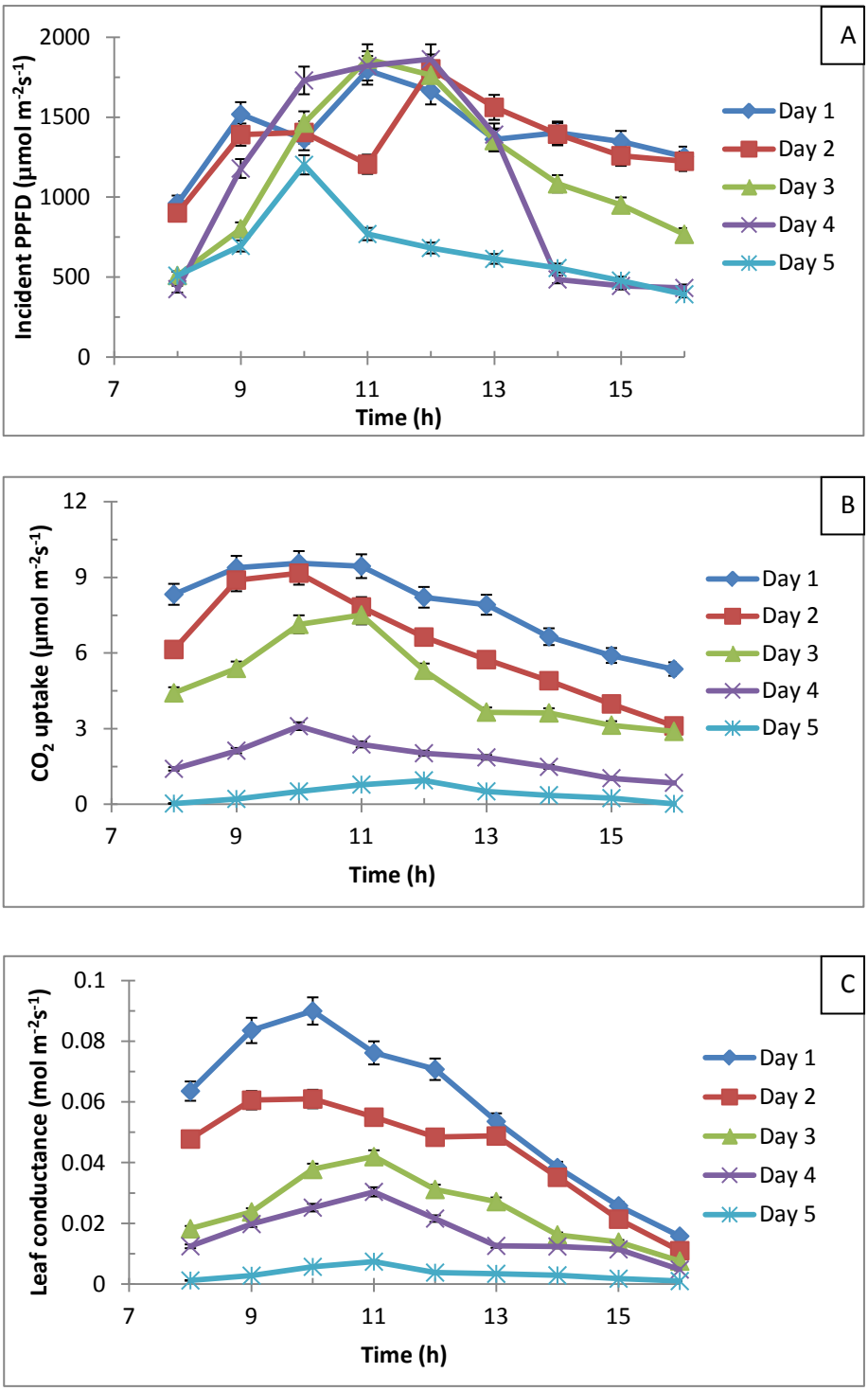


Fig. 1. Diurnal changes in incident photosynthetic photon flux density (A), CO_2 uptake (B) and leaf conductance (C) of *Chromolaena odorata* leaves one to five days after being sprayed with glyphosate. Each point is the mean value of five independent readings. Vertical bars represent standard error. Bars not visible indicate standard error smaller than the symbol.

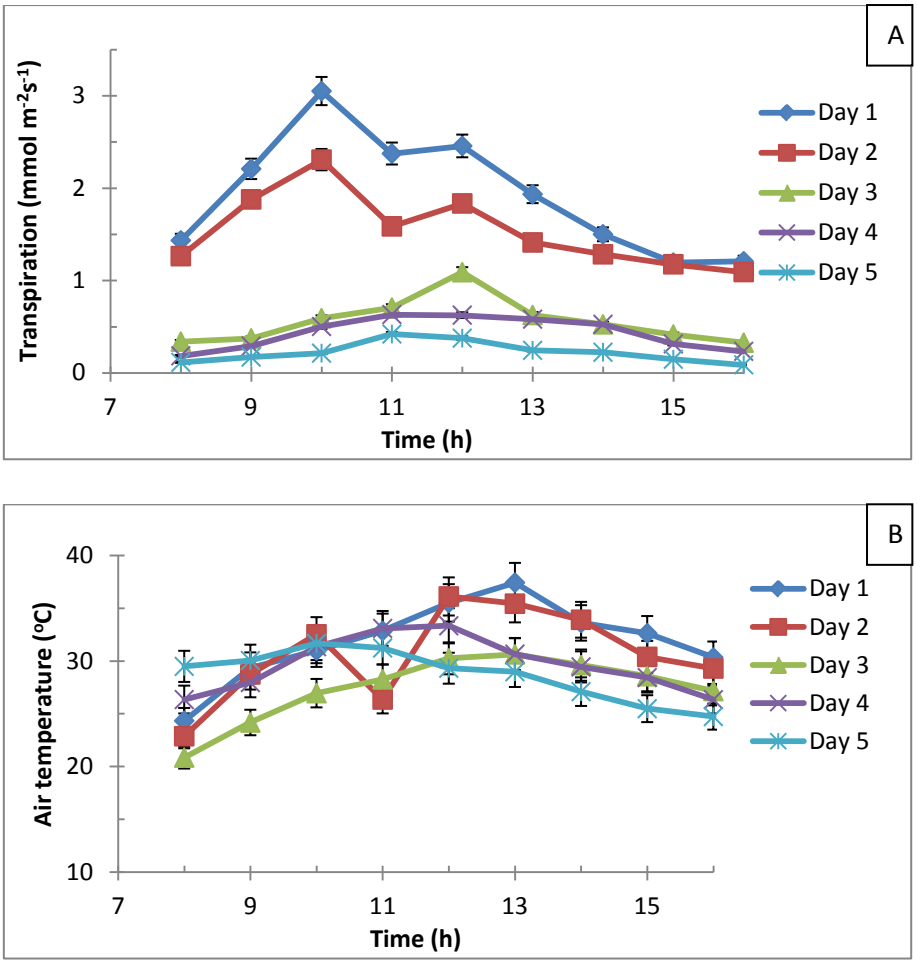


Fig. 2. Diurnal changes in transpiration (A) and ambient temperature (B) of *Chromolaena odorata* leaves one to five days after being sprayed with glyphosate. Each point is the mean value of five independent readings. Vertical bars represent standard error. Bars not visible indicate standard error smaller than the symbol.

Leaf conductance decreased progressively from day one to day five (Fig. 3B) after herbicide treatment and contributed to decreases in CO_2 uptake (Fig. 3A) and transpiration (Fig. 3C). Photosynthetic quantum yield decreased from 0.54 on day one to 0.025 on day five (Fig. 4A). Intrinsic photochemical efficiency of PSII followed a pattern similar to photosynthetic quantum yield (Fig. 4C). Photosynthetic quantum yield and F_v/F_m decreased after herbicide application from day one to day five, being close to zero at the end of the treatment (Figs. 5A, C).

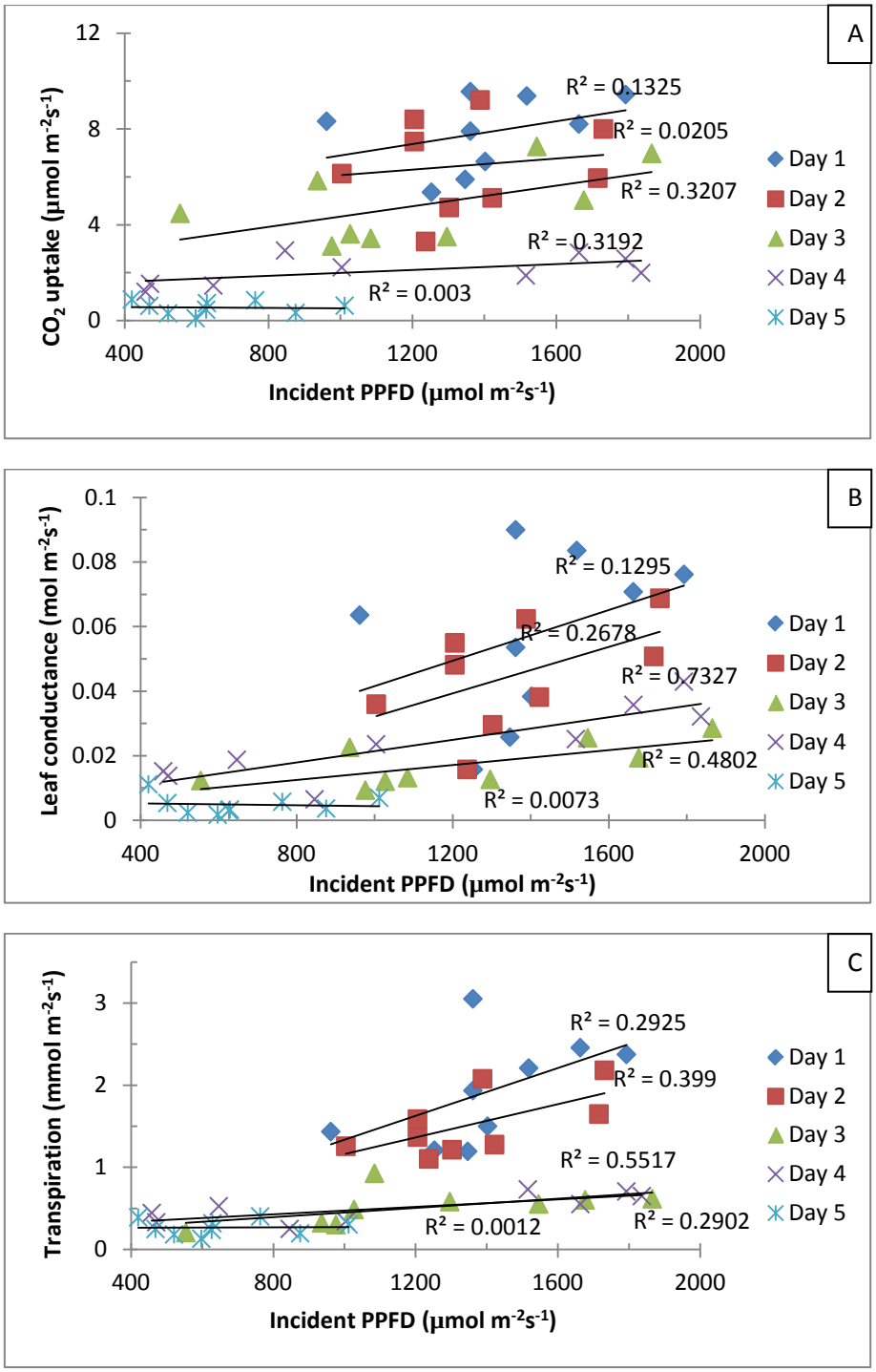


Fig. 3. Relationship between CO_2 uptake and incident photosynthetic photon flux density (A), leaf conductance and incident photosynthetic photon flux density (B) and transpiration and incident photosynthetic photon flux density (C) in healthy single stem of *Chromolaena odorata* plants one to five days after being sprayed with glyphosate. Each point is the mean of five independent readings. The regression lines are indicated by R^2 values.

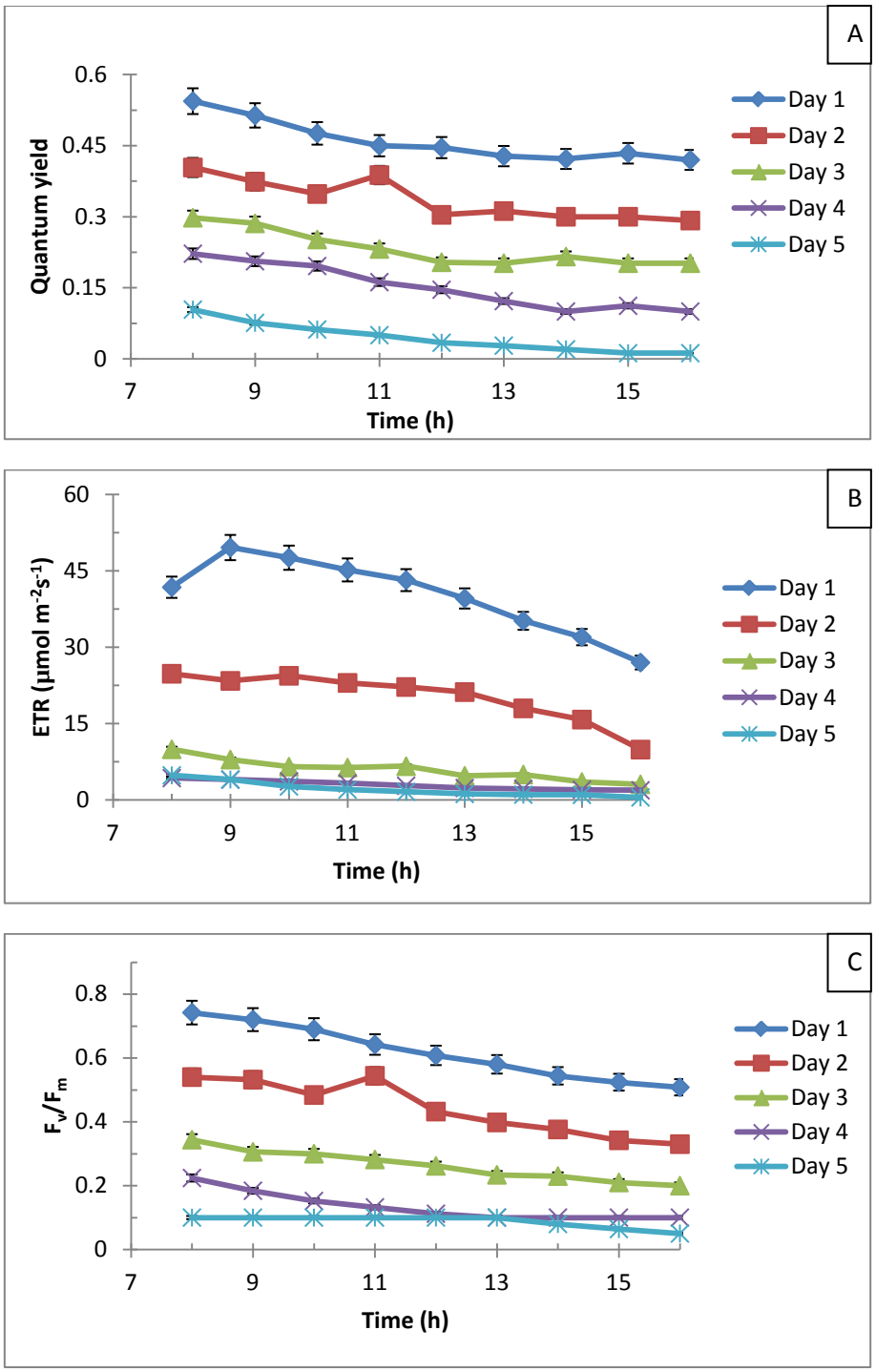


Fig. 4. Diurnal changes in photosynthetic quantum yield (A), electron transport rate through PSII (B) and the potential quantum yield of PSII photochemistry in the dark adapted state (F_v/F_m) (C) in healthy single stem of *Chromolaena odorata* plants one to five days after being sprayed with glyphosate. Each point is the mean value of five independent readings. Vertical bars represent standard error. Bars not visible indicate standard error smaller than the symbol.

