UNIVERSIDADE FEDERAL DE PERNAMBUCO
CENTRO DE CIÊNCIAS BIOLÓGICAS
DEPARTAMENTO DE BOTÂNICA
PROGRAMA DE PÓS-GRADUAÇÃO EM BIOLOGIA VEGETAL

KARLA VIVIANE DE FIGUEIREDO

EFEITO DO DÉFICIT HÍDRICO E DA REMOÇÃO DA CERA EPICUTICULAR NAS TROCAS GASOSAS EM *Jatropha mollissima* E *Jatropha curcas*

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Dissertação submetida ao Programa de Pós-Graduação em Biologia Vegetal do Centro de Ciências Biológicas da Universidade Federal de Pernambuco, como requisito parcial para a obtenção do grau de Mestre em Biologia Vegetal.

Orientador: Prof. Dr. Mauro Guida dos Santos

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“O único lugar onde sucesso vem antes do trabalho é no dicionário”. (Albert Einstein)

Dedico a minha mãe (Louise) pelo amor, carinho e dedicação e motivação para continuar lutando mesmo com as adversidades.

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LISTA DE FIGURAS

LISTA DE TABELAS

SUMÁRIO

1. APRESENTAÇÃO

Devido às mudanças climáticas globais é esperado que haja aumento da aridez nas regiões áridas e semiáridas do globo. Restrição na disponibilidade de água afeta severamente o crescimento, desenvolvimento e produtividade dos vegetais, e a sobrevivência de plantas nessas áreas dependerá do grau de tolerância à seca apresentado por elas. Assim, a compreensão dos mecanismos que as plantas utilizam para tolerar à seca é fundamental para o desenvolvimento de cultivares tolerantes.

Nesse contexto, o desenvolvimento de uma barreira que evite a perda excessiva de água e que seja capaz de refletir parte da radiação que chega a superfície da folha foi de suma importância para manutenção do processo vital às plantas: a fotossíntese. As ceras epicuticulares, camada da cutícula que representa a principal interface da planta com o ambiente, representam um importante fator de adaptação a estresses ambientais incluindo seca e alta irradiância.

No entanto, é sabido que apenas a cutícula não é suficiente para manter o status hídrico da planta, assegurando seu metabolismo, quando a água é um fator limitante. Assim, outros mecanismos de adaptação à falta de água fazem-se necessários como eficiente controle estomático.

Em geral, as plantas nativas ou exóticas adaptadas às regiões semiáridas apresentam um eficiente controle estomático e maior deposição de cera. Entretanto, ainda há uma lacuna no que diz respeito ao conhecimento de como as ceras epicuticulares podem influenciar os mecanismos de trocas gasosas em espécies dessa região.

A escolha de culturas tolerantes à seca visa encontrar plantas que consigam manter alta a atividade fotossintética com perda mínima de água durante a transpiração. Assim, o conhecimento das alterações nos mecanismos de trocas gasosas e a eficiência das principais resistências foliares, isto é, estomática e cuticular são de suma importância para entender os aspectos de tolerância quando estas espécies estão sujeitas ao déficit hídrico.

Dessa forma, o objetivo principal desse trabalho foi avaliar o papel do controle estomático e o papel da composição e teor da cera epicuticular sobre a tolerância à seca em duas espécies do mesmo gênero, porém, uma nativa e outra exótica a flora brasileira. Para isso foram avaliadas as relações hídricas, trocas gasosas e algumas variáveis bioquímicas em plantas de *Jatropha mollissima* e *Jatropha curcas* sob déficit hídrico.

2. FUNDAMENTAÇÃO TEÓRICA

2.1 Mecanismos de tolerância à seca

Dos estresses bióticos e abióticos aos quais as plantas são frequentemente expostas, a seca é uma das condições mais severas para crescimento e desenvolvimento dos vegetais (MANAVALAN et al., 2009). Restrição na disponibilidade de água acarreta uma série de mudanças morfológicas, fisiológicas, bioquímicas e moleculares em todos os órgãos da planta afetando negativamente crescimento e produtividade (WANG et al., 2001). Assim, a compreensão dos mecanismos que as plantas utilizam para tolerar à seca é fundamental para o desenvolvimento de cultivares tolerantes.

Para assegurar a sobrevivência, as plantas podem desenvolver mecanismos de adaptação à falta de água como: fechamento dos estômatos, ajustamento osmótico, ajustamento da parede celular, produção de folhas menores, redução da área foliar, aumento na densidade e profundidade de raízes e espessamento da cutícula (MATTOS et al, 1999).

Nesse aspecto, a fotossíntese é um dos processos do metabolismo primário dos vegetais que é mais afetado pela restrição hídrica (LIU & HUANG, 2008). Diminuição da difusão de CO_2 da atmosfera para o sítio de carboxilação é geralmente considerada a principal causa da diminuição da fotossíntese em planta sob déficit hídrico moderado (FLEXAS et al., 2004).

Segundo Cornic (2000) a limitação estomática é aceita como o principal fator redutor da fotossíntese. Isso porque ao absorverem CO_2, inevitavelmente, as plantas perdem água pelas folhas através dos estômatos, que apresentam mecanismos para controlar o seu grau de abertura. Abertura e fechamento dos estômatos dependem da ação coordenada do mecanismo que controla a turgescência das células-guarda e da regulação de hormônios como o Ácido abscísico (ABA), Etileno, Giberelinas (fechamento) e Citocininas (abertura) (CHAVES et al., 2003). Assim, os estômatos apresentam papel fundamental nas trocas gasosas, controlando a perda de água por transpiração e a assimilação do CO_2 pelas plantas (JONES, 1998).

Um controle estomático eficiente sobre as trocas gasosas pode reduzir a perda de água quando as plantas estão sobre baixa disponibilidade hídrica e elevada taxa evaporativa atmosférica assim, evitam que ocorra uma severa desidratação das folhas (ARCOVERDE et al., 2011). Entretanto, quando o estresse torna-se mais intenso a planta também recorre a controles não-estomáticos, como acúmulo de solutos orgânicos (MARQUES DA SILVA & ARRABAÇA, 2004) e produção de metabólitos secundários (ALAM, 2010).

Outra resposta das plantas à baixa disponibilidade de água é a inibição de crescimento aéreo e radicular que, apesar de diminuir a área transpiracional, acaba reduzindo também a radiação interceptada durante todo o período vegetativo e, finalmente, ocorre diminuição da produção de biomassa (PEREIRA & CHAVES, 1993). Isso acompanhado por fechamento estomático e, conseqüentemente redução de gás carbônico (CO_2) captado para fotossíntese, acaba induzindo um estresse oxidativo, que pode afetar o metabolismo normal através de danos oxidativos a lipídios, proteínas e ácidos nucléicos (POMPELLI et al, 2010). Condições de seca severa pode ainda levar a interrupção do desenvolvimento reprodutivo, senescência, murcha, dessecação e morte de folhas imaturas (SCHULZE, 1986).

Além disso, a perda de turgor causado pelo déficit hídrico nas plantas pode parar o crescimento vegetal, além de afetar diversos processos bioquímicos como a diminuição na síntese de proteínas (HSIAO, 1973; RODRIGUES et al., 2011) e aminoácidos (RODRIGUES et al, 2011).