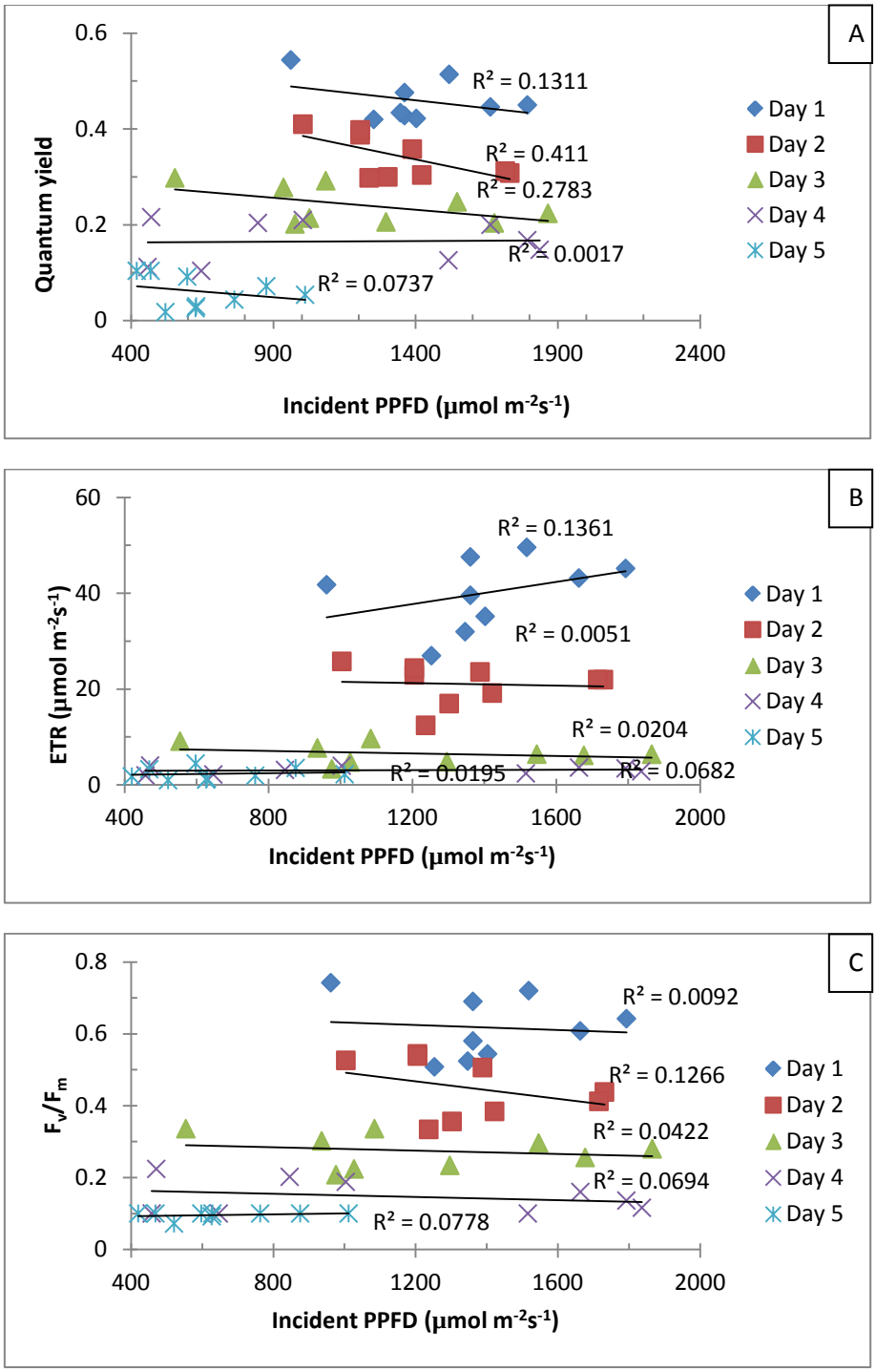


Fig. 5. Relationship between photosynthetic quantum yield and incident photosynthetic photon flux density (A), electron transport rate through PSII and incident photosynthetic photon flux density (B) and the potential quantum yield of PSII photochemistry in the dark adapted state (F_v/F_m) and incident photosynthetic photon flux density (C) in healthy single stem of *Chromolaena odorata* plants one to five days after being sprayed with glyphosate. Data are the mean values of five measurements. The regression lines are indicated by R^2 values.

Pearson's coefficient of determination for all five days is presented in Table 1. Significant correlations existed between CO_2 uptake and transpiration, ETR and quantum yield and transpiration and F_v/F_m.

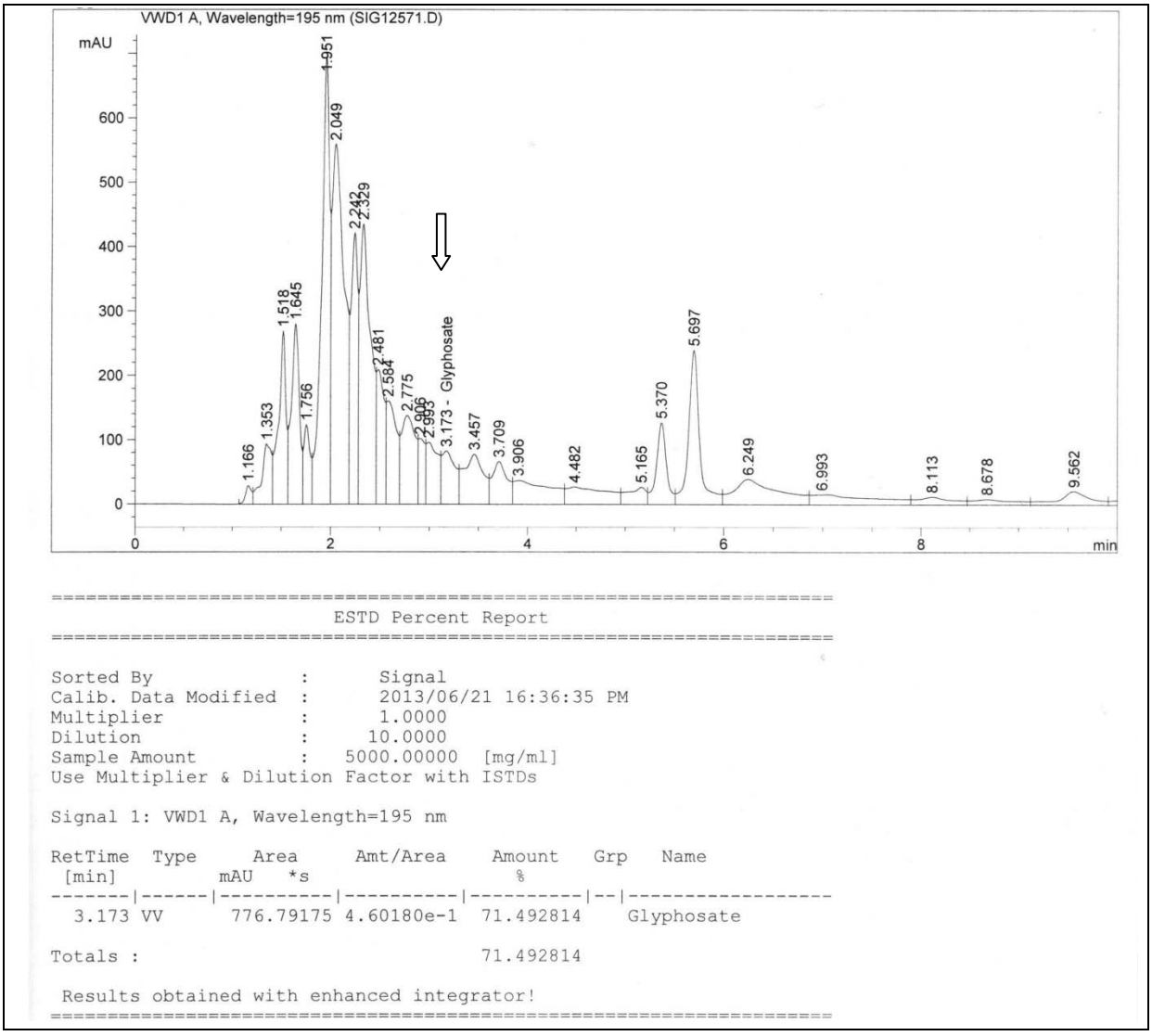
Table 2 indicates the percent of glyphosate detected in *C. odorata* tissue on five consecutive days, 24 h after application. Glyphosate concentration was high in leaves (days 1 and 2) and stems (days 1, 2 and 3) immediately after application. After day three, most of the glyphosate was located in the roots which suggested that the herbicide was transported from the aerial parts to below ground (Table 2).

Figures 6-20 represent individual chromatograms indicating the concentration of glyphosate detected in *C. odorata* tissue using HPLC analysis. Zero readings indicate that glyphosate was present in quantities that were too small to be determined by the chromatograph detector.

Table 1. Pearson's correlation coefficients for various variables after herbicide treatment (values in bold are significant at $P \leq 0.05$).

	A	PPFD	*g*	*E*	Temp.	ETR	Yield	F_v/F_m
A	1							
PPFD	0.584	1						
g	0.644	0.486	1					
E	**0.842**	0.595	0.596	1				
Temp.	0.261	0.647	0.259	0.530	1			
ETR	**0.886**	0.466	0.470	**0.830**	0.311	1		
Yield	**0.866**	0.502	0.476	**0.762**	0.224	**0.940**	1	
F_v/F_m	**0.899**	0.464	0.504	**0.820**	0.238	**0.975**	**0.971**	1

Abbreviations {*A*=CO_2 uptake, PPFD=photosynthetic photon flux density, *g*=leaf conductance, *E*=transpiration, Temp.=ambient temperature, ETR=electron transport rate, F_v/F_m= ratio of variable to maximal fluorescence}



ESTD Percent Report

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Dilution : 10.0000
Sample Amount : 5000.00000 [mg/ml]
Use Multiplier & Dilution Factor with ISTDs

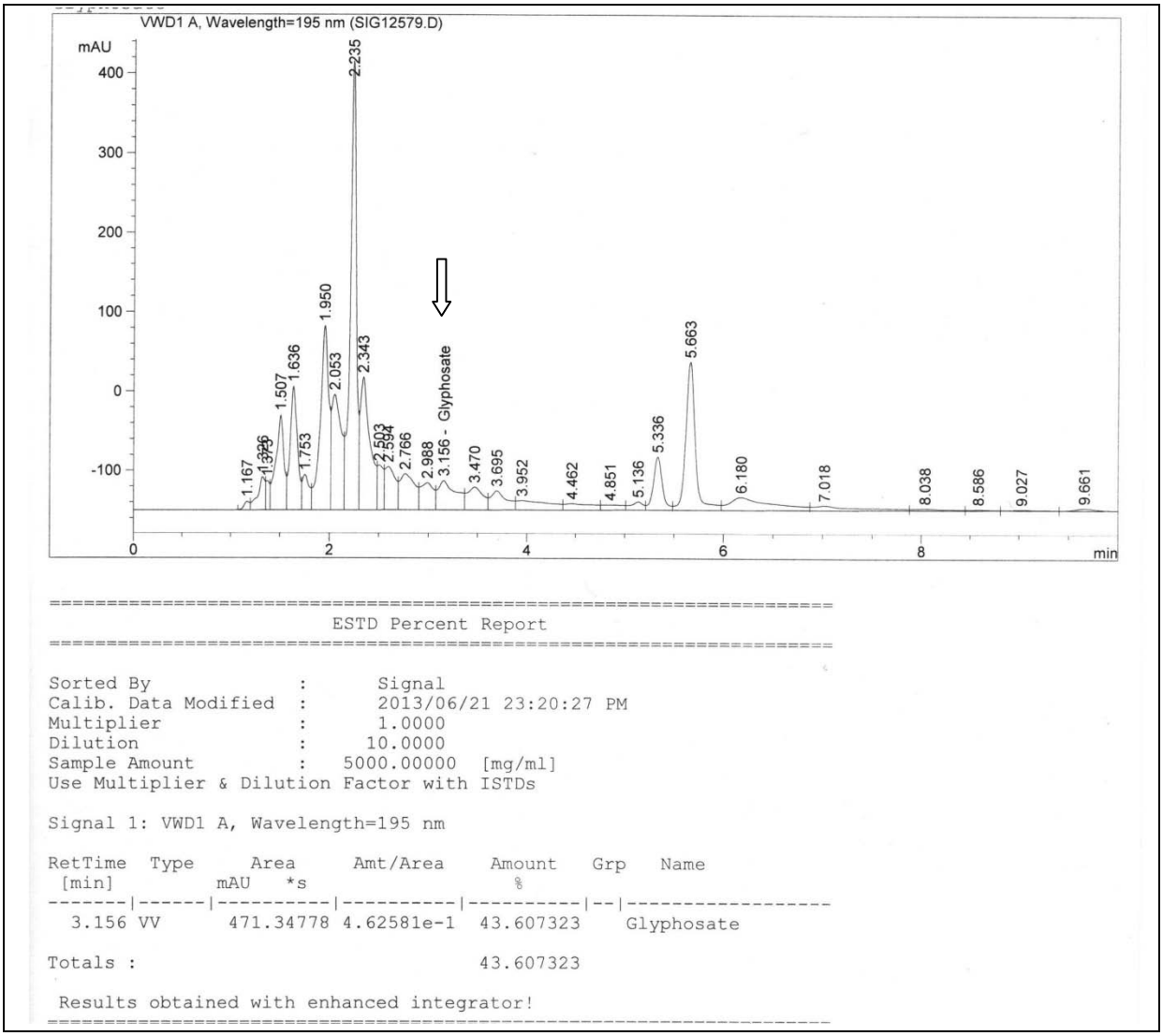
Signal 1: VWD1 A, Wavelength=195 nm

RetTime [min]	Type	Area mAU *s	Amt/Area	Amount %	Grp	Name
3.173	VV	776.79175	4.60180e-1	71.492814		Glyphosate

Totals : 71.492814

Results obtained with enhanced integrator!

Fig. 6. Chromatogram indicating glyphosate concentration in leaf samples of *C. odorata* on day 1. Absorbance (mAU=milliabsorbance units) is plotted against retention time (min). Glyphosate peak is labelled with a retention time of 3.173 min on the chromatogram. Other chromatographic peaks represent separated compounds detected in the herbicide solution.