Depois da fotossíntese, um dos processos mais afetados durante a seca é o metabolismo de carboidratos, devido à diminuição de oferta de CO_2 para as enzimas carboxilases, como consequência do fechamento estomático. Análises bioquímicas sugerem que alterações no processo de quebra de carboidratos sob estresse hídrico seja uma resposta adaptativa (ALAM et al, 2010). Em alguns casos quando o nível de água atinge valores muito baixos observando-se não somente diminuição na taxa de metabolismo de carbono fotossintético, mas também mudanças qualitativas (MARQUES DA SILVA & ARRABAÇA, 2004).

Ainda, teor e composição da cutícula também podem conferir proteção à planta uma vez que sua função fisiológica primária é limitar a perda de água por controle não-estomático, e essa função tem sido frequentemente relacionada às ceras epicuticulares (BAUR, 1998). Outras funções também são atribuídas a essa estrutura: proteção contra a luz ultravioleta (CHARLES et al., 2008) e a moderação da troca de gasosa (JEFFREE et al., 1996).

Em se tratando de plantas da Caatinga, estudos sobre as propriedades físico-químicas da cera epicuticular são de alta relevância (OLIVEIRA et al, 2003). Quando se propõe cultivo de plantas para esta região, o sucesso do empreendimento depende em alto grau da resistência da cultura ao estresse hídrico. A presença de uma cutícula muito impermeável tem sido descrita como uma característica que pode indicar tolerância à seca (JAMAUX et al, 1997) assim, as plantas com baixas taxas de transpiração cuticular podem conservar uma alta quantidade relativa de água em condições de déficit hídrico.

2.2 Ceras cuticulares

Há mais de 400 milhões de anos a aquisição de uma cobertura lipídica, a cutícula, possibilitou a colonização do continente pelas plantas (KENRICK & CRANE, 1997). A cutícula está presente em todos os órgãos com crescimento primário, estando ausente nas raízes e em órgãos com crescimento secundário. Ela desempenha um importante papel na fisiologia e ecologia das plantas, sendo a principal interface entre as células epidérmicas e a atmosfera (BUSCHHAUS et al., 2007), além de ser uma eficiente barreira protetora, uma vez que atua controlando a perda de água, íons e nutrientes, e reduz a infecção por patógenos (GUHLING et al., 2005).

A cutícula é uma camada bastante heterogênea constituída de cutina e ceras (JENKS et al., 2001). Segundo Müller & Riederer (2005), as ceras são, primeiramente, biossintetizadas para conferir proteção contra as proteases de fungos, e podem ser distinguidas pela sua origem, ultraestrutura e composição químicasó posteriormente a matriz é formada. A cutina é um biopolímero do tipo poliéster que forma a matriz da cutícula. Já as ceras, podem estar embebidas na matriz (intracuticular) ou sobrepostas (epicuticular) (KOCH & ENSIKAT, 2008). As ceras epicuticulares representam a primeira superfície de contato das plantas com o ambiente, e sua estrutura e composição podem variar entre espécies de plantas, órgãos, estágio de crescimento (JEFFREE, 1996), no entanto, são constituídas, majoritariamente, por *n*-alcanos de cadeia carbônica longa (C_{21}-C_{35}), alcoóis primários (C_{22}-C_{40}), ácidos graxos (C_{20}-C_{24}), aldeídos (C_{24}-C_{36}), alcoóis secundários (C_{21}-C_{35}), cetonas (C_{21}-C_{35}) e ésteres (C_{36}-

C_{60}) Estas ainda podem apresentar triterpenóides e metabólitos secundários menores como esteróis e flavonóides (KUNST & SAMUELS, 2003).

As propriedades físicas e químicas da cera cuticular determinam funções vitais para planta (BUSCHHAUS et al., 2007). Além de reduzir a perda de água, resguarda a superfície de folhas, caules, flores e frutos do acúmulo de partículas da atmosfera (KERSTIENS, 2006). Além disso, é eficiente na proteção contra bactérias e fungos patogênicos (GENTRY & BARBOSA, 2006), bem como atenua os efeitos deletérios da radiação ultravioleta (CHARLES et al., 2008). As ceras também intermediam várias interações inseto-planta (KERSTIENS, 2006).

2.2.1 Cera epicuticular foliar e seu papel na tolerância à seca

A superfície hidrofóbica das folhas confere algumas vantagens à planta. A repelência à água impede que, após o período chuvoso, a água fique retida na superfície, promovendo assim, a condução de solutos orgânicos do interior para fora da planta. A retenção dessa água também criaria um ambiente favorável à proliferação de microrganismos potencialmente prejudiciais, como bactérias e fungos patogênicos (HEREDIA & DOMINGUEZ, 2009). Além disso, as propriedades físico-químicas das ceras epicuticulares das folhas criam um mecanismo de auto-limpeza na superfície da folha, removendo micropartículas como poeira, pólen, esporos e micróbios (KOCH & ENSIKAT, 2008). O comportamento de forrageamento de muitos insetos, bem como a preferência alimentar, também depende da composição química e estrutura das ceras epicuticulares (MARKSTÄDTER et al., 2000).

Ainda, muitos estudos têm sugerido que as ceras epicuticulares representam um importante fator de adaptação à estresses ambientais, incluindo seca e alta irradiância (JORDAAN & KRUGER, 1992; RAVEH et al., 1998; WEIGLIN & WINTER, 1991). Sanchez et al. (2001) e Yang et al. (2011), por exemplo, verificaram o acúmulo de cera em folhas de *Pisum sativum* L. (ervilha) e *Arabidopsis thaliana* (L.) Heyhn, respectivamente, ocorrendo em resposta ao estresse hídrico. Samdur et al. (2003) sugeriram que o aumento no teor de cera foliar é uma resposta de aclimatação ao déficit hídrico para reduzir a perda de água cuticular, melhorando a eficiência no uso da água na folha. Entretanto, Olivera et al.

(2003) admitem que a cera epicuticular por si só não é suficiente para garantir a sobrevivência das plantas a longos períodos de estiagem.

Um mecanismo protetor contra o excesso de luminosidade no complexo e sensível aparato fotossintético, também é gerado pela presença das ceras epicuticulares. Ela controla o grau de difusão e reflexão, e torna tolerável a intensidade de radiação que alcança o fotossistema ativo nos tecidos no interior das folhas (RIEDERER, 2006). Grant et al. (1995) e Jeffree et al. (1971) sugerem que as cera epicuticulares exercem papel fundamental na reflectância da luz nas superfícies das folhas. Já Mauseth (1988) verificou que a cera epicuticular foi capaz de refletir 25% da luz incidente sobre as folhas de *Echeveria* sp. e Reicosky & Hanover (1978) verificaram que a cera da folha do pinheiro é capaz de reduzir a absorção de comprimentos de ondas longos, reduzindo a temperatura foliar. Além disso, ao aumentar a reflectância, a cera pode diminuir o risco de sobre-excitação dos centros de reação do fotossistema II, evitando assim, que ocorra danos foto-oxidativos (ROBINSON et al., 1993)

Por fim, trabalho realizado por Mohammadian et al. (2007) com folhas de *Leucadendron lanigerum* H. Buek ex Meisn. (Proteaceae) indica que a cera diminui a perda de água, mas tem pouco impacto sobre a reflectância da folha. Além disso, a deposição de cera sobre os estômatos aumenta a resistência à difusão de gases, acarretando em diminuição da transpiração estomática, condutância e fotossíntese. Dessa forma, a produção de cera pode colaborar para evitar danos causados pelo excesso de luz, e esta propriedade pode beneficiar plantas que se desenvolvem em ambientes áridos e com alta radiação solar.