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Sample Amount : 5000.00000 [mg/ml]
Use Multiplier & Dilution Factor with ISTDs

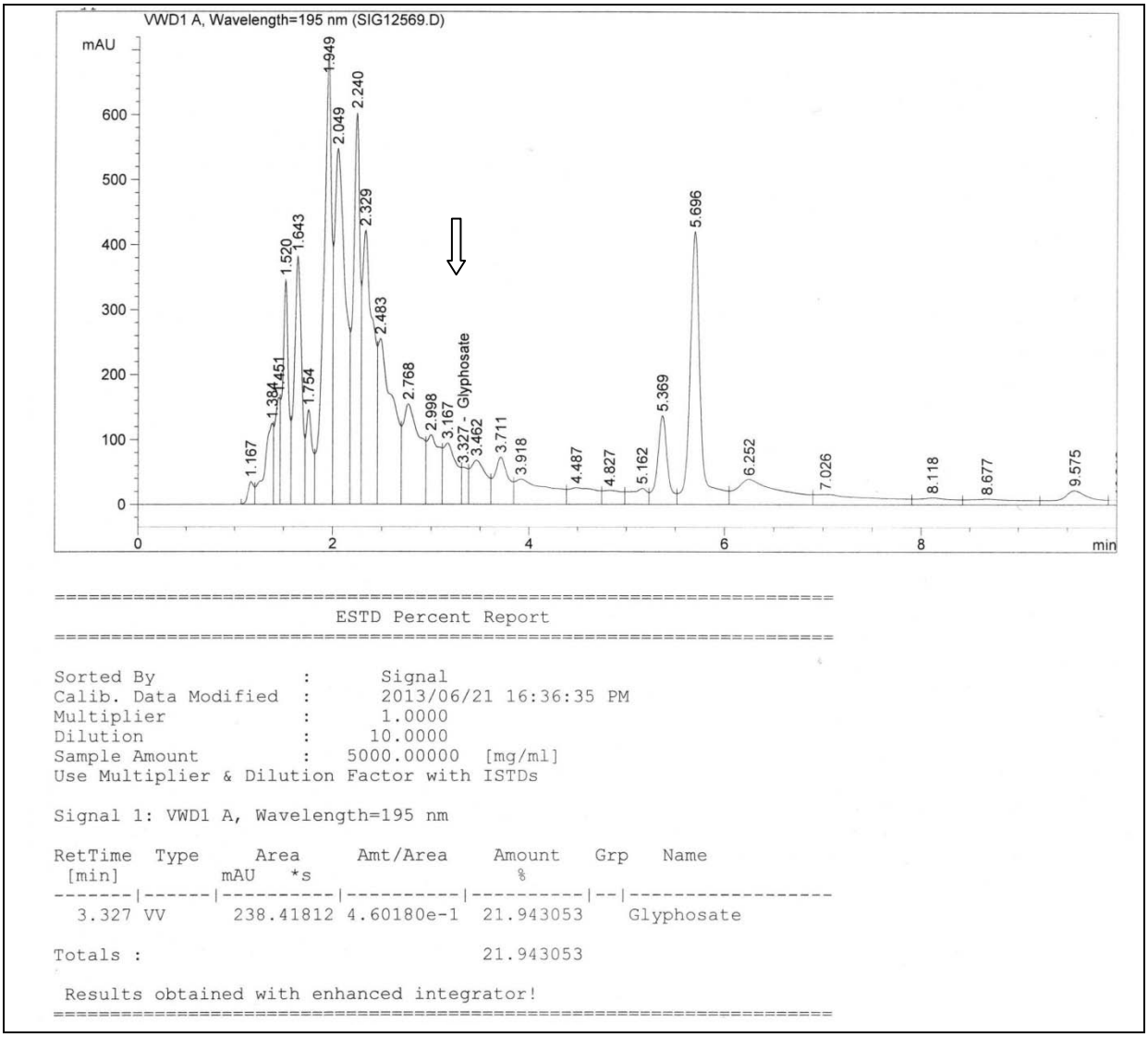
Signal 1: VWD1 A, Wavelength=195 nm

RetTime [min]	Type	Area mAU *s	Amt/Area	Amount %	Grp	Name
3.156	VV	471.34778	4.62581e-1	43.607323		Glyphosate

Totals : 43.607323

Results obtained with enhanced integrator!

Fig. 7. Chromatogram indicating glyphosate concentration in leaf samples of *C. odorata* on day 2. Absorbance (mAU=milliabsorbance units) is plotted against retention time (min). Glyphosate peak is labelled with a retention time of 3.156 min on the chromatogram. Other chromatographic peaks represent separated compounds detected in the herbicide solution.



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Dilution : 10.0000
Sample Amount : 5000.00000 [mg/ml]
Use Multiplier & Dilution Factor with ISTDs

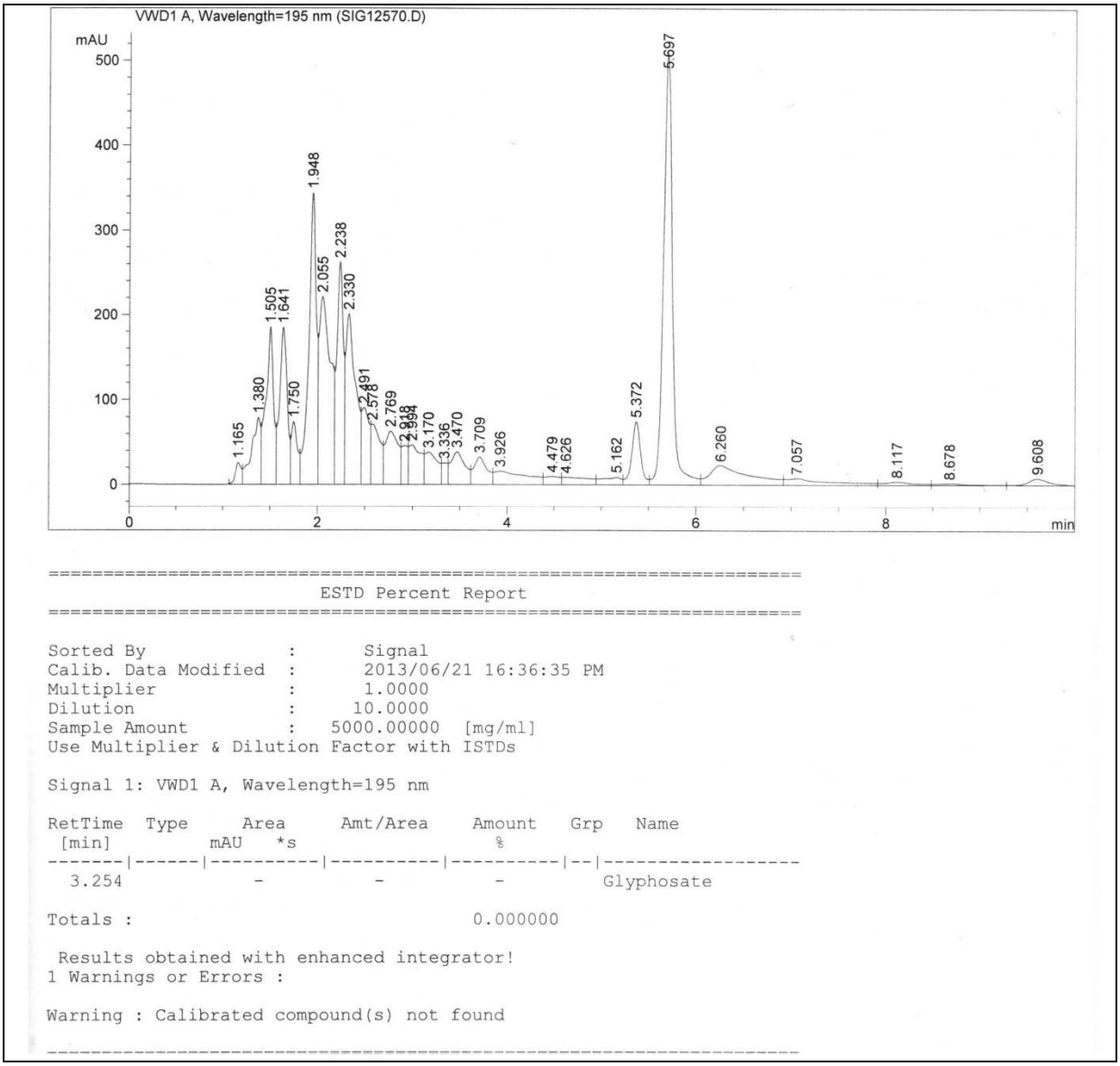
Signal 1: VWD1 A, Wavelength=195 nm

RetTime [min]	Type	Area mAU *s	Amt/Area	Amount %	Grp	Name
3.327	VV	238.41812	4.60180e-1	21.943053		Glyphosate

Totals : 21.943053

Results obtained with enhanced integrator!

Fig. 8. Chromatogram indicating glyphosate concentration in leaf samples of *C. odorata* on day 3. Absorbance (mAU=milliabsorbance units) is plotted against retention time (min). Glyphosate peak is labelled with a retention time of 3.327 min on the chromatogram. Other chromatographic peaks represent separated compounds detected in the herbicide solution.



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Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, Wavelength=195 nm

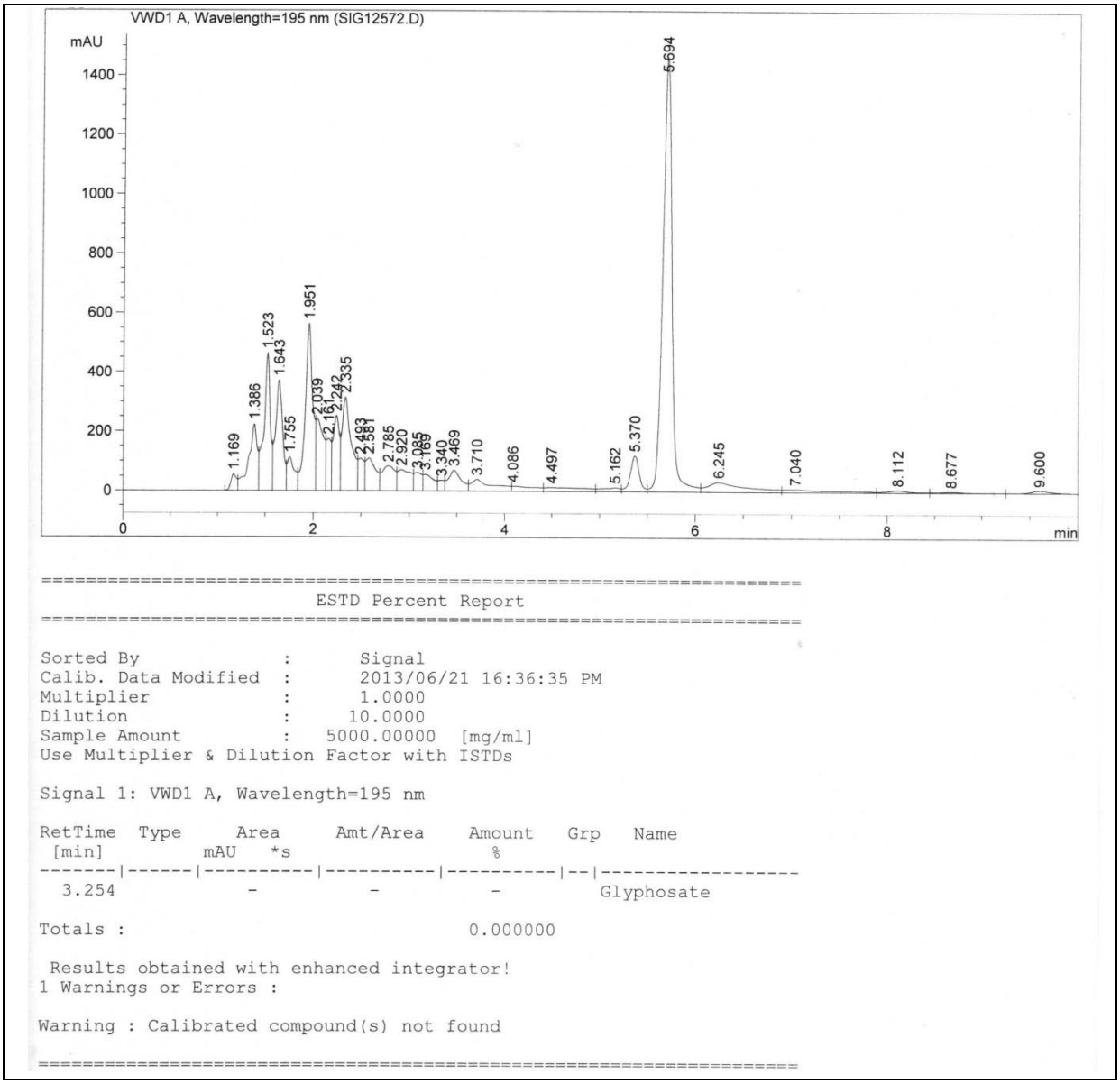
RetTime [min]	Type	Area mAU *s	Amt/Area	Amount %	Grp	Name
3.254		-	-	-		Glyphosate

Totals : 0.000000

Results obtained with enhanced integrator!
1 Warnings or Errors :

Warning : Calibrated compound(s) not found

Fig. 9. Chromatogram indicating glyphosate concentration in leaf samples of *C. odorata* on day 4. Absorbance (mAU=milliabsorbance units) is plotted against retention time (min). Glyphosate concentration was too low to be detected at a retention time of 3.254 min. Other chromatographic peaks represent separated compounds detected in the herbicide solution.

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Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, Wavelength=195 nm

RetTime  Type     Area      Amt/Area    Amount   Grp   Name
 [min]          mAU   *s                  %
-------|------|----------|----------|----------|--|------------------
  3.254            -          -          -         Glyphosate

Totals :                                0.000000

 Results obtained with enhanced integrator!
1 Warnings or Errors :

Warning : Calibrated compound(s) not found

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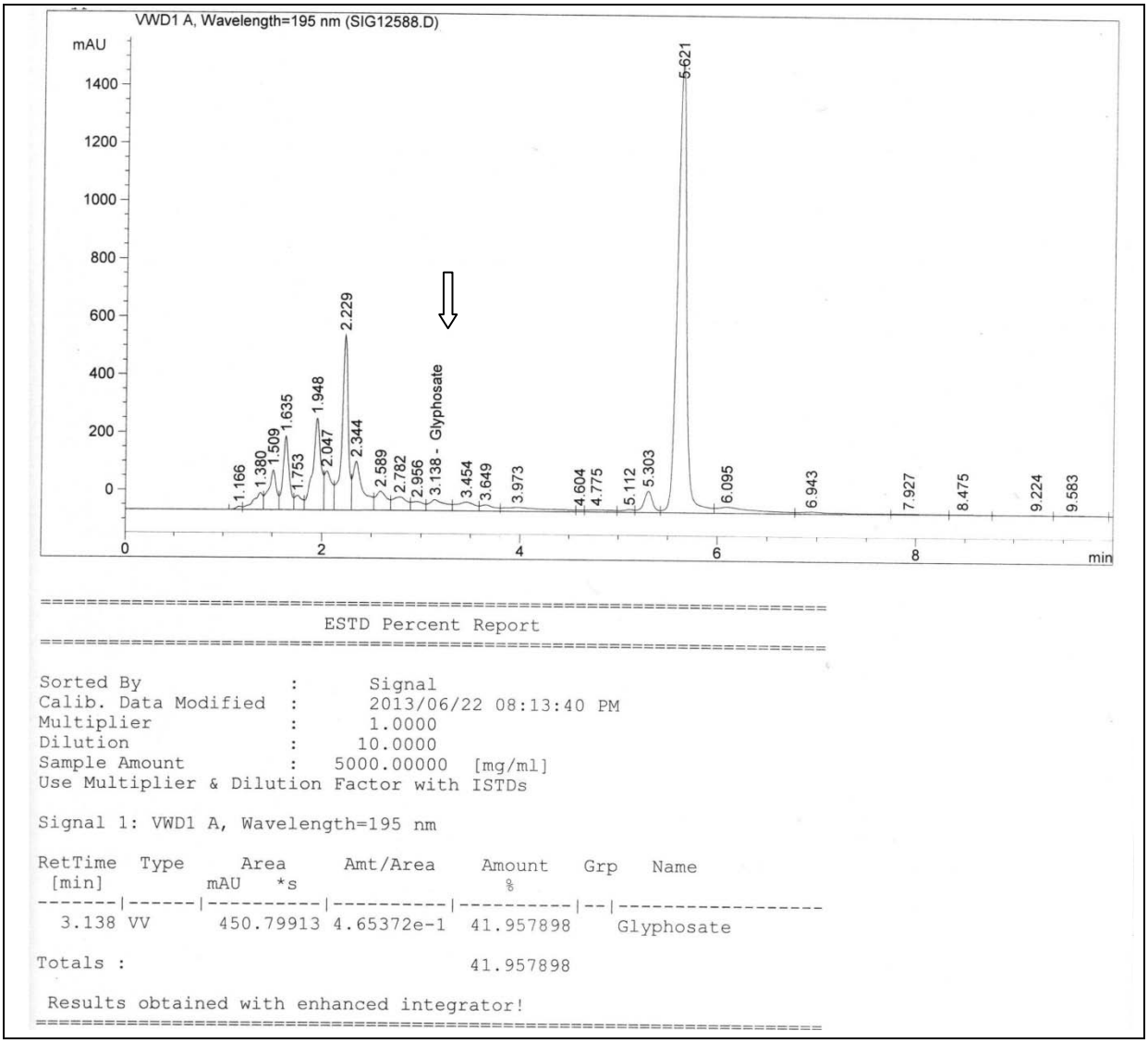
Fig. 10. Chromatogram indicating glyphosate concentration in leaf samples of *C. odorata* on day 5. Absorbance (mAU=milliabsorbance units) is plotted against retention time (min). Glyphosate concentration was too low to be detected at a retention time of 3.254 min. Other chromatographic peaks represent separated compounds detected in the herbicide solution.