2.2.2. Cera epicuticular em Euphorbiaceae

Estudos com lipídios cuticulares foliares de Euphorbiaceae são restritos a poucos gêneros, e em sua maioria tratam de interação biológica ou da química. Em espécies de *Macaranga* Thouars foram identificados triterpenos cuticulares como β-amirina, fridelano e taraxerol, parecendo serem responsáveis pela interação planta-formiga (mimercofilia) (JÜRGENS et al., 2006; MARKSTÄDTER et al., 2000). Já Guhling et al. (2005) encontraram ácidos graxos, alcoóis primários, aldeídos, além de α-amirina, β-amirina e lupeol compondo a cera epicuticular de *Macaranga tanarius* (L.) Müll. Arg.. Triterpenos também foram caracterizados em *Manihot* Mill. (MARKSTÄDTER et al., 2000) e em caule de *Ricinus communis* L. (GUHLING et al., 2006). Em todos os casos, a ocorrência de triterpenos foi relacionada à presença de cristais na cera epicuticular.

Nas folhas da mamona foram identificados *n*-alcanos, alcoóis primários, aldeídos, ácidos graxos e triterpenos (lupeol, α-amirina e β-amirina) (VERMEER et al., 2003) e, no extrato foliar de *Cnidoscolus aconitifolius* (Miller) I.M. Johnston foram identificados, além de α-amirina e β-amirina, amirenona e *n*-alcanos (ESCALANTE-EROSA et al., 2004).

Nenhum dos trabalhos, no entanto, trata da importância do teor e composição das ceras epicuticulares nos mecanismos de trocas gasosas e manutenção do status hídrico das plantas.

2.3 *Jatropha curcas* L. e *Jatropha mollissima* (Pohl) Baillon.

Jatropha curcas L. e *Jatropha mollissima* (Pohl) Baillon. são espécies exótica e nativa, respectivamente, encontradas em todo o nordeste brasileiro, sobretudo em Pernambuco. Em um ambiente naturalmente árido ou semi-árido, é comum a exposição dessas espécies às condições de alta demanda evaporativa e déficit hídrico, o que acarreta um desenvolvimento limitado. Diversos fatores de estresse, tal como excessiva temperatura, luz e evaporação, bem como pouca disponibilidade hídrica, restringem a assimilação de CO_2 destas espécies (KHEIRA & ATTA, 2009; POMPELLI et al., 2010).

Sobre as espécies propostas neste estudo não se têm muitos dados literários. Os únicos dados encontrados tratam de plântulas de *J. curcas* cultivadas sob condições de casa de vegetação expostas à diferentes regimes de déficit hídrico (ARCOVERDE et al., 2011; POMPELLI et al., 2010). Em se tratando de condições de campo, Santos (2008) avaliou comparativamente o comportamento fenológico, bem como trocas gasosas foliares e eficiência fotoquímica na Zona da Mata e Agreste do Estado de Alagoas. Porém, não há qualquer registro de trabalhos com ecofisiologia de espécies nativas nas duas principais condições climáticas ocorrentes na Caatinga: as estações chuvosa e seca.

Jatropha curcas é uma planta rústica, com uma grande plasticidade, resistente a longas estiagens, bem como à pragas e doenças, sendo adaptável à condições edafoclimáticas muito variáveis. Possivelmente é nativa de áreas tropicais da América do Sul e, atualmente, encontra-se amplamente cultivada em áreas tropicais da África e Ásia (OPENASHAW, 2000). Desenvolve-se bem tanto em regiões tropicais secas como nas zonas equatoriais úmidas, assim como em solos pedregosos, arenosos e salinos, podendo suportar longos períodos de seca. Há ainda os benefícios de produção que se inicia a partir do segundo ano do plantio e perdura de quarenta a cinquenta anos, produzindo de quatro a cinco quilos de semente por individuo a partir do quinto ano de plantio (KUMAR & SHARMA, 2008).

Jatropha curcas cada vez mais tem atraído atenção para o uso de suas sementes como matéria-prima na produção de biodiesel. Suas sementes contêm 30 a 35% de óleo com as características necessárias para a produção do biocombustível (AGARWAL & AGARWAL, 2007). O teor de óleo de *J.curcas* é superior ao de oleaginosas tradicionais como oliva (25-30%), soja (18-20%) e algodão (18-20%) (MORETTO & FETT, 1998). Além disso, o pinhão-manso tem sido usado na recuperação de terras pouco férteis, prevenção e controle da erosão, como cerca viva em áreas de pastagem (KUMAR & SHARMA, 2008)

Embora relevante economicamente, deve-se considerar os efeitos negativos que seu cultivo poder promover. Em muitos casos *J. curcas* é considerada uma espécie exótica (SAHOO et al., 2009) e o seu crescimento pode causar efeitos negativos para a biodiversidade. Heller (1996) chama a atenção para o efeito fitotóxico da torta de sementes usada como fertilizante, na germinação de espécies locais. Já Sahoo et al. (2009) mostrou que a possibilidade de qualquer efeito alelopático do pinhão-manso é remota, uma vez que a espécie suporta uma variedade considerável de meso-fauna e microbiota, além de permitir o crescimento de outras espécies nativas como também de interesse econômico.

Jatropha mollissima é um arbusto nativo ocorrente na Caatinga. Possui inflorescência terminal cimosa, monóica, flores pentâmeras, pistiladas e estaminadas do tipo prato e produtoras de néctar e frutos do tipo esquizocárpicos, secos, com três cocas globosas de deiscência explosiva e, consequentemente, dispersão primária por autocoria (NEVES et al., 2010). O pinhão-bravo destaca-se pelo potencial oleaginoso apresentado por suas sementes (38%) (MAYWORM et al., 1998), mas dependendo das características intrínsecas do fruto, sua forma de cultivo e colheita, esses teores podem ser significativamente inferiores (TEIXEIRA, 1987). Embora haja estudos sobre a ecologia (LEAL et al., 2007) e fenologia de *J. mollissima* (NEVES et al., 2010), são raros os trabalhos que abordem características de ordem ecofisiológica ou mesmo produtiva da espécie.

Diante do potencial econômico que *J. curcas* e *J. mollissima* apresentam e a vantagem de serem bem adaptadas à baixa disponibilidade hídrica, além de se desenvolverem bem em áreas abandonadas e degradadas, ambas podem representar uma alternativa de cultivo para o sertanejo, diminuindo a pressão sobre as espécies nativas e assim, contribuindo tanto para recuperação de áreas inférteis como para a conservação de áreas preservadas de vegetação nativa.

Além disso, trabalhos envolvendo a ecofisiologia e a bioquímica de plantas nativas e exóticas da Caatinga e que apresentam potencial econômico como oleaginosas ajudariam a compreender os diferentes fatores que determinam a habilidade dessas espécies em tolerar à seca. O conhecimento da eficiência da cutícula como barreira antitranspirante é de suma importância para entender os aspectos de tolerância quando estas espécies estão sujeitas ao déficit hídrico (BUSCHHAUS et al., 2007; GUHLING et al., 2005).

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4. MANUSCRITO

Epicuticular wax removal influences gas exchange and water relations in leaves of exotic and native species from Brazilian semi-arid region under greenhouse induced drought stress

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Running head: Leaf epicuticular wax removal decreases water content

Abstract

The primary physiological function of the cuticle is to limit leaf water loss, thus, in semi-arid region has great importance. In this study, we examined the hypothesis that the quality and content of the epicuticular wax between different species influences the leaf gas exchange. Plants of *Jatropha mollissima*, a Brazilian semi-arid native, and *Jatropha curcas*, an exotic species, were subjected to a water deficit in the presence or absence of epicuticular wax. Plants were grown in 10-L pots under greenhouse conditions. The relative water content, gas exchange parameters and primary carbon metabolism were measured at 21 days after the irrigation was reduced to induce a water deficit. The well-watered plants of both species showed recovery of gas exchange days after the removal of epicuticular wax. Furthermore, under drought, a gradual increase in transpiration rates was only observed in native species leaves without wax, although the stomatal conductance did not differ between both species. High relative water content was maintained, except in leaves under drought and without wax from the 13th day when compared with all other treatments. The wax production was induced in both species under water shortage. Nevertheless, the native species showed a higher content of long-chain *n-alkanes*. In fact, the barrier to water vapour under reduced stomatal conductance was highest in the native species.

Introduction

Native or exotic plants adapted to semi-arid regions exhibit efficient stomatal control (Souza *et al*. 2010; Arcoverde *et al*. 2011; Rodrigues *et al*. 2011) and have a thick waxy layer and/or composition with different water permeability (Oliveira and Salatino 2000; Oliveira *et al*. 2003). *Jatropha mollissima* and *Jatropha curcas* are native and exotic shrubs from Brazilian semi-arid, respectively, which are located in open areas of the Caatinga, a seasonally dry tropical forest in Brazil. Both are rich in oil, approximately 40% of the oil is contained within the seeds (Maywor *et al*. 1998). The characteristics of this show great potential for use as biofuel (Maes et al. 2009).

Drought is a major abiotic factor that affects the success of plants (Manavalan *et al*. 2009; Pinheiro and Chaves 2011). The rapidly changing global climate has increased the aridity in semi-arid regions of the world, which ultimately restricts the growth and survival of plants in these areas (Chaves *et al*. 2002; Passioura 2007). Water deficits initially affect photosynthesis because the diffusion of atmospheric CO_2 to the site of carboxylation is limited (Chaves *et al*. 2003; Santos *et al*. 2006). In most cases, plants respond to water stress through stomatal control (Souza *et al*. 2010). Stomatal control reduces water loss, which can provide the maintenance of relative water content under low water availability and high atmospheric evaporation (Arcoverde *et al*. 2011; Pinheiro and Chaves 2011). On the other hand, the stomatal closure may increase the leaf temperature due to low transpiration rate, specially under field conditions in semi-arid region (Chaves *et al*. 2002; Frosi *et al*. 2012).

Another adaptive mechanism is a hydrophobic layer that coats the epidermis of all aerial plant organs. The primary physiological function of the cuticle is to limit water loss through non-stomatal control, and this role has often been associated with its content and composition, i.e., epicuticular waxes (EW) (Yang *et al*. 2011). In fact, the cuticular conductance for atmospheric carbon dioxide (CO_2) and water vapour is low, which is ignored in most situations, except when stomatal conductance is extremely low. The cuticle is composed of three important constituents: waxes, polysaccharide microfibrils and cutin. Cutin is a three-dimensional polymer network of esterified fatty acids. Thus main barrier for diffusion is located within a waxy skin, which is less than 1 μm thick (Kerstiens 1996). The epicuticular wax is a complex mixture of straight chain C20 to C60 aliphatics that might include cyclic metabolites, such as triterpenoids and flavonoids (Baker *et al*. 1979). The structure and composition of epicuticular wax could also serve as a mechanical barrier against various other factors, such as insects, pathogens, UV-B radiation, and extreme temperatures (Barnes and Cardoso-Vilhena 1996).

We hypothesised that removing the epicuticular wax alters gas exchange in both species, thereby increasing the transpiration rate in well-watered plants but not in plants under drought stress. It is known that the wax content is related to increased drought tolerance in some plant species (Yang *et al*. 2011). Moreover, several studies have shown that under drought, the cuticular wax content increases in the leaves of different species, such as cotton (Bondada *et al*. 1996), peanut (Samdur *et al*. 2003) and *Arabidopsis thaliana* (Yang *et al*. 2011).

Thus, the primary objective of this study was to evaluate the role of epicuticular wax in gas exchange, leaf water relations and some leaf biochemical parameters of *J. mollissima* and *J. curcas* under mild water deficit.

Material and methods

Plant material and growth conditions

Jatropha mollissima (Pohl) Bailon and *Jatropha curcas* L. (Euphorbiaceae) plants were propagated from seeds under greenhouse conditions in 10 L pots containing 9.0 kg of substrate: a 3:1:1 mixture of black earth, red clay and washed sand, respectively. After 125 days of cultivation under optimal hydration, the water quantity in the soil was varied: 100% (control, 560 mL) and 25% (stress) of the pot capacity during 21 days. During the experiment the average temperature in the greenhouse ranged from 24 to 33°C, and the maximum photosynthetic photon flux density (PPFD) was approximately 700 µmol m-2 s-1. The vapor pressure deficit (VPD) variations are shown (Fig. 1).

Leaf relative water content (RWC) *and soil* (SRWC)

The relative water content (RWC), which characterises the maximum hydration of the tissue, was measured at predawn during the 15 days of water treatment. The RWC was calculated using the formula: [(FW-DW)/(TW-DW)] x 100, where FW is the leaf fresh weight, TW is the turgid weight and DW is the dry weight at 80°C after 48 h (Barrs and Weatherley 1962; Souza *et al*. 2010). The soil relative water content (SRWC) was calculated using the formula (FW − DW)/DW.

Mechanical wax removal

Two leaves per individual from each species subjected to different water treatments were choosed and EW was removed. The removal was performed using an aqueous solution of gum arabic (Sigma-Aldrich) (1.5 g mL^{-1}) applied with a brush on the abaxial and adaxial leaf surfaces at a rate of 0.1 mL^{-1} cm^2. After 24 h of soaking, the newly formed film was removed with tweezers (Jetter and Schäffer 2001).

Epicuticular wax content

At maximal stress in 21st day, undamaged and fully expanded leaves of both species were subjected to extraction using two successive 30 s treatments with dichloromethane. The average epicuticular wax content was calculated as the ratio between the amount of wax obtained and the dry leaf weight, which was expressed as % dry matter. The classes of compounds were identified using Thin Layer Chromatography (TLC) with silica gel (Fluka 60760 Kieselgel G) containing a 0.02% sodium fluorescein salt (Fluka F6377) and *n*-hexane/dichloromethane (73:27 v/v) as the mobile phase followed by visualisation under long wave UV.

Leaf gas exchange

The gas exchange was measured using an infrared gas analyser (IRGA, model LCpro Analytical Development Co. Ltd, Hoddesdon, UK), which measures the stomatal conductance (g_s), net CO_2 assimilation (P_N) and transpiration (E). The measurements were performed using fully expanded and healthy leaf from six plants per treatment. Two leaves with and without EW were measured from each plant (removed at 48 h before water restriction). All gas exchange measurements were made between 0930 and 1130 h with the IRGA leaf chamber PPFD set at 700 µmol m-2 s-1. Leaf temperature was measured at the same time as leaf gas exchange using an infrared thermometer.