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Sample Amount         :   5000.00000  [mg/ml]
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, Wavelength=195 nm

RetTime  Type     Area      Amt/Area    Amount    Grp   Name
 [min]         mAU   *s                   %
-------|------|----------|----------|----------|--|------------------
  3.138 VV      450.79913 4.65372e-1  41.957898     Glyphosate

Totals :                              41.957898

 Results obtained with enhanced integrator!
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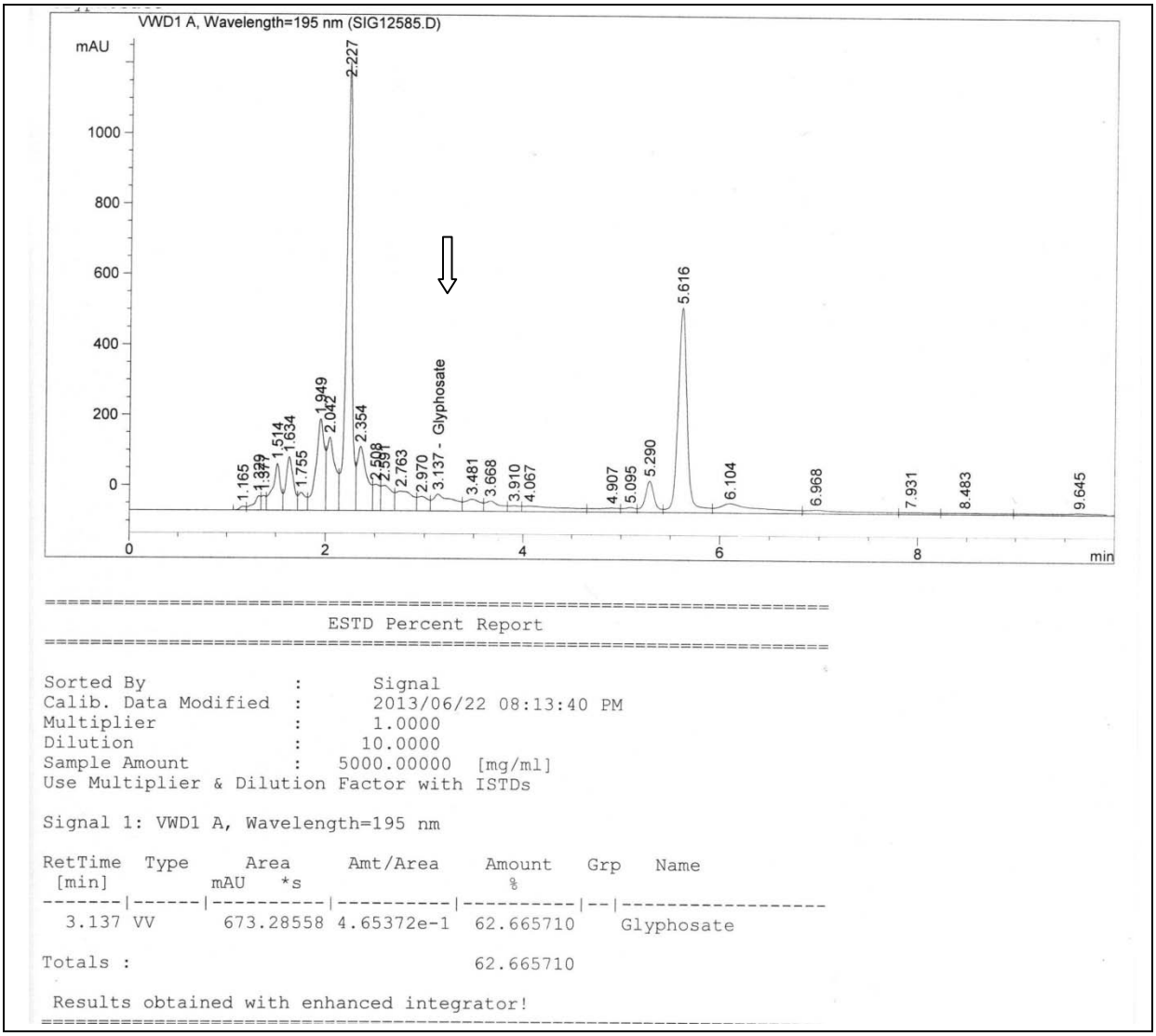
Fig. 11. Chromatogram indicating glyphosate concentration in stem samples of *C. odorata* on day 1. Absorbance (mAU=milliabsorbance units) is plotted against retention time (min). Glyphosate peak is labelled with a retention time of 3.138 min on the chromatogram. Other chromatographic peaks represent separated compounds detected in the herbicide solution.



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Sample Amount : 5000.00000 [mg/ml]
Use Multiplier & Dilution Factor with ISTDs

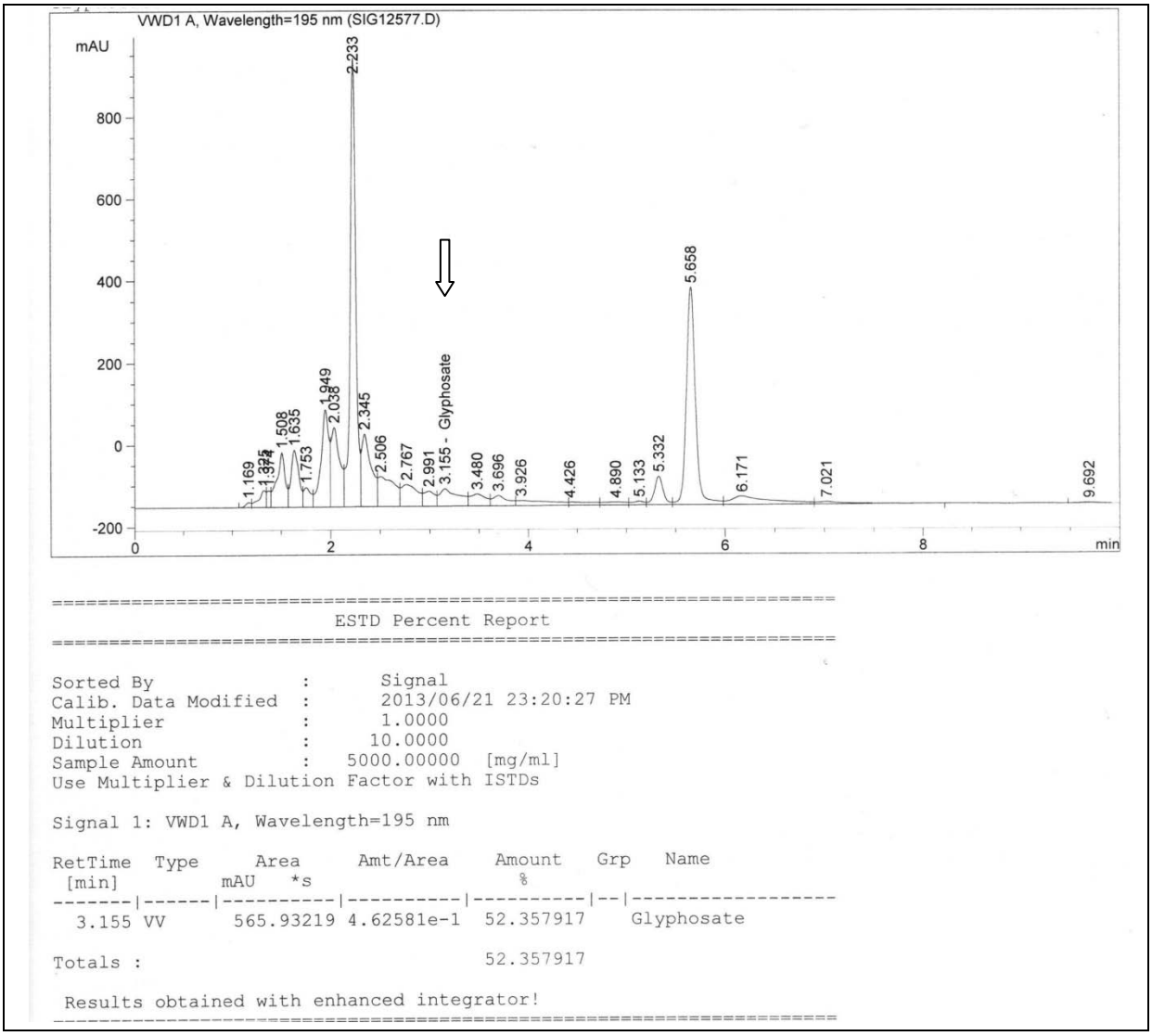
Signal 1: VWD1 A, Wavelength=195 nm

RetTime [min]	Type	Area mAU *s	Amt/Area	Amount %	Grp	Name
3.137	VV	673.28558	4.65372e-1	62.665710		Glyphosate

Totals : 62.665710

Results obtained with enhanced integrator!

Fig. 12. Chromatogram indicating glyphosate concentration in stem samples of *C. odorata* on day 2. Absorbance (mAU=milliabsorbance units) is plotted against retention time (min). Glyphosate peak is labelled with a retention time of 3.137 min on the chromatogram. Other chromatographic peaks represent separated compounds detected in the herbicide solution.



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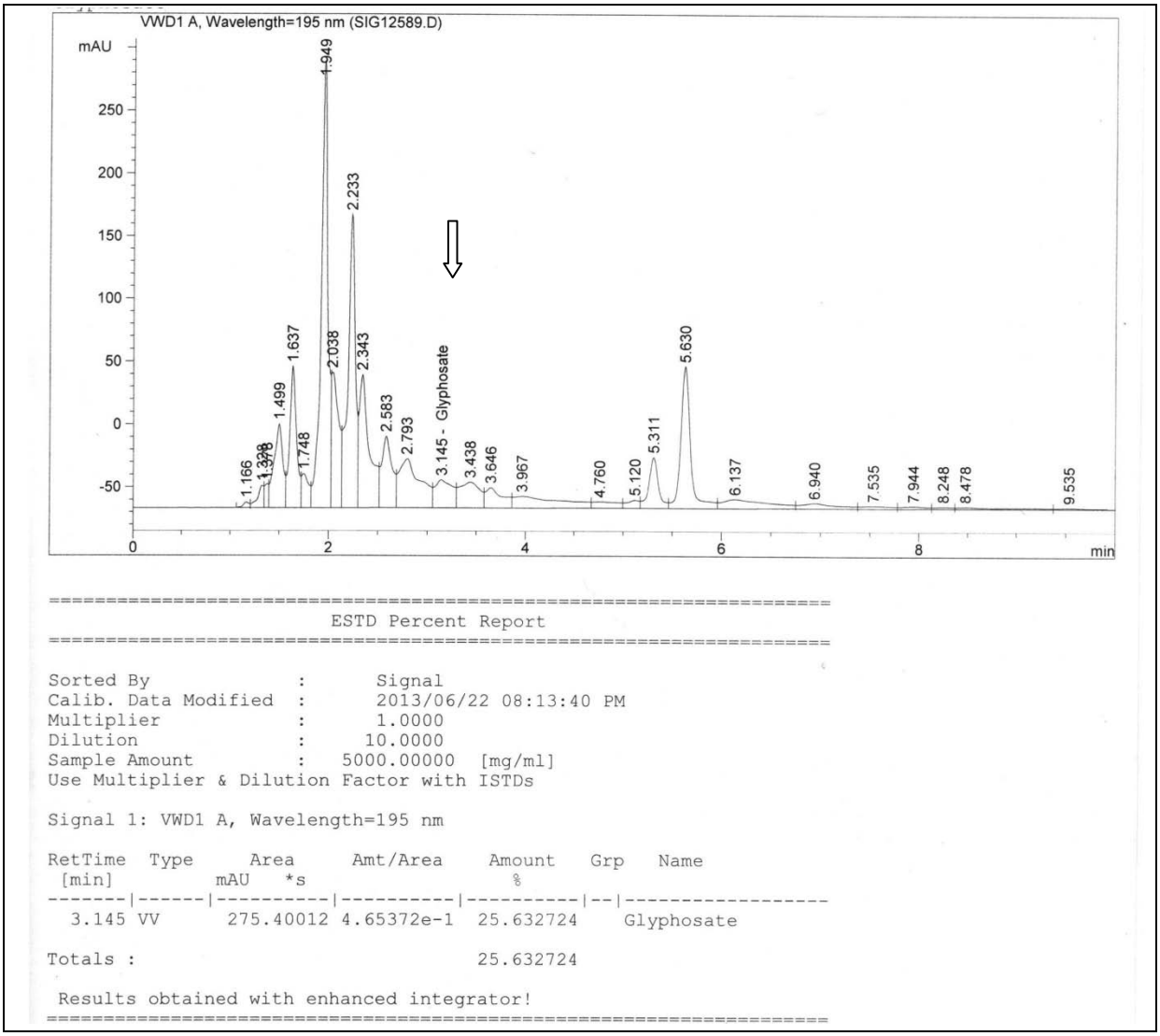
Sample Amount : 5000.00000 [mg/ml]
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, Wavelength=195 nm

RetTime [min]	Type	Area mAU *s	Amt/Area	Amount %	Grp	Name
3.155	VV	565.93219	4.62581e-1	52.357917		Glyphosate
Totals :				52.357917		

Results obtained with enhanced integrator!

Fig. 13. Chromatogram indicating glyphosate concentration in stem samples of *C. odorata* on day 3. Absorbance (mAU=milliabsorbance units) is plotted against retention time (min). Glyphosate peak is labelled with a retention time of 3.155 min on the chromatogram. Other chromatographic peaks represent separated compounds detected in the herbicide solution.