Biochemistry analysis

The fully expanded leaves of control and drought stressed plants were collected in the middle of afternoon (14:30 h) of 21st day after water shortage. These leaves were wrapped in aluminium foil, immersed in liquid nitrogen and stored in a freezer -20°C. These leaves were used to determine the total soluble carbohydrates (TSC), soluble proteins (SP), total free amino acids (FA) and total chlorophyll (CHL). The fresh leaf samples were examined following the methodologies of Dubois *et al*. (1956), Bradford (1976), Moore and Stein (1948) and Lichthenthaler (1987), respectively.

Statistical analysis

The experiment was conducted in a completely randomised factorial using two species, two water regimes, two leaves per individual (with and without epicuticular wax) and seven replicates. The data were subjected to analysis of variance and the mean values of the treatments were compared with the Student-Newman-Keuls test at 5% significance when needed, using the Statistica software package ver.7.0 (Statsoft Inc., Tulsa, OK).

Results

The leaf RWC of *J. mollissima* was approximately 10 % higher ($p < 0.05$) than that of *J. curcas* in all treatments (Fig. 1a-b). . There was no difference between the RWC values for the control and stressed. *J. mollissima* plants ($p > 0.05$), regardless of the treatment day, except in leaves with both stress associated with: drought and without wax. The RWC of the *J. curcas* leaves differed between treatments ($p < 0.05$) and days of stress ($p <0.05$). The *J.*

curcas leaves under stress and without wax showed a lower RWC from the 13th day after water shortage, but the *J. mollissima* leaves under the same treatment at same condition only exhibited a lower RWC at maximal stress (Fig. 1*a*, *b*). The soil relative water content (SRWC) under water stress was 50% lower maximum stress (Fig. 1*c*).

The stomatal conductance (g_s) reflected a significant association between the water availability and presence of epicuticular wax in *J. mollissima* ($p < 0.05$) (Fig. 2*a*). At 24 h after the initiation of stress, a strong stomatal control was induced in *J. mollissima* plants subjected to water restriction, regardless of the wax presence. In well-watered plants, a lower g_s was observed after the wax was removed than when a waxy coverage was present. After the 10th day of stress, the leaves without wax showed higher g_s than the intact leaves in well-watered treatment (Fig. 2*a*). However, the *J. curcas* plants showed a decreased g_s due to the water regime ($p < 0.05$), but the absence of wax had no effect on this parameter (Fig. 2*d*).

As a consequence of stomatal closure, both species showed a decrease in the net CO_2 assimilation rate (P_N) under water stress (Fig. 2*b*, *e*). Similarly, the P_N in the leaves of both species was influenced by water deficit and wax removal ($p < 0.05$); plants under stress with or without wax showed P_N values lower than the controls, but the P_N of leaves without wax was decreased approximately 75% during the first days of stress or at 48 h after wax removal (Fig. 2*b*, *e*). Well-watered plant leaves without wax showed a lower P_N, around 30%, compared with intact well-watered leaves (Fig. 2*b*, *e*) 48 h after wax has been removed. The well-watered leaves from *J. mollissima* plants after the 15th day without wax showed g_s and P_N values that were comparable with the control. However, the same values in the *J. curcas* well-watered leaves were recovered only at 21st day after wax removal (Fig. 2*b*, *e*).

The transpiration rates (*E*) in the leaves of *J. mollissima* with and without epicuticular wax differed under both water regimes in native species (Fig. 2*c*). Under maximal water stress conditions at 21th day, *J. mollissima* leaves without wax exhibited ten-fold higher water loss rates. On the other hand, *J. curcas* did not show this behaviour (Fig. 2*f*). Under water deficit, both species decreased g_s, P_N and E values from the 5th day after water shortage.

Both species maintained leaf temperatures (6-8°C) that were higher than the air temperature (Fig. 3), even with changes in VPD throughout the experiment (Fig. 1). In leaves of *J. mollissima* and *J. curcas*, there was a variation in the leaf temperature (T_l) throughout the experiment ($p < 0.05$). From the 12th day for *J. mollissima* and the 7th day for *J. curcas*, the temperature in the leaves under water deficit conditions was 2°C higher than in the leaves of the well-watered plants. The temperature in the leaves without wax was not different from that in the wax-covered leaves (Fig. 3).

Epicuticular wax content increased 68% in *J. mollissima* and 67% in *J. curcas*.when subjected to water restriction (Table 1). The total wax content in *J. mollissima* and *J. curcas* plants under watered and stressed conditions was not different. The wax for both species was predominantly comprised of *n-alkanes*, triterpenes, fatty alcohols and fatty acids. However, only *J. mollissima* showed a predominance of long chain *n-alkanes*, and the same amount was present in both well-watered and drought-stressed leaves (Table 1).

There were no significant differences in the leaf soluble carbohydrates (LSC), total protein (TP) and leaf free amino acids (FA) in both species. The total chlorophyll (CHL) contents increased under water deficit, firstly in native species (Fig. 4*d*, *h*).

Discussion

The results showed that water deficit, absence of epicuticular wax and the combination of both factors induced changes in gas exchange in young *J. mollissima* and *J. curcas* plants native and exotic from a semi-arid region in Brazil (Fig. 2*a*-*f*), respectively. Nevertheless, both species under well-watered and without wax leaves treatment, recovered g_s and P_N values in the end of experiment.

Decreasing in P_N and E in young water-stressed plants of both species was associated with lower g_s. In *J. curcas* plants, the control of stomatal aperture has been associated with an immediate response to low soil water availability (Arcoverde *et al*. 2011; Rodrigues *et al*. 2011; Pompelli *et al*. 2010; Silva *et al*. 2010). Moreover, *J. mollissima* plants under both water regimes maintained the highest RWC (Fig. 1*a*) throughout the experiment period, potentially reflecting the adaptation of native *J. mollissima* plants to water deficit, a condition common to semi-arid Brazilian regions. However, in *J. mollissima* leaves that EW was removed the RWC decreased at maximal drought stress (Fig. 1*a*-*b*), showing barrier efficacies against leaf transpiration under mild water shortage conditions. *J. curcas* plants with EW also maintained a stable RWC under stress, which is consistent with results of Arcoverde *et al*. (2011), where a reduction in this parameter was observed only under severe water deficit. However, in the present study, even under mild water shortage, the leaves under drought and without wax (Fig. 1*b*) showed a decreased RWC, which shows the importance of EW under drought stress, when the closed stomata reduces gas exchange. However, even the transpiration rate has not increased (Fig. 2*f*), we could realize the importance of wax for this species also. Perhaps no increase of E values was a consequence of the lower RWC in the end of drought treatment (Fig. 1*b*).

Under greenhouse conditions, two situations should be observed for gas exchange: In the first situation, well-watered leaves without wax in both species exhibited lower P_N values after the first day of water deficit (48 h after wax removal), with a greater intensity in *J. mollissima*. Nevertheless, after 5 days, *J. mollissima* leaves without wax began to recover g_s and P_N (Fig. 2*a*-*b*) compared with the control. This recovery did not occur in *J. curcas* (Fig.

2*e*), which maintained a reduced P_N in well-watered leaves without wax until 21st day when compared to well-watered leaves with wax. In the second situation, the g_s, P_N and E were decreased under drought stress in both leaf types and species. The results of this study are contrary to the findings of Mohammadian *et al*. (2007), where in epicuticular wax removal increased transpiration and photosynthetic rates in *Leucadendron lanigerum*, but under field conditions, which might have led to the differences in the responses obtained in our study. Thus, EW influences gas exchange differently, depending on the species and conditions of hydration. If there is sufficient water availability in the soil, there is no restriction on the g_s; however, the absence of wax in the cuticle can increase stomatal control. The EW might have a different composition and content between species (Mohammadian *et al*. 2007; Grantz 1990; Oliveira *et al*. 2003) and could increase (Table 1) with decreasing g_s to restring leaf water vapour, demonstrating a tolerance mechanism to water stress.

Several plants have shown an acclimatory response to water deficit through increasing the epicuticular wax load on the leaves during the first 30 days, (Bondada *et al*. 1996; Hauke and Schreiber, 1998; Samdur *et al*. 2003). In our study, the epicuticular wax content increased after 21 days under water shortage in both species (Table 1). These results suggest that the production of wax was increased in both species under drought, which has been shown in previous studies (Grantz 1990; Premachandra *et al*. 1992; Yang *et al*. 2011). The leaves of well-watered plants without wax showed a decreased g_s and P_N, which was recovered after a few days (Fig. 3*c*-*d*). Thus, accumulation of waxes reflects a rapid adaptation of the species to low soil moisture conditions. Grantz (1990) showed that the deposition of wax on stomatal guard cells and other cells was absent under high humidity and induced under low humidity. The formation of wax around the stomata might be necessary to increase the efficiency of stomatal closure, reduce transpiration, and mediate drought tolerance (Yang *et al*. 2011). Wax deposition must start as early as a few days after the perception of stress (Premachandra *et al*. 1992), and tolerant species often have thicker foliar wax (Shepherd and Griffiths 2006).

The EW quality was different between species. Thus, *J. mollissima* leaves showed a predominance of long-chain *n-alkanes* under both water treatments (Table 1). The predominance of long-chain *n-alkanes* is associated with water stress. Oliveira *et al*. (2003) discussed that native plants of Caatinga has different chemistry of the wax constituents and and this factor was shown important to determine the degree of resistance to water vapour, in addition, *n-alkanes* and alcoholic triterpenes were the most efficient barriers. In this study, higher RWC was observed throughout the experimental period in native plants from a Brazilian semi-arid region compared with the exotic species under greenhouse conditions. In this way, when the EW was removed from the treatment under stress, the *E* in the *J. mollissima* leaves increased from the 15th day of water restriction compared with *J. curcas* leaves under the same treatment (Fig. 2*c*, *f*); moreover, the g_s was extremely low in both species (Fig. 2*a*, *d*). The barrier established by EW becomes evident when the g_s was low (Kerstiens 1996). Under maximum drought stress (21st day), the *J. mollissima* leaves without wax showed a higher *E* than the leaves with wax (Fig. 2*c*); again the g_s was the same for both water conditions (Fig. 2*a*). Thus, the RWC was decreased in leaves without wax under stress (Fig. 1*a*, *b*) compared with the other treatments in both species. These results suggested that long-chain *n-alkanes* can lead to lower *E* in leaves with wax of the native species under drought conditions.

Leaf temperature was not affected by EW in any treatment. Our results showed that leaf temperature is determined by soil water availability. Plants under water stress had higher leaf temperature with or without wax. Even when there was a high transpiration rate, as observed in *J. mollissima*, since the water availability in the soil was not sufficient to replace the water lost to atmosphere, which can be seen in the lower RWC of the stressed and without wax leaves (Fig. 1a, b). Both species exhibited leaf temperatures higher than the air temperature; however, the irrigated plants exhibited temperatures 2.5°C lower than in the stressed plants (Fig. 3). In semi-arid regions, excessive radiation and high temperature generally accompany a water deficit, which results in multiple stress factors for the species; however, the biggest factor is the water restriction, as shown in *J. curcas* (Silva *et al*. 2010). According to Riederer (2006), wax controls the degree of diffusion and reflection of UV radiation to increase the tolerance to radiation. The reduction of leaf temperature (Jenks and Ashworth 1999) is another role attributed to EW, which this study did not indicate (Fig. 3). In fact, it has been argued that EW reduces water loss but does little to reflect sunlight, and the deposition of wax around the stomata increases resistance to gas diffusion, consequently lowering E and P_N (Mohammadian *et al*. 2007).

Under moderate water stress, the leaf gas exchange was decreased and the leaf temperature was increased; however the RWC was not changed, except in leaves under drought without wax. Other parameters of the leaf primary metabolism were not altered (Fig. 4), except photosynthetic pigments such as total chlorophyll under water deficit. These results were consistent with those of Arcoverde *et al*. (2011), where in *J. curcas* exhibited changes in these parameters only under severe water shortage. These results confirm the drought tolerance of this species and help to demonstrate the importance of EW for the control of transpiration rates, particularly when stomatal conductance is strongly decreased under drought conditions.

In conclusion, the present study showed that the young plants of *J. mollissima* and *J. curcas* were able to tolerate low water availability by maintaining high water status through strong stomatal control. Which does not discards the possibility of occur osmotic adjustment in these species, a hypothesis that can be tested in future studies. The integrity of the EW was essential for the maintenance of gas exchange for both species, as its removal reduced g_s and P_N. Under drought and without wax, E increased in *J. mollissima* leaves after 15 days, even the g_s was near zero, which suggests an important role for the EW in the native species.

Acknowledgements

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Table legend

Table 1. Changes to the n-alkane content of cuticles from young Jatropha mollossima and Jatropha curcas plants under water deficit. The irrigation was reduced to 25% of the control to induce water stress. Values are the mean (± s.e.) of three replications. Alkanes are listed according to their carbon numbers. Values within a species followed either by the same lower-case letter or the same upper-case letter are not significantly different from each other (Student–Newman– Keuls test at 5%)

Figure legends

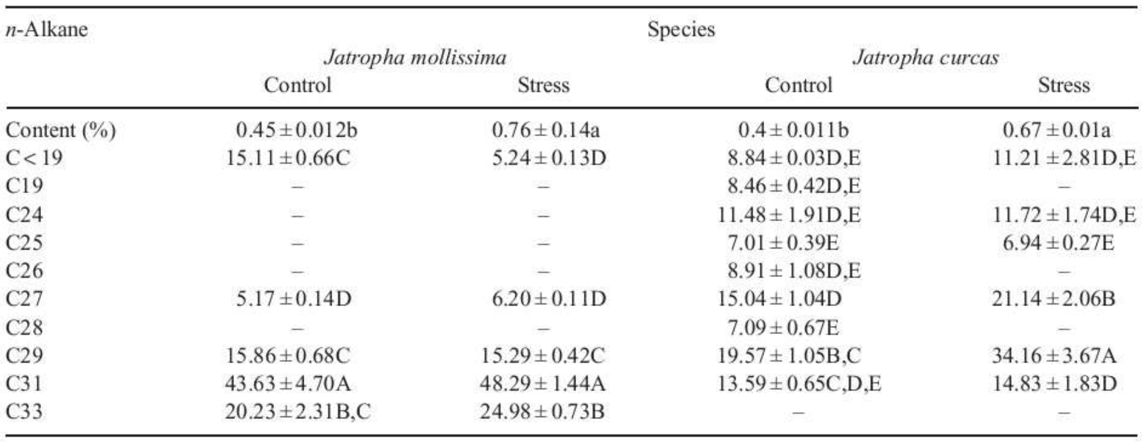
Fig. 1. Relative water content (RWC) in *J. mollissima* (*a*), *J. curcas* (*b*), substrate moisture (*c*) and variation of vapor pressure deficit (VPD) (*d*) under greenhouse conditions. The irrigation was reduced to 25% of the control to induce water stress, and the epicuticular wax was removed at 48 h before inducing water stress. Each symbol represents the mean value of five replications (±S.E.).