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Dilution : 10.0000
Sample Amount : 5000.00000 [mg/ml]
Use Multiplier & Dilution Factor with ISTDs

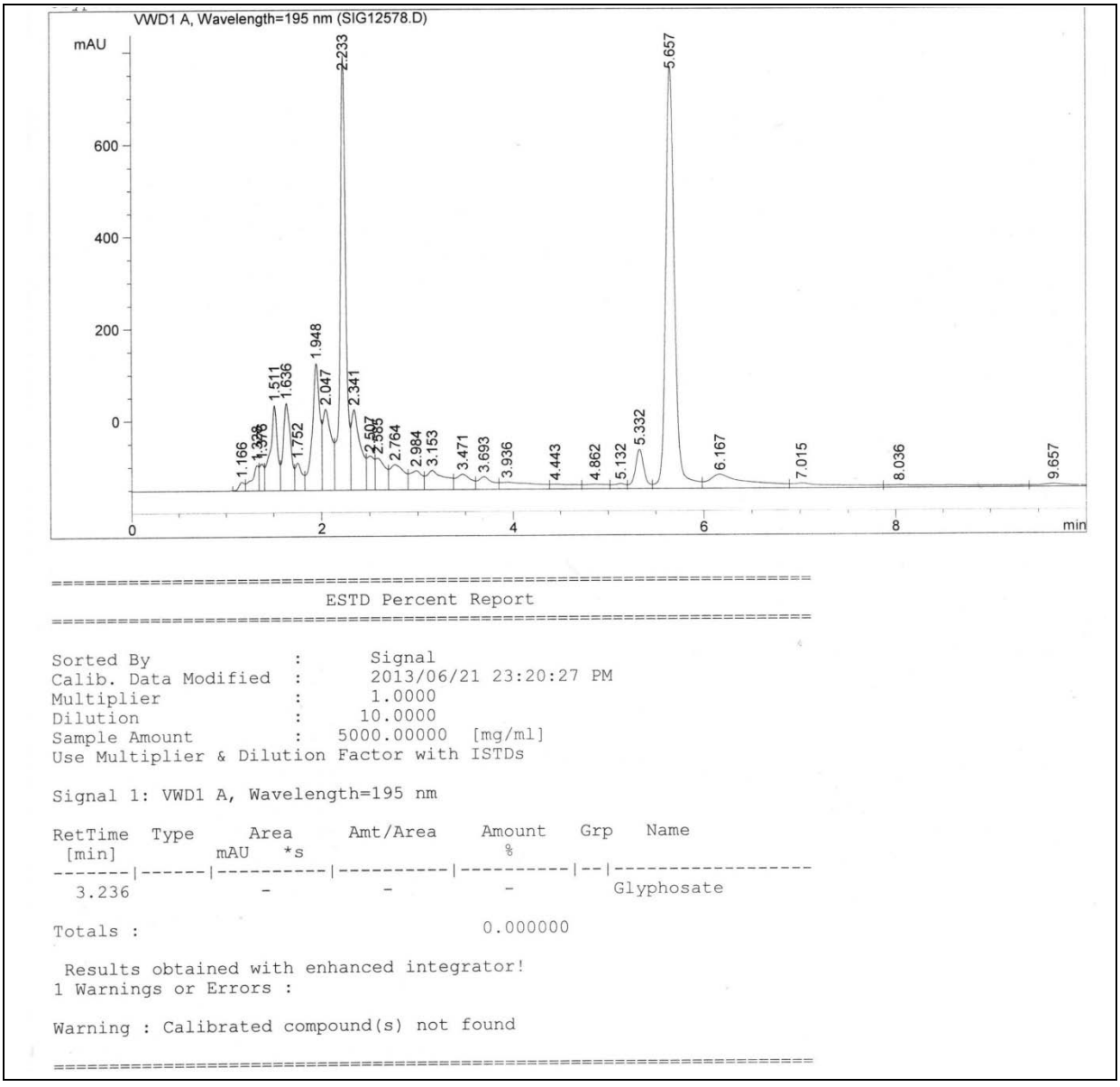
Signal 1: VWD1 A, Wavelength=195 nm

RetTime [min]	Type	Area mAU *s	Amt/Area	Amount %	Grp	Name
3.145	VV	275.40012	4.65372e-1	25.632724		Glyphosate

Totals : 25.632724

Results obtained with enhanced integrator!

Fig. 14. Chromatogram indicating glyphosate concentration in stem samples of *C. odorata* on day 4. Absorbance (mAU=milliabsorbance units) is plotted against retention time (min). Glyphosate peak is labelled with a retention time of 3.145 min on the chromatogram. Other chromatographic peaks represent separated compounds detected in the herbicide solution.



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Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, Wavelength=195 nm

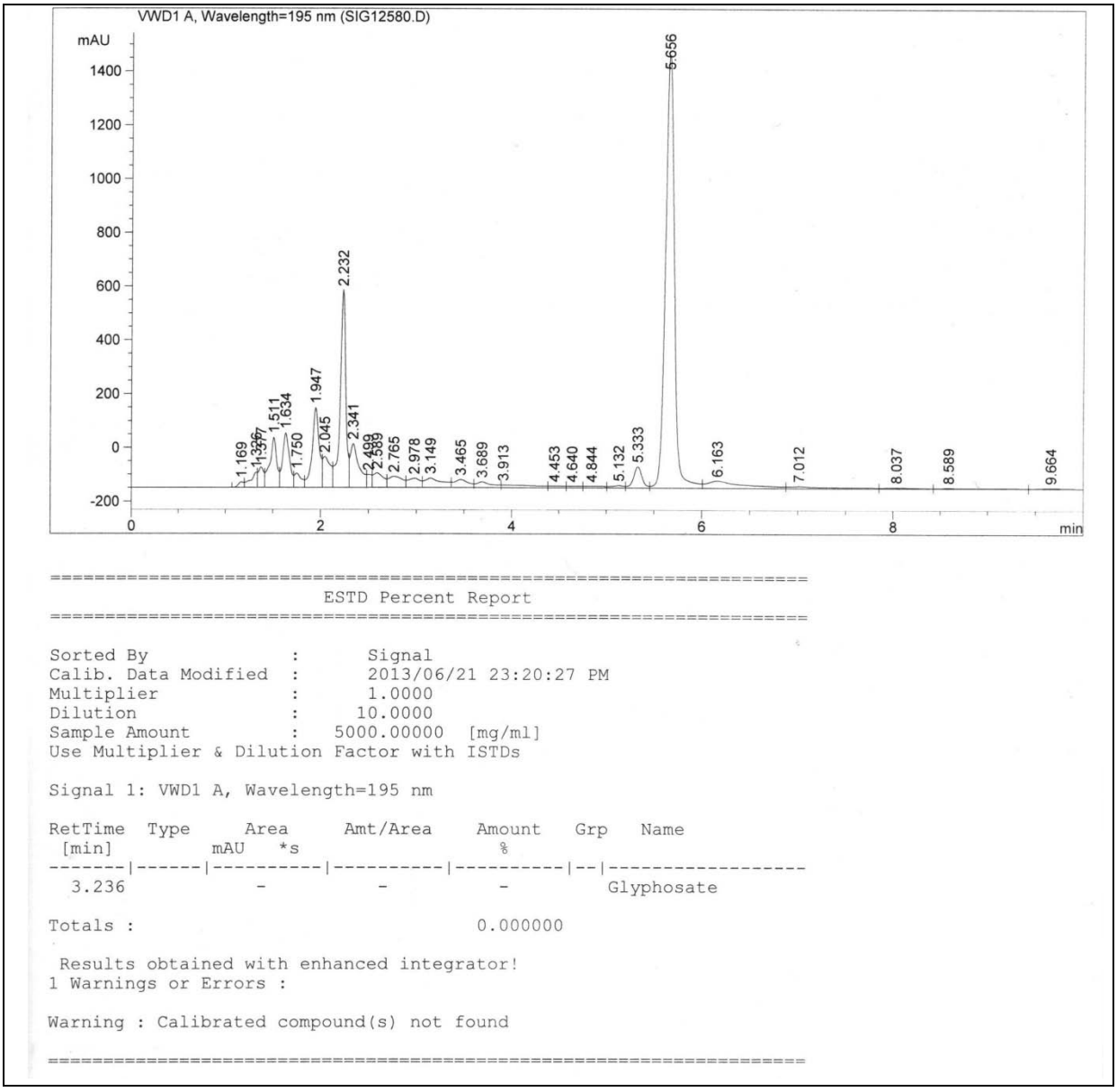
RetTime [min]	Type	Area mAU *s	Amt/Area	Amount %	Grp	Name
3.236		-	-	-		Glyphosate

Totals : 0.000000

Results obtained with enhanced integrator!
1 Warnings or Errors :

Warning : Calibrated compound(s) not found

Fig. 15. Chromatogram indicating glyphosate concentration in stem samples of *C. odorata* on day 5. Absorbance (mAU=milliabsorbance units) is plotted against retention time (min). Glyphosate concentration was too low to be detected at a retention time of 3.236 min. Other chromatographic peaks represent separated compounds detected in the herbicide solution.

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Dilution              :     10.0000
Sample Amount         :   5000.00000  [mg/ml]
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, Wavelength=195 nm

RetTime  Type     Area      Amt/Area    Amount   Grp   Name
 [min]          mAU   *s                  %
-------|------|----------|----------|----------|--|------------------
  3.236            -          -          -          Glyphosate

Totals :                                0.000000

 Results obtained with enhanced integrator!
1 Warnings or Errors :

Warning : Calibrated compound(s) not found

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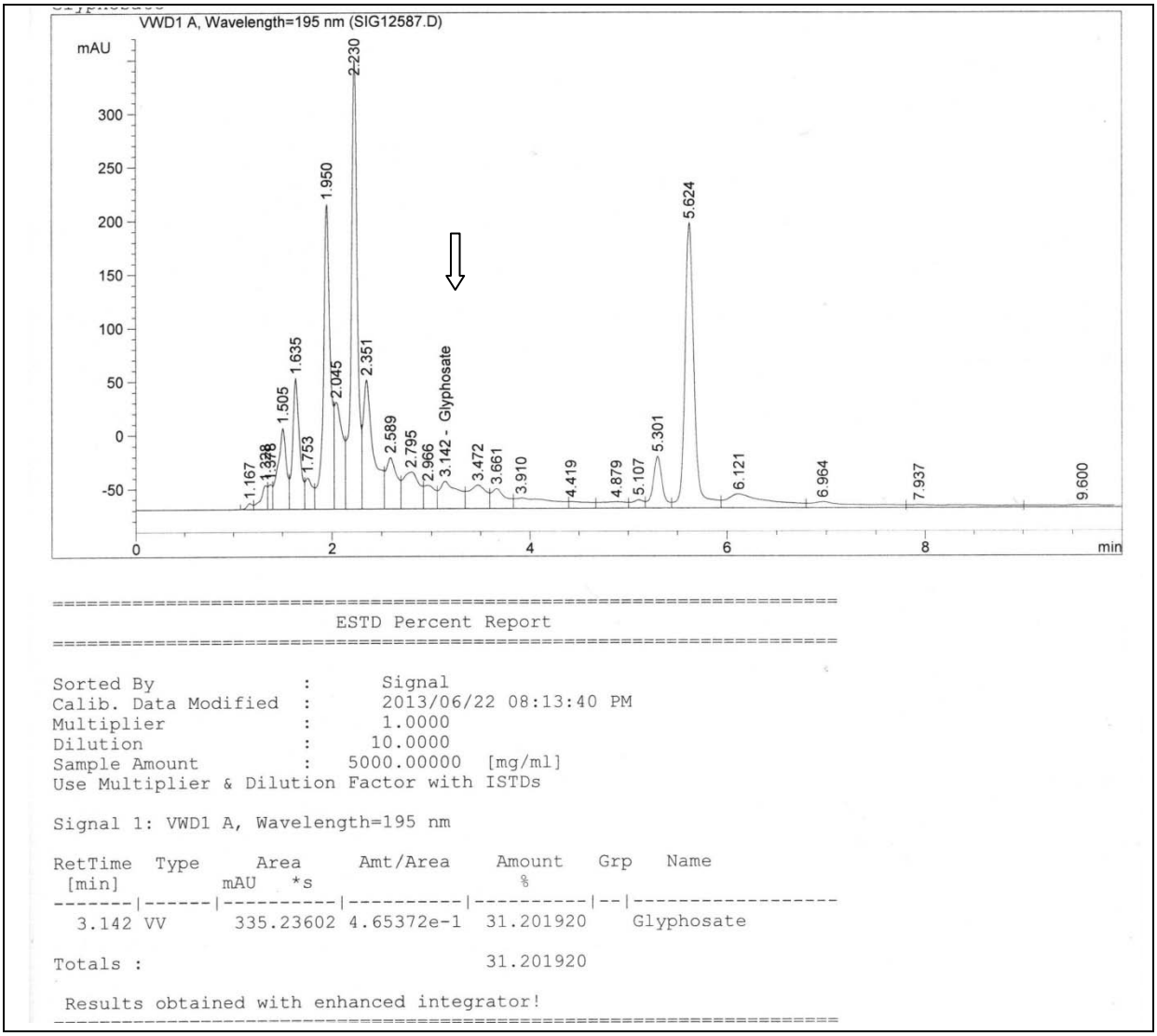
Fig. 16. Chromatogram indicating glyphosate concentration in root samples of *C. odorata* on day 1. Absorbance (mAU=milliabsorbance units) is plotted against retention time (min). Glyphosate concentration was too low to be detected at a retention time of 3.236 min. Other chromatographic peaks represent separated compounds detected in the herbicide solution.



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Dilution : 10.0000
Sample Amount : 5000.00000 [mg/ml]
Use Multiplier & Dilution Factor with ISTDs

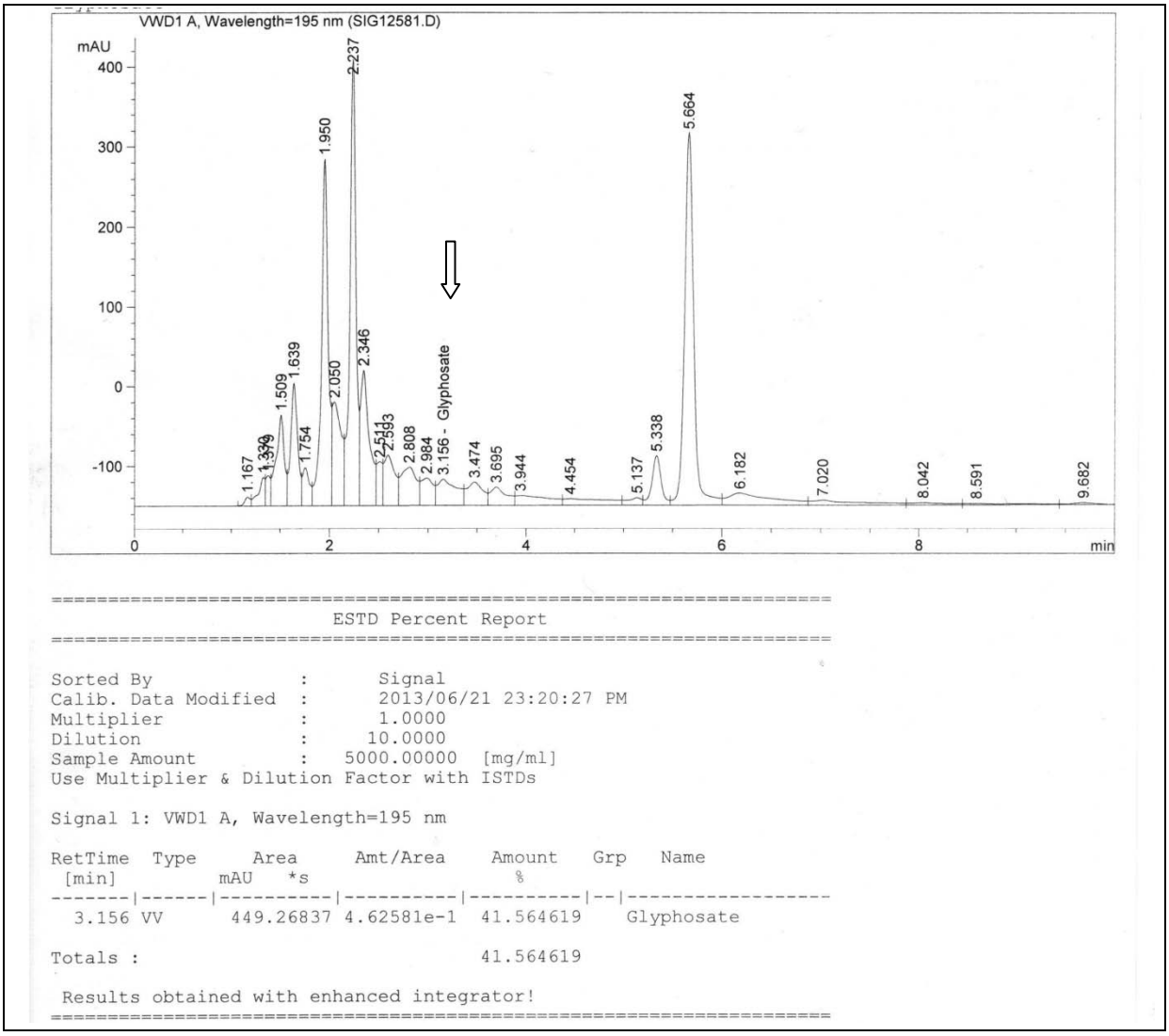
Signal 1: VWD1 A, Wavelength=195 nm

RetTime [min]	Type	Area mAU *s	Amt/Area	Amount %	Grp	Name
3.142	VV	335.23602	4.65372e-1	31.201920		Glyphosate

Totals : 31.201920

Results obtained with enhanced integrator!

Fig. 17. Chromatogram indicating glyphosate concentration in root samples of *C. odorata* on day 2. Absorbance (mAU=milliabsorbance units) is plotted against retention time (min). Glyphosate peak is labelled with a retention time of 3.142 min on the chromatogram. Other chromatographic peaks represent separated compounds detected in the herbicide solution.



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Dilution : 10.0000
Sample Amount : 5000.00000 [mg/ml]
Use Multiplier & Dilution Factor with ISTDs

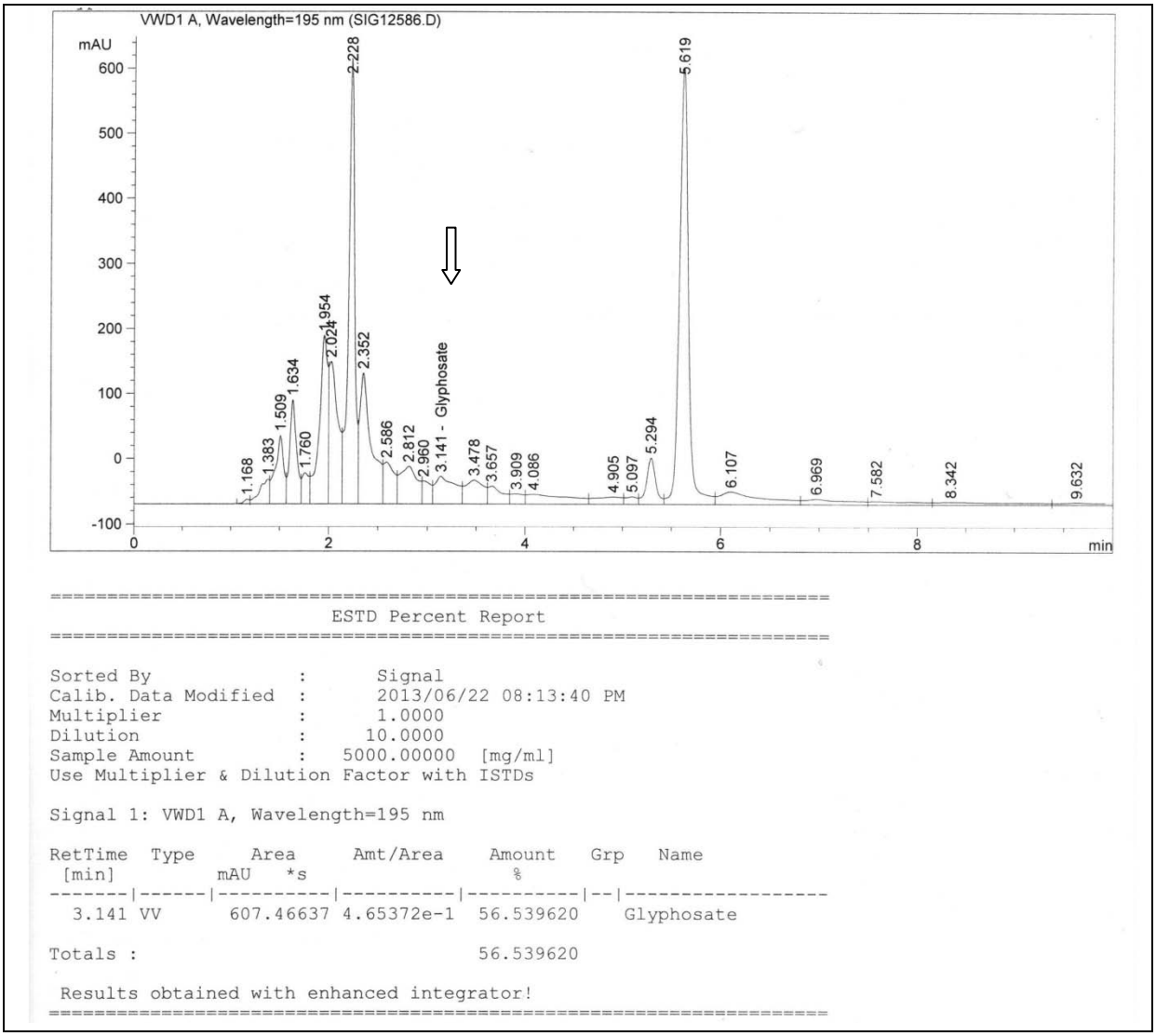
Signal 1: VWD1 A, Wavelength=195 nm

RetTime [min]	Type	Area mAU *s	Amt/Area	Amount %	Grp	Name
3.156	VV	449.26837	4.62581e-1	41.564619		Glyphosate

Totals : 41.564619

Results obtained with enhanced integrator!

Fig. 18. Chromatogram indicating glyphosate concentration in root samples of *C. odorata* on day 3. Absorbance (mAU=milliabsorbance units) is plotted against retention time (min). Glyphosate peak is labelled with a retention time of 3.156 min on the chromatogram. Other chromatographic peaks represent separated compounds detected in the herbicide solution.



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Dilution : 10.0000
Sample Amount : 5000.00000 [mg/ml]
Use Multiplier & Dilution Factor with ISTDs

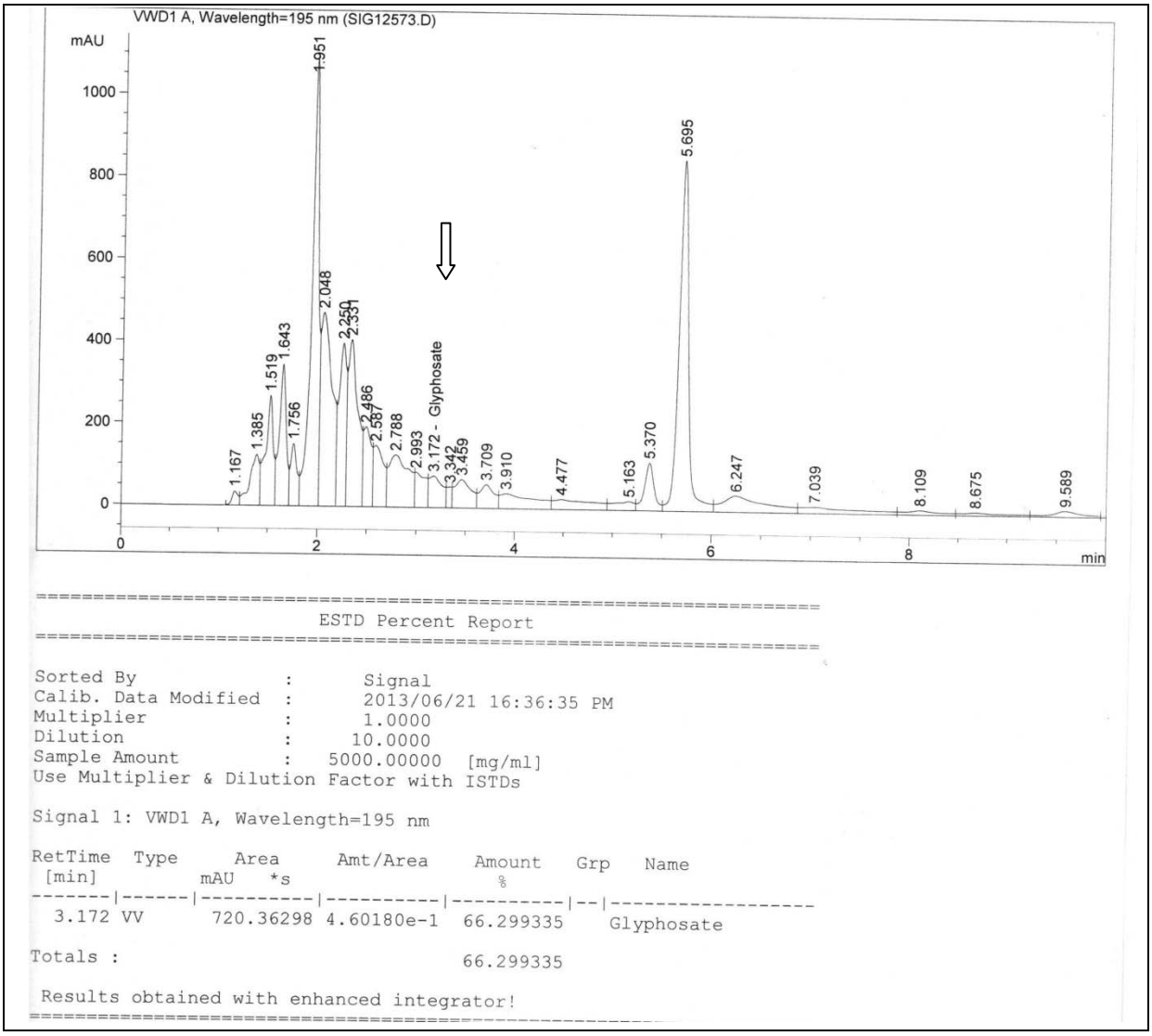
Signal 1: VWD1 A, Wavelength=195 nm

RetTime [min]	Type	Area mAU *s	Amt/Area	Amount %	Grp	Name
3.141	VV	607.46637	4.65372e-1	56.539620		Glyphosate

Totals : 56.539620

Results obtained with enhanced integrator!

Fig. 19. Chromatogram indicating glyphosate concentration in root samples of *C. odorata* on day 4. Absorbance (mAU=milliabsorbance units) is plotted against retention time (min). Glyphosate peak is labelled with a retention time of 3.141 min on the chromatogram. Other chromatographic peaks represent separated compounds detected in the herbicide solution.



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Dilution : 10.0000
Sample Amount : 5000.00000 [mg/ml]
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, Wavelength=195 nm

RetTime [min]	Type	Area mAU *s	Amt/Area	Amount %	Grp	Name
3.172	VV	720.36298	4.60180e-1	66.299335		Glyphosate

Totals : 66.299335

Results obtained with enhanced integrator!

Fig. 20. Chromatogram indicating glyphosate concentration in root samples of *C. odorata* on day 5. Absorbance (mAU=milliabsorbance units) is plotted against retention time (min). Glyphosate peak is labelled with a retention time of 3.172 min on the chromatogram. Other chromatographic peaks represent separated compounds detected in the herbicide solution.

Table 2. Glyphosate concentration in *Chromolaena odorata* tissue over five days after herbicide treatment.

	Glyphosate concentration (%)		
	Leaves	**Stems**	**Roots**
Day 1	71.5	42.0	0
Day 2	43.6	62.7	31.2
Day 3	21.9	52.4	41.6
Day 4	0	25.6	56.5
Day 5	0	0	66.3

6.5. Discussion

The use of a surfactant is recommended to enhance absorption of glyphosate into aerial tissue. In this study, young, single stem plants were chosen as they usually have more actively growing tissue and absorb more herbicide than older plants. High concentrations of glyphosate were detected in leaf and stem tissue 24 h after application (Table 2). The decrease in glyphosate concentration in leaves (from day one) and stems (from day two) and subsequent accumulation in the roots suggested that the herbicide was transported from the aerial parts to below ground.

Glyphosate caused a reduction in CO_2 uptake in leaves from day one to day five probably by disrupting the capacity of the leaves to photosynthesise. Inhibition of photosynthetic CO_2 uptake (Vivancos *et al*., 2011) occurs through the inhibition of 5-enolpyruvylshikimate-3-phosphate synthase, leading to a reduction of amino acids required for protein synthesis (Olesen & Cedergreen, 2010). Studies have shown that CO_2 uptake starts to decrease within a few hours of glyphosate application (Geiger *et al*., 1999).

A simultaneous decrease in leaf conductance and CO_2 uptake was observed in barley when sprayed with glyphosate (Olesen & Cedergreen, 2010). According to Zobiole *et al*. (2010) glyphosate induced stomatal closure is an important factor contributing to decreased CO_2 uptake. The decrease in transpiration may possibly be associated with a decrease in leaf conductance as well as epidermal and stomatal damage (Huang *et al*., 2012). Correlation between CO_2 uptake and leaf conductance was not significant (Table 1) suggesting that carbon assimilation was not directly affected by the latter parameter. This is in agreement with other studies that have shown that decrease in CO_2 uptake was primarily due to a reduction in ribulose bisphosphate regeneration rather than leaf conductance (Geiger *et al*., 1999).

Damage to *Lolium perenne* L. chloroplasts by glyphosate resulted in decreased chlorophyll concentrations (Yanniccari *et al*., 2012). In this study glyphosate probably led to damage to photosystem II and resulted in decreased F_v/F_m (Fig. 4C). Decrease in F_v/F_m may possibly be due to reduction in chlorophyll synthesis or degradation (Huang *et al*., 2012). These results contradict findings of Olesen & Cedergreen (2010) who found no consistent pattern for

chlorophyll fluorescence parameters in glyphosate treated barley plants (*Hordeum vulgare* L.). However, our work is consistent with findings by Christensen *et al*. (2003) who found changes in chlorophyll fluorescence parameters in sugar beet (*Beta vulgaris* L.) and white mustard (*Sinapis alba* L.) within 24 h after glyphosate treatment. The contradiction in study results is probably due to differences in glyphosate concentrations and species used.

The damage to PSII reaction centres was probably progressive and irreparable and associated with decreases in the photosynthetic quantum yield and ETR through PSII. Effective PSII quantum yield represents the capacity of the plant to convert photon energy into chemical energy, once a steady state of electron transport has been achieved (Genty *et al*., 1989). The progressive decrease in photosynthetic quantum yield (Fig. 4A) indicated that PSII reaction centres were probably damaged by the herbicide. Furthermore, glyphosate can lower the activity of enzymes of the carbon cycle resulting in a decrease in the rate of electron transport (Yanniccari *et al*., 2012). The decrease in the ETR through PSII may be due to blocking of primary and secondary electron acceptors (Huang *et al*., 2012; Tan *et al*., 2012).

The herbicide was highly mobile suggested by its accumulation in the roots within two days. The toxic levels of glyphosate in the roots probably damaged meristematic tissue and interfered with the ability of the weed to absorb water (Yanniccari *et al*., 2012). Evidence for this conclusion was observed in *C. odorata* plants that exhibited increasing degrees of wilting from day two. The transport of glyphosate from the aerial to below ground tissue resulted in the zero readings of glyphosate in leaf samples on days four and five and in stem samples on day five.

A study by Huang *et al*. (2012) found that 1% glyphosate was the most effective concentration compared to 0.3, 0.5 and 2% for the control of *Imperata cylindrica* (L.). It is therefore likely that glyphosate concentrations below 1% may not be effective in managing the spread of *C. odorata*. Spraying the weed using a 1% glyphosate solution resulted in 100% mortality within six days. Further observations, up to four weeks after spraying, revealed complete mortality with no evidence of resprouting.

6.6. Conclusion

Glyphosate (1%) resulted in a 100% mortality of *C. odorata* within six days of spraying. The herbicide, when combined with a surfactant, was easily absorbed through leaves and decreased gas exchange and chlorophyll fluorescence characteristics probably by damaging PSII reaction centres. The herbicide rapidly decreases leaf conductance leading to a decrease in CO_2 uptake and transpiration. After day three, most of the glyphosate was located within the roots, suggesting that the herbicide was transported from the aerial parts to below ground. This study demonstrated that this broad spectrum herbicide was effective in the control of *C. odorata* plants in the field.