Fig. 2. Changes in stomatal conductance (g_s) (*a*, *d*), net CO_2 assimilation (P_N) (*b*, *e*) and transpiration (E) (*c*, *f*) in young *J. mollissima* and *J. curcas* plants under greenhouse conditions. The irrigation was reduced to 25% of the control to induce water stress, and the epicuticular wax was removed at 48 h before inducing water stress. Each symbol represents the mean value of five replications (±S.E.).

Fig. 3. Changes in leaf temperature (T_L) (°C), in young *Jatropha mollissima* (*a*) and *Jatropha curcas* (*b*) plants under greenhouse conditions. The irrigation was reduced to 25% of the control to induce water stress, and the epicuticular wax was removed at 48 h before inducing water stress. Each symbol represents the mean value of five replications (±S.E.).

Fig. 4. Changes in leaf content of total soluble carbohydrates (LSC) (*a*, *e*), total soluble proteins (TP) (*b*, *f*), total free amino acids (LFA) (*c*, *g*) and total chlorophyll (*d*, *h*) in young *J. mollissima* and *J. curcas* plants under greenhouse conditions. The irrigation was reduced to 25% of the control to induce water stress. Each symbol represents the mean value of five replications (±S.E.).

Table 1.



n-Alkane	Species			
	Jatropha mollissima		*Jatropha curcas*	
	Control	Stress	Control	Stress
Content (%)	0.45 ± 0.012b	0.76 ± 0.14a	0.4 ± 0.011b	0.67 ± 0.01a
C < 19	15.11 ± 0.66C	5.24 ± 0.13D	8.84 ± 0.03D,E	11.21 ± 2.81D,E
C19	–	–	8.46 ± 0.42D,E	–
C24	–	–	11.48 ± 1.91D,E	11.72 ± 1.74D,E
C25	–	–	7.01 ± 0.39E	6.94 ± 0.27E
C26	–	–	8.91 ± 1.08D,E	–
C27	5.17 ± 0.14D	6.20 ± 0.11D	15.04 ± 1.04D	21.14 ± 2.06B
C28	–	–	7.09 ± 0.67E	–
C29	15.86 ± 0.68C	15.29 ± 0.42C	19.57 ± 1.05B,C	34.16 ± 3.67A
C31	43.63 ± 4.70A	48.29 ± 1.44A	13.59 ± 0.65C,D,E	14.83 ± 1.83D
C33	20.23 ± 2.31B,C	24.98 ± 0.73B	–	–

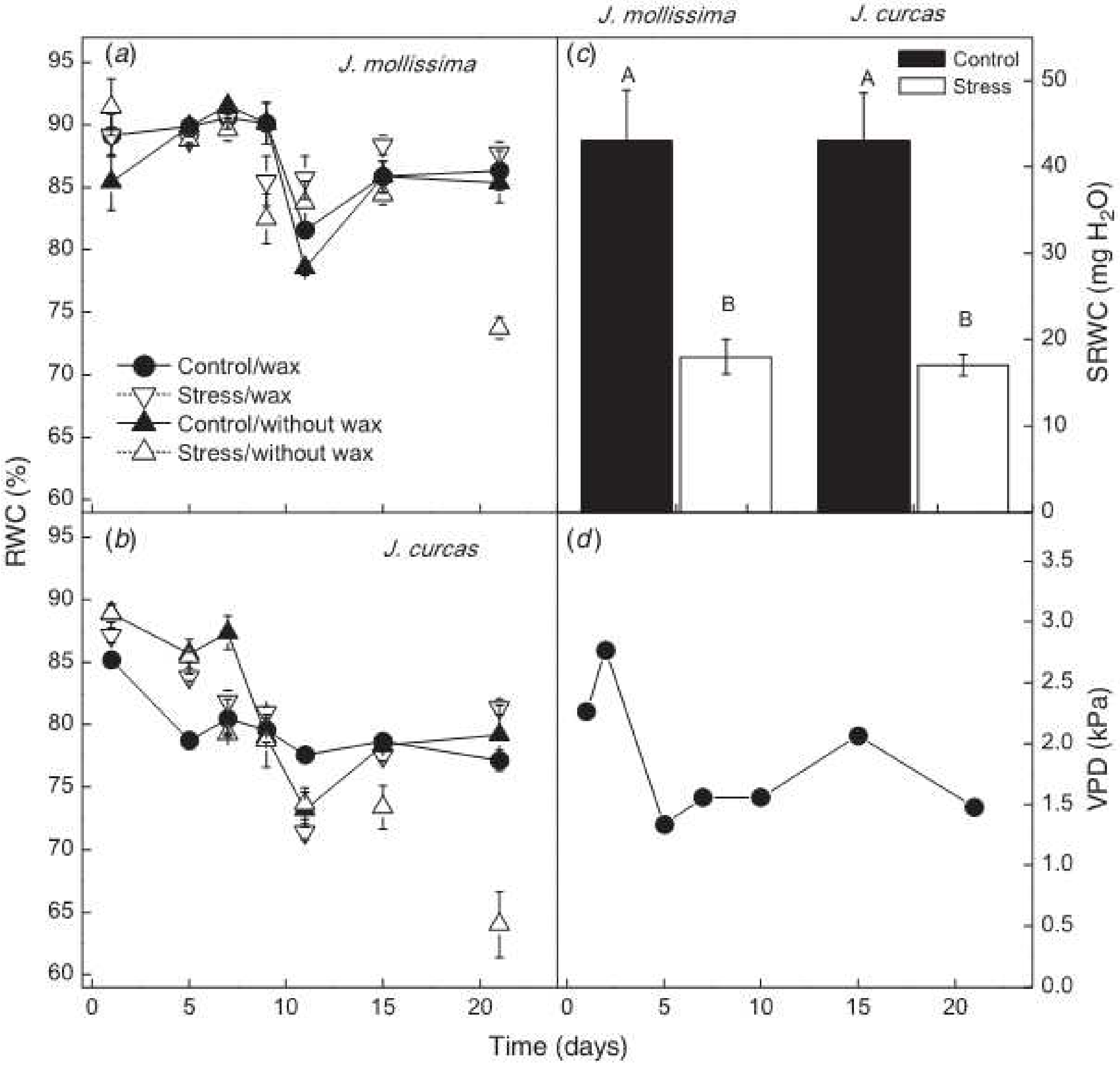

J. mollissima
J. curcas
(a)
J. mollissima
(b)
J. curcas
(c)
(d)
RWC (%)
SRWC (mg H2O)
VPD (kPa)
Time (days)
Control/wax
Stress/wax
Control/without wax
Stress/without wax
Control
Stress
A
B
A
B
95
90
85
80
75
70
65
60
0
5
10
15
20
50
40
30
20
10
0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0