6.7. References

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7. Antimicrobial properties

7.1. Abstract

Chromolaena odorata is classified as an invasive weed in southern Africa and is a serious threat to indigenous biodiversity. The species has been noted to have some medicinal value. The aim of this investigation was to evaluate the potential antimicrobial benefit of *C. odorata* against selected bacteria and fungi. Bacterial and fungal assays were performed on extracts derived from the leaves and stems using the minimum inhibitory concentration (MIC) and disc diffusion assays respectively. Both the methanol and ethanol extracts were more effective against bacteria and fungi than aqueous extracts. The methanol leaf extracts were effective against all gram positive bacteria (*Bacillus subtilis, Bacillus cereus, Staphylococcus aureus, Staphylococcus epidermidis*) as well as one gram negative bacterium, *Escherichia coli*. The stem extract was not as effective as the leaf extract. However, some activity was noted for stem samples using ethyl acetate and methanol extracts for all gram positive bacteria tested. The potency of the leaf and stem extracts were low based on their relatively high MIC values. High zones of inhibition were noted in the ethanol extracts using a 1: 10 concentration compared to higher dilutions. Phytochemical analysis of selected compounds in the leaf extract of *C. odorata* revealed the presence of tannins, phenolic compounds, sterols, triterpenoids, alkaloids, flavonoids and saponin glycosides. The antimicrobial effect of the leaf extract may be due to the presence of phenolic, alkaloid and flavonoid compounds. The results showed that the weed has limited potential as an antimicrobial agent.

7.2. Introduction

Chromolaena odorata is a perennial shrub belonging to the family Asteraceae. It is mainly a perennial weed of plantation crops and pastures of southern Asia and western Africa (Phan *et al*., 2001). A decoction of the leaf is used as a cough remedy and as an ingredient with lemongrass and guava leaves for the treatment of malaria (Vital & Rivera, 2009). In Vietnam, a formulation prepared from the aqueous extract of the leaves of *C. odorata* has been licensed for clinical use (Phan *et al*., 2001). Traditionally, fresh leaves or decoctions have been used throughout Vietnam as well as other tropical countries for the treatment of leech bite, soft

tissue wounds, burn wounds, skin infection and dento–alveolitis (Truong, 1989; Nghiem, 1992). Clinical studies using aqueous extracts from *Chromolaena* leaves have shown antimicrobial and anticoagulation effects, as well as the promotion of wound healing (Phan *et al*., 1998). Other medicinal uses include anti-diarrheal, astringent, antispasmodic, antihypertensive, anti-inflammatory and diuretic properties (Iwu *et al*., 1999). Furthermore, decoctions of the leaves of *C. odorata* are used to treat diseases such as ulcers and skin eruptions (Baez *et al*., 1998) and have been documented to possess antiprotozoal activity (Taleb-Contini *et al*., 2004). Previous investigations of the leaves and stems of *C. odorata* revealed the presence of essential oils (Lamaty *et al*., 1992; Chowdhury, 2002), steroids (Ahmad & Nabi, 1967), triterpenes (Tarapatra *et al*., 1977) and flavonoids (Hai *et al*., 1995; Wollenweber *et al*., 1995; Wollenweber & Roitman, 1996).

Flowers of the weed have also been subjected to investigation for essential oils (Baruah & Leclercq, 1993), fats (Baruah & Pathnak, 1993) and alkaloids (Biller *et al*., 1994; Suksamrarn *et al*., 2004). A decoction of flowers is used as an antipyretic and heart tonic (Bunyapraphatsara & Chokechaijaroenporn, 2000). Work on *Chromolaena moritziana*, based on interviews with herbalists, indicated that leaves and flowers of the plant are taken orally to remove mucous from the respiratory tract (Baez *et al*., 1998).

There is a continuous and urgent need to discover plants with antimicrobial properties, especially in rural Africa, where most dwellers are too impoverished to afford specialised health care. The low socio-economic standing of the great majority of people in KwaZulu-Natal, especially in rural areas, suggests that many use traditional methods of healthcare (Hirst, 1990). Traditional healers therefore play a crucial role in providing health care to the majority of the population. The role of traditional medicines is increasingly appreciated for the prevention and treatment of many human ailments (Janardhanan & George, 2006). Medicinal plants have been extensively studied due to the wide acceptance of traditional medicine as an alternative form of healthcare and the alarming increase in the incidence of new and re-emerging infectious diseases (Vital and Rivera, 2009). Herbal remedies have also become the focus of intense scrutiny, not only from a conservation perspective but in order to gauge whether their use is supported by actual pharmacological effects (Jager *et al*., 1996; Rabe & van Staden, 1997).

The aim of this investigation was to evaluate the potential antimicrobial benefit of *C. odorata* against selected bacteria and fungi. Extracts of *C. odorata*, used as antimicrobial remedies, may reduce harvesting pressure on other critically endangered medicinal species.

7.3. Materials and methods

7.3.1. *Antibacterial assay*

Leaf materials from wild populations were separated into three portions of 500 g and dried in an oven at 60^{o}C for 72 h. Stems were subjected to a similar collection and drying procedure. Each portion of dried material was weighed before being ground to a powder using a dry mill. Powdered material was placed into separate conical flasks containing one of three extracting media, i.e., water, ethyl acetate and methanol. The media were left for 72 h in an orbital shaker at 20 shakes per minute. After 72 h the extracts were filtered and concentrated using a rotary evaporator (Labotec).

The plant extracts were tested against four strains of gram-positive {*Bacillus subtilis* (ATCC 11744), *Bacillus cereus* (ATCC 11778), *Staphylococcus aureus* (ATCC 29737) and *Staphylococcus epidermidis* (ATCC 12228)} and four strains of gram negative bacteria {*Escherichia coli* (ATCC 13706), *Proteus vulgaris* (ATCC 49132), *Enterobacter aerogenes* (ATCC 35029) and *Shigella sonnei* (ATCC 9290)}. Base plates were prepared by pouring 10 mL Mueller-Hinton agar (Biolab) into sterile 9 cm Petri dishes and allowed to set. The molten agar (48^{o}C) was inoculated with a broth culture (10^{6} bacteria/mL) of the test organism and poured over the base plates forming a uniform top layer. Ten microlitres of *C. odorata* extract were applied per filter paper disc (Whatman No. 3, 6 mm diameter) so that each disc contained 1 mg of material. The Whatman discs were air dried and placed onto the top layer of the agar plates. Each extract was tested in triplicate with chloramphenicol and streptomycin sulfate, serving as controls. The plates were incubated at 37^{o}C for 18 h. Antibacterial activity was expressed as the ratio of the inhibition zone (mm) produced by the extract and the inhibition zone caused by the control (Vlietinck *et al*., 1995). Minimum inhibitory concentration (MIC) values were taken as the lowest concentration of extract that completely inhibited bacterial growth after 18 h of incubation at 37^{o}C (Nascimento *et al*., 2000). Clear inhibition zones around discs indicated the presence of antimicrobial activity.

7.3.2. *Antifungal assay*

Leaves and stems of *C. odorata* (1 kg) were crushed separately in a homogeniser (Labotec). The plant materials were soaked in ethanol (95% v/v) and distilled water for two weeks in two litre conical flasks at room temperature. After two weeks, the extracts (ethanol and distilled water) were evaporated to a residue using a rotary evaporator (Labotec). Extracts for testing methanol and aqueous extracts were prepared in three different concentrations. Stock solutions of the residue were prepared by dissolving the extract in ethanol and distilled water to obtain a concentration of 100 mg/mL. Further dilutions to the respective stock solutions (1:10, 1:100, 1:500) were made and antifungal activity was evaluated in a phosphate buffer at pH 6.0 (Champion *et al*., 1992). Extracts were tested for antifungal activity against five fungal strains i.e. *Aspergillus flavus, Aspergillus glaucus, Candida albicans, Candida tropicalis and Trichophyton rubrum.* Plates containing potato dextrose agar served as controls. Assays were performed in triplicate.

7.3.3. *Phytochemicals*

7.3.3.1. *Preparation of extracts*

Healthy leaves of *C. odorata* were collected in the morning from wild populations and dried in an oven at 45^{o}C for 48 h. Dried leaves were then ground into a fine powder using an electric mill. Five hundred millilitres of ethyl acetate were poured into a flask containing 100 g of powder. The flask was gently shaken using an orbital shaker for 1 h (20 shakes per minute). The extract was then reduced to a semi solid suspension using a rotary evaporator (Labotec). This procedure was repeated using methanol and 50% ethanol. A rotary evaporator was used to dry the final suspension into a powder. The powder was stored in an air tight container until needed for the phytochemical analysis. For each phytochemical test, 10 mg of the powder were dissolved in deionised water to obtain a final volume of 100 mL. The procedure for all phytochemical tests was similar to those suggested by Kumar *et al*. (2012).

7.3.3.2. *Tannins and phenolic compounds*

Approximately 5 mL of diluted iodine solution were added to the extract in a test tube. Tannins and phenolic compounds were indicated by a red colour change.

7.3.3.3. *Steroids and triterpenoids*

Five millilitres of chloroform were added to the extract in a test tube. Three drops of concentrated sulphuric acid were then added to the mixture and the contents allowed to stand for 30 min. Sterols and triterpenoids were indicated by a distinctive red and yellow layer respectively.

7.3.3.4. *Alkaloids*

Three drops of a potassium mercuric iodide solution were added to the leaf extract in a test tube. Alkaloids were indicated by a white colour change.

7.3.3.5. *Flavonoids*

Five millilitres of lead acetate were added to the leaf extract in a test tube. The solution was allowed to stand for 5 min. Flavonoids were indicated by a yellow colour change.

7.3.3.6. *Saponin glycosides*

Ten millilitres of deionised water were added to the leaf extract in a test tube. The test tube was then sealed with parafilm and shaken for 30 s. Saponin glycosides were indicated by the appearance of froth.

7.4. Results

Table 1. Minimal inhibitory concentration (MIC) of crude extract of *Chromolaena odorata* tested against selected gram positive and negative bacteria (n=3). Chloramphenicol (Chlor.) and streptomycin sulfate (Strept.) were used as controls. (Na= no activity)

Bacteria	**Gram**	**Medium (MIC) (mg/mL)**						**Control µg/mL**	
10^6 Bacteria/mL	**+/-**	**Water**		**Ethyl acetate**		**Methanol**		**Chlor.**	**Strept.**
		Leaves	**Stem**	**Leaves**	**Stem**	**Leaves**	**Stem**		
Bacillus subtilis	+	Na	Na	7.0	9.0	8.0	9.0	<2.0	<2.0
Bacillus cereus	+	Na	Na	8.0	8.5	7.5	8.5	<2.0	<2.0
Staphylococcus aureus	+	Na	Na	8.0	Na	8.0	Na	<2.0	<2.0
Staphylococcus epidermis	+	Na	Na	7.0	8.0	8.0	8.5	<2.0	<2.0
Escherichia coli	-	Na	Na	Na	Na	8.5	Na	<2.0	<2.0
Proteus vulgaris	-	Na	Na	Na	Na	Na	Na	<2.0	<2.0
Shigella sonnei	-	Na	Na	Na	Na	Na	Na	<2.0	<2.0
Enterobacter aerogene	-	Na	Na	Na	Na	Na	Na	<2.0	<2.0

All gram positive bacteria were inhibited by the ethyl acetate leaf extracts. Similarly, extracts from the stem inhibited all gram positive bacteria except for *S. aureus* (Table 1). Methanol extracts derived from the leaves and stems produced a similar effect when compared to ethyl acetate extracts. However, methanol extracts were effective in the inhibition of *E. coli*, a gram negative bacterium (Table 1). Aqueous extracts did not produce any inhibitory effect on the selected bacteria.

Table 2. Effect of ethanol and aqueous extracts obtained from *Chromolaena odorata* on selected fungal species. Stock solutions were diluted using a ratio of 10, 100 and 50 (n=3). Potato dextrose agar was used as a control.

Fungal species	**Ethanol extract**						**Aqueous extract**					
	Leaf	**Stem**	**Leaf**	**Stem**	**Leaf**	**Stem**	**Leaf**	**Stem**	**Leaf**	**Stem**	**Leaf**	**Stem**
	1:10	1:10	1:100	1:100	1:500	1:500	1:10	1:10	1:100	1:100	1:500	1:500
Aspergillus flavus	++	+	+	-	-	-	+	-	-	-	-	-
Aspergillus glaucus	++	+	++	-	-	-	++	+	-	-	-	-
Candida albicans	+++	+	++	-	-	-	+	+	-	-	-	-
Candida tropicalis	++	++	+	+	-	-	+	+	-	-	-	-
Trichophyton rubrum	+	+	+	-	-	-	+	-	-	-	-	-

- = Negative antifungal activity

+ = Positive antifungal activity (low inhibition)

++ = Positive antifungal activity (medium inhibition)

+++ = Positive antifungal activity (high inhibition)

++++ = Positive antifungal activity (very high inhibition)

N.B. The control did not inhibit any fungal species.

The antifungal assay indicated greater activity when extracts were more concentrated (Table 2 and 3). The 1: 10 dilution produced the greatest antifungal activity. Higher dilution rates of extracts proved ineffective as antifungal agents. It was also clear that ethanol was more effective than water as an extraction medium (Table 2). Furthermore, extracts from leaves contained more antifungal activity than stems. Only one fungus i.e. *C. albicans* was highly inhibited by extracts from leaves (Table 2). The zone of inhibition was recorded as very high (++++), high (+++), medium (++), and low (+), which corresponded to a zone of inhibition of between 41–50, 31–40, 21–30, and 11–20 mm, respectively.

Table 3. Minimal inhibitory concentration observed in different dilutions prepared from a stock solution of 100 mg/mL of aqueous and ethanol extracts of *Chromolaena odorata* (n=3).

Fungal species	Ethanol extract		Aqueous extract	
	Stems	Leaves	Stems	Leaves
Aspergillus flavus	1:10	1:100	-	1:10
Aspergillus glaucus	1:10	1:100	1:10	1:10
Candida albicans	1:10	1:100	1:10	1:10
Candida tropicalis	1:100	1:100	1:10	1:10
Trichophyton rubrum	1:10	1:100	1:10	-

Phytochemical analysis of selected compounds in the leaf extract revealed the presence of tannins, phenolic compounds, steroids, triterpenoids, alkaloids, flavonoids and saponin glycosides (Table 4).

Table 4. Phytochemical analysis of leaf extracts of *Chromolaena odorata.*

Phytochemical/s	Present/Absent
Tannins and phenolic compounds	Present
Steroids and triterpenoids	Present
Alkaloids	Present
Flavonoids	Present
Saponin glycosides	Present

7.5. Discussion

Leaf and stem extracts were not effective due to their high MIC values (Table 1). High MIC values suggested that active compounds were present in low concentrations which may limit their use as prospective pharmaceutical drugs. Methanolic extracts from various medicinal plants from KwaZulu-Natal, tested by Rabe & van Staden (1997), also exhibited high MIC values. Those researchers concluded that many compounds may probably not be pharmaceutically useful. Previous testing of medicinal plants for antibacterial and antifungal activities have shown activity against gram positive bacteria (Coopoosamy & Magwa, 2007; Grierson & Afolayan, 1999; Kelmanson *et al.*, 2000; Rabe & van Staden, 1997; Shamim *et al*., 2004; Vlietinck *et al*., 1995) whilst gram negative bacteria were unaffected. Studies by Vlietinck *et al*. (1995) and Coopoosamy *et al*. (2010) concluded that the low inhibition of gram negative bacteria is due to their higher resistance compared to gram positive strains. Gram positive bacteria often cause human diseases such as colds, wounds and sores (Waihenya *et al.*, 2002). Extracts of *C. odorata* are useful in the treatment of superficial wounds, abrasions, cuts and infections caused by gram positive bacteria (Phan *et al*., 1998). However, based on the findings of this study, extracts from the weed is unlikely to substantially benefit patients and its use is probably based on folklore rather than medicinal merit.

Ethanol was found to be more effective than aqueous extracts in inhibiting fungi (Tables 2 and 3). However, higher leaf antifungal activities than those of the stem were evident against the test organisms. Higher zones of inhibition were noted in the ethanol extracts (1:10 concentration) compared to others. Plant use as an antimicrobial source may be confined to poor rural communities because of limited access to westernised medication. The low concentration of antimicrobial compounds in *C. odorata* may limit benefit to pharmaceutical companies as a viable drug source.

Aqueous extraction was not as effective as alcohol in preventing bacterial and fungal growth (Table 1 and 2). Traditionally, plant extracts are prepared with water (e.g. infusions, decoctions, poultices), so it would seem unlikely that traditional healers extract those compounds that are responsible for antimicrobial activity. It has been suggested that boiling of leaves by traditional healers may help to release active compounds and, in the process, provide necessary ingredients for traditional cures (Coopoosamy *et al.*, 2010). However,

boiling has been shown to significantly reduce phytochemicals, minerals and nutrient content in many plants e.g. *Sesamum indicum* L. (Momoh *et al*., 2012). Therefore, boiled extracts from *C. odorata* are unlikely to benefit traditional users.

The antimicrobial effect of the leaf extract may be due to the presence of phenolic and flavonoid compounds (Table 4). Phenolic compounds, e.g. eugenol are considered bacteriostatic and have been shown to have antifungal properties (Cowan, 1999). Furthermore, flavonoids are possibly synthesised in plants in response to infection (Cowan, 1999) and may inhibit certain microorganisms. Tannins may protect plants against herbivory (Cowan, 1999). It has been suggested that consuming tannin containing beverages can cure or prevent a variety of illnesses (Momoh *et al*., 2012). However, since these phytochemicals are present in low concentrations, isolating and incorporating these metabolites into pharmaceutical drugs may prove costly compared to cheaper alternatives that are presently available.

7.6. Conclusion

Extracts from the weed are unlikely to substantially benefit patients due to low concentrations of active compounds. It is likely that any benefit derived from leaf extracts may be due to the presence of selected phytochemicals. However, it is likely that medicinal use of the weed is based on folklore rather than scientific merit. Other antibiotics e.g. penicillin is more effective for the treatment of specific microbial infections than those derived from *C. odorata*.

7.7. References

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8. General discussion and conclusions

Chromolaena odorata, a prolific exotic weed, causes ecosystem destruction and biodiversity loss in KwaZulu-Natal. Despite increased interest in the control and spread of the species, little is known of its photosynthetic characteristics under field conditions. The rapid proliferation of the plant in recent years suggests that it has high ecophysiological tolerance to stressors and the ability to outcompete indigenous flora. The aim of this work was to investigate the ecophysiological attributes of *C. odorata* that contribute to its invasive success. Photosynthetic performance was evaluated by monitoring diurnal changes in gas exchange, chlorophyll *a* fluorescence and plant water relations. Gas exchange characteristics of plants growing in exposed and shaded environments were compared and seasonal patterns evaluated. Susceptibility to drought stress and its effects on ecophysiological characteristics were also determined.

Gas exchange data (chapter 2) indicated that *C. odorata* possesses relatively high CO_2 assimilation rates compared to a wide range of C_3 plants (Kattge & Knorr, 2007). Gas exchange patterns have been shown to contribute to the success of invasive species (Yamashita *et al*., 2000). There was no incident PPFD limitation to CO_2 uptake at values up to 2000 µmol $m^{-2}s^{-1}$. Acclimation to high incident PPFD probably maximises carbon gain and ensures rapid growth. High growth rates probably aid in colonising ecosystems and competitively excluding species with lower productivity. The characteristic multi-stem growth habit probably ensures alternative sinks for extra photoassimilates (El-Sharkawy, 2004). Greater photoassimilate demand has been shown to enhance photosynthesis and lead to greater total biomass and yield (El-Sharkawy, 2012). High water use efficiency (WUE) probably allows photosynthetic enzymes to maintain high carbon assimilation despite reduced leaf conductance. Moreover, high WUE may be a mechanism to increase the efficiency of water absorption thereby ensuring greater growth and reproduction.

Comparative responses of sun and shade plants growing in adjacent stands indicated that traits and physiological responses differed significantly (Chapter 3). Shade plants had a significantly higher specific leaf area than sun plants. High surface leaf area is important in contributing to productivity under reduced light conditions. Greater investment in

photosynthetic tissues increases the capacity for light capture and allows acclimation across a broader range of incident PPFD regimes. In high PPFD conditions, reduced SLA of sun compared to shade leaves may maintain higher carbon assimilation and reduce transpiration. Reduced SLA may thus be viewed as a water conservation strategy in sunny environments.

The leaves of *C. odorata* exhibit high phenotypic plasticity by efficiently regulating stomatal conductance. Analyses by Farquhar & Sharkey (1982) found that higher photosynthetic rates of sun leaves were as a result of both increased stomatal conductance and intrinsic photosynthetic capacity of the mesophyll tissue. The decrease in quantum yield from dawn to midday in sun plants may be a response to increased water loss. However, there was a significant correlation between leaf conductance and transpiration and between F_v/F_m and Ψ, suggesting that efficient stomatal control reduces dehydration stress. Water use efficiency was greater in shade than sun plants, suggesting that stomatal function was tightly coupled to CO_2 uptake, transpiration and ETR. Compared to sun plants, higher WUE in shade plants may possibly be an avoidance strategy to reduce excessive water loss.

Higher concentrations of chlorophylls and carotenoids in shade plants suggested that there was reduced photooxidative damage under low light conditions. Reduced SLA and photosynthetic pigments in sun leaves may be adaptations to prevent excessive light absorption thus protecting photosystems from photodamage. A significantly higher proline concentration in sun compared to shade leaves may be an adaptation to protect enzymes from dehydration injury (El-Sharkawy, 2012). Higher photosynthetic pigment concentrations in shade compared to sun leaves may be a strategy to harvest light more efficiently under low light conditions.

There were no significant seasonal differences in CO_2 uptake (chapter 4) suggesting a high capacity for maintaining carbon gain in winter and summer. Rapid growth, maintained throughout the year, may be an efficient strategy to outperform co-occurring indigenous species for available resources. In summer, cloud cover creates natural disparity in incident PPFD, whilst its absence in winter ensures smaller variation in gas exchange parameters. Results from this study indicated that incident PPFD drives leaf conductance, CO_2 uptake and transpiration in both seasons. Reduced F_v/F_m, from dawn to midday in both seasons, indicated that photoinhibition probably occurred when excessive PPFD decreased quantum efficiency (chapter 4). Cloud cover in summer lowers transpiration and increases WUE. Lower WUE in

winter is probably caused by evapotranspiration stress due to reduced soil moisture. Decreased F_v/F_m at midday, in both seasons, was reversible with values at dawn closely approximating those at dusk thus indicating lack of permanent damage to the photosynthetic apparatus.

The ability to acclimate to low PPFD may aid seedlings to establish and persist under the canopy of trees for extended periods. Shade tolerance has been suggested as a potential mechanism of invader success (Jones & McLeod, 1990). Low light tolerant species may undergo rapid growth once gaps within the canopy emerge. Visual inspections confirmed that species growing in the shade possessed single stems compared to multi-stem sun plants. Plants overlapping both stands, i.e. those exposed to intermittent light, displayed characteristics of both communities. This suggests a wide tolerance to incident PPFD.

Water stress significantly reduced CO_2 uptake, lowered water potential and caused leaf wilting (chapter 5). Wilting may represent a physiological strategy to minimise water loss and reduce excessive incident PPFD. Leaf wilting is an adaptive response to prevent dehydration injury and was usually associated with an increase in proline. The significant increase in this metabolite in water stressed plants suggested that it serves an adaptive osmoprotective role. In addition, loss of turgidity in leaves may reduce light stress and photoinhibition. With increase in duration of stress, leaf shedding occurred, suggesting a strategy to lower energy loads and conserve water. Severe water stress (after day eight) led to almost complete stomatal closure.

Although well watered plants exhibited signs of photoinhibition, full recovery in photosynthetic quantum yield and F_v/F_m occurred at the end of the day. The midday depression in F_v/F_m in well watered plants (0.75- 0.77), was probably caused by high PPFD (chapter 5). However, decreases in F_v/F_m in water stressed plants suggested that chronic photoinhibition rather than a down regulation of photochemistry occurred, as there was no recovery in intrinsic photochemical efficiency at dusk. Stomatal closure and the subsequent reduction in water loss prevented damage to the photosystems. Increase in WUE in water stressed plants may be interpreted as an adaptive response to progressive water scarcity. High WUE may be a trait contributing to increased quantum yield under stress conditions.

The ability to recover rapidly from low quantum yield values at midday may be a mechanism that enables the weed to be more successful than other C_3 species in colonising disturbed habitats. The study showed that *C. odorata* is susceptible to water stress but that this response may be delayed by leaf wilting and shedding strategies. Tolerance to water stress in *C. odorata* is similar to other typical C_3 species. The higher prevalence of the weed along the east coast of KwaZulu-Natal compared to the drier inland may be attributed to the greater rainfall.

Antimicrobial screening of leaf and stem extracts of *C. odorata* revealed greater efficacy against selected fungi and gram positive than negative bacteria (chapter 7). The effectiveness of ethanol and methanol extracts was low, suggesting that active compounds were present in low concentrations. Leaf decoctions to treat ailments in traditional medicine are debatable. Extracts with alcohol were more effective than those with water in preventing bacterial and fungal growth. Greater efficacy of leaf, compared to stem extracts, may probably be due to higher concentration of phenolic, alkaloid and flavonoid compounds. In addition, tannins, sterols, triterpenoids and saponin glycosides were detected in leaf tissues. The presence of phytochemicals may possibly be a strategy to deter herbivorous predators. Pyrrolizidine alkaloids in aqueous root and seed extracts of *C. odorata* were shown to possess nematicidal (Thoden *et al*., 2007), antibacterial (Khoa *et al*., 2011), antifungal (Ngane *et al*., 2006) and antiprotozoal (Vital & Rivera, 2009) activities.

Chromolaena displays considerable morphological variation within the environment (Ambika, 1998). In open areas, it forms a compact shrub up to three metres whereas, under trees or forest margins, it can overgrow trees up to 10 m (Goodall & Erasmus, 1996). The highly plastic morphology may be a strategy to smother and eliminate potential competitors. Many invasive species have high reproductive rates (Rejmanek & Richardson, 1996). The dispersal success of the weed may be attributed to its high reproductive capacity i.e. both by vegetative means and by massive seed production (Ambika, 1998). Each plant is capable of producing up to one million seeds per season (Gautier, 1992). Up to 80% of seeds produced are capable of germinating (Ambika & Jayachandra, 1990). The small seeds (cypsela) are light and attached to a pappus which can be dispersed long distances by animals and wind (Kriticos *et al*., 2005).

In addition, *C. odorata* produces phytotoxins which are allelopathic and, when released into the soil, these toxins may reduce the growth of indigenous flora (Ambika, 1998; Lorenzo *et al*., 2013). Furthermore, symbiotic nitrogen fixing bacteria are associated with the roots of *Chromolaena*, ensuring growth in low nutrient soils (Ambika, 1998; Yang *et al*., 2013). Moreover, *Chromolaena* fulfils all of the criteria suggested by Baker (1965) in his interpretation of the ideal weed (Table 1).

Table 1. Characteristics of an ideal weed (adapted from Baker, 1965).

CHARACTERISTICS
1. Effective seed dispersal mechanism
2. Longevity of seed
3. Rapid seedling growth
4. High seed output
5. Maintains seed production
6. No special germination requirement
7. Can self pollinate
8. Cross pollination by insects or wind
9. Flowers after short vegetative period
10. Vigourous vegetative growth
11. Ability to regenerate from root stock
12. High tolerance to climatic variables
13. Ability to compete by special means e.g. phytotoxin production, smothers competitors etc.

The broad spectrum herbicide, glyphosate, was effective in killing young plants within a week of spraying (chapter 6). Most of the glyphosate was present within the plant within 24 h after treatment, suggesting that an efficient uptake is a major determinant in herbicide efficacy (Feng *et al*., 2000). Although the herbicide is non-selective, detection within the root

two days after spraying, suggested transport from leaves to the roots. Greater initial CO_2 uptake probably enhanced glyphosate transport from leaves to other tissues. Glyphosate application decreased CO_2 uptake and photosynthetic quantum yield from day one to day five, suggesting disruption in photosynthetic capacity. The toxic levels of glyphosate in the root probably affected water absorption, resulting in progressive wilting from day two. Significant correlation existed between all chlorophyll fluorescence parameters (quantum yield, ETR, F_v/F_m) and CO_2 uptake, suggesting that these parameters are useful in assessing glyphosate efficacy.

Plant responses to environmental constraints are complex and complemented by a variety of biochemical, anatomical and physiological traits. In the natural environment, there is competition among plants for resources and species interactions may exist. In addition, gas exchange measurements rely on the interaction between various environmental parameters which are dynamic in nature. Controlling these variables is impossible; therefore the study has inherent limitations.

Due to time constraints, certain experiments (e.g. herbicide application) were performed in winter. However, the outcome of these experiments may have been better validated if these studies were repeated in summer. Furthermore, an important disadvantage of closed gas exchange systems is that air tight chambers may not possible so that gas leakage may have lead to incorrect readings. However, closed systems are portable, ensure rapid measurements and yield reliable data if properly calibrated.

Furthermore, this study attempted to simulate field conditions (chapter 5) by allowing three months acclimation in the open before the imposition of water stress; it is possible that the restrictions imposed on the plants may have adversely affected photosynthetic performance. It is likely, for instance, that root development was restricted in pots, affecting growth and subsequently gas exchange measurements. Subjecting plants to water stress in the field is more difficult.

Future research incorporating population and ecophysiological studies that focus on genetic and evolutionary dynamics may prove useful in devising effective control measures to limit spread of *C. odorata*. Quantitative genetic analysis could shed more light on how phenotypic plasticity enhances colonising and reproductive ability. Transgenic manipulation, for

instance, can be used to suppress flowering in *C. odorata* and effectively control spread. Moreover, genetic manipulation may be an effective strategy to limit achene production and regulate new growth. A major limitation to glyphosate use is its non-selectivity when treating weeds in the field. However, genetically engineering glyphosate resistant crops is possible thereby increasing herbicide selectivity when applied under field conditions.

Glyphosate, when used alone, can be very effective in controlling the spread of *C. odorata*. However, the effectiveness of the herbicide may be enhanced by combining the herbicide with another. A combination of herbicides may prove more effective than a single one. Although it is recommended that most herbicides be sprayed on actively growing herbaceous plants, mostly in summer (Feng *et al*., 2000) glyphosate proved effective even in winter in this study.

Understanding the physiological characteristics of its invasive success, in particular its photosynthetic performance, is important to control and manage effectively the spread of weed species. Plant distribution models can be developed based on the weed's high CO_2 uptake and plasticity. These models may help to identify vulnerable ecosystems for potential invasion and spread. An integrated approach that incorporates distribution patterns and ecophysiological based models is needed to reduce the impact of *Chromolaena* in KwaZulu-Natal.

Ecophysiological characteristics that enhance spread and invasive potential of *Chromolaena odorata* include:

a) higher photosynthetic rates than other C_3 species,

b) high WUE that maximises CO_2 uptake and minimises water loss,

c) acclimation to high and low incident PPFD,

d) tight coupling of incident PPFD, leaf conductance and CO_2 uptake,

e) leaf wilting and shedding as effective photoprotective strategies that reduce energy loads,

f) efficient mechanisms to prevent dehydration injury and,

g) phytochemicals such as antimicrobials, present at low concentrations, which probably serve to reduce herbivory.

8.1. References

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