Fig. 1

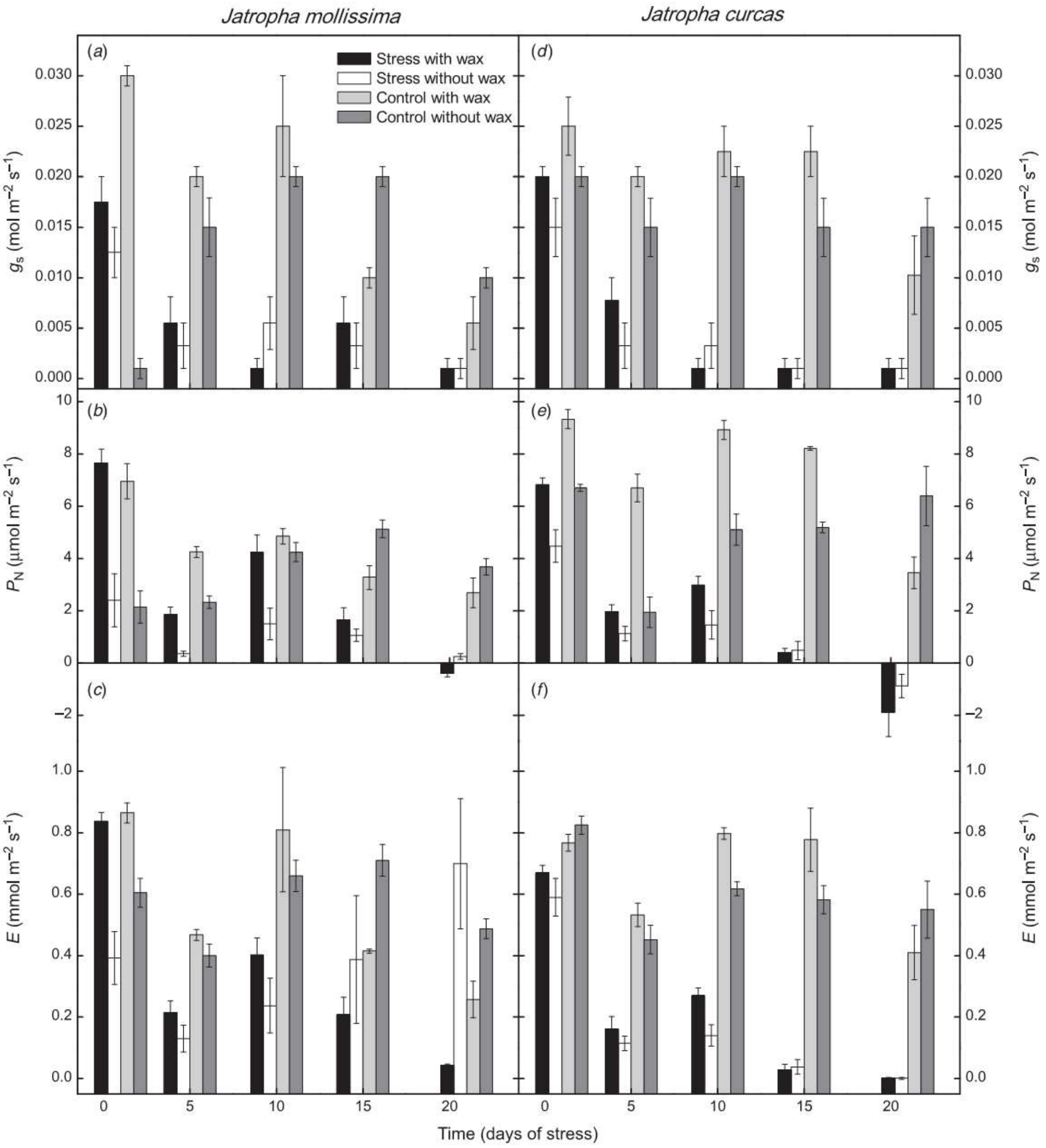

Jatropha mollissima
Jatropha curcas
(a)
(b)
(c)
(d)
(e)
(f)
Stress with wax
Stress without wax
Control with wax
Control without wax
g_s (mol m^{-2} s^{-1})
0.030
0.025
0.020
0.015
0.010
0.005
0.000
P_N (μmol m^{-2} s^{-1})
10
8
6
4
2
0
−2
E (mmol m^{-2} s^{-1})
1.0
0.8
0.6
0.4
0.2
0.0
0
5
10
15
20
Time (days of stress)

Fig. 2

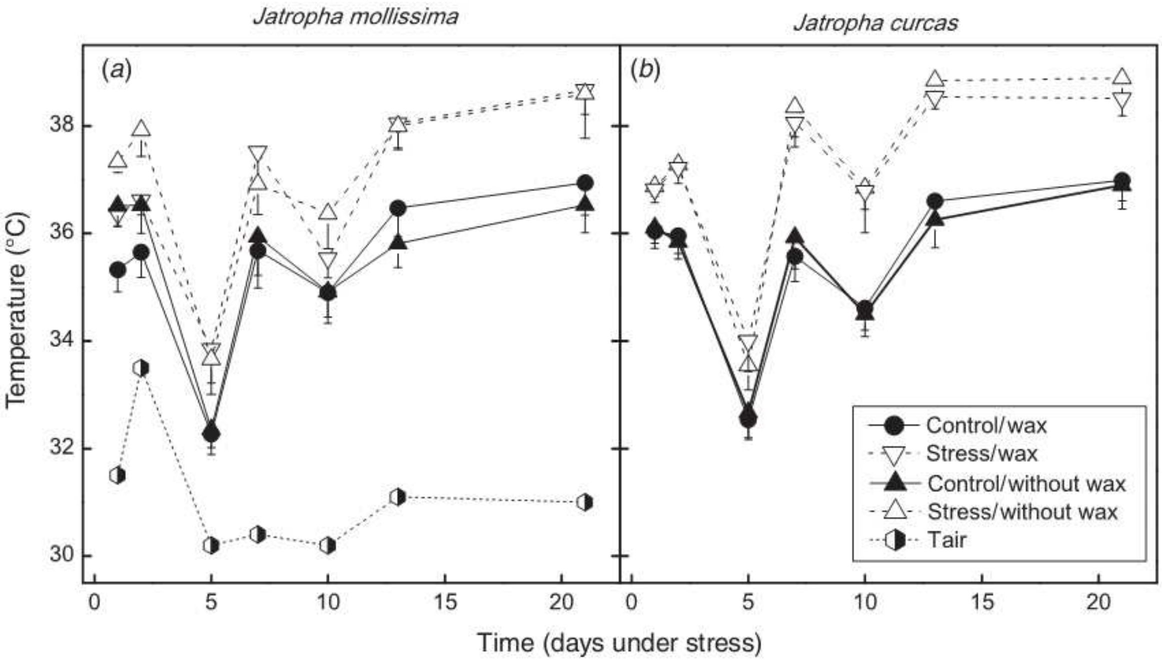

Jatropha mollissima
Jatropha curcas
(a)
(b)
Temperature (°C)
30
32
34
36
38
0
5
10
15
20
Time (days under stress)
Control/wax
Stress/wax
Control/without wax
Stress/without wax
Tair

Fig. 3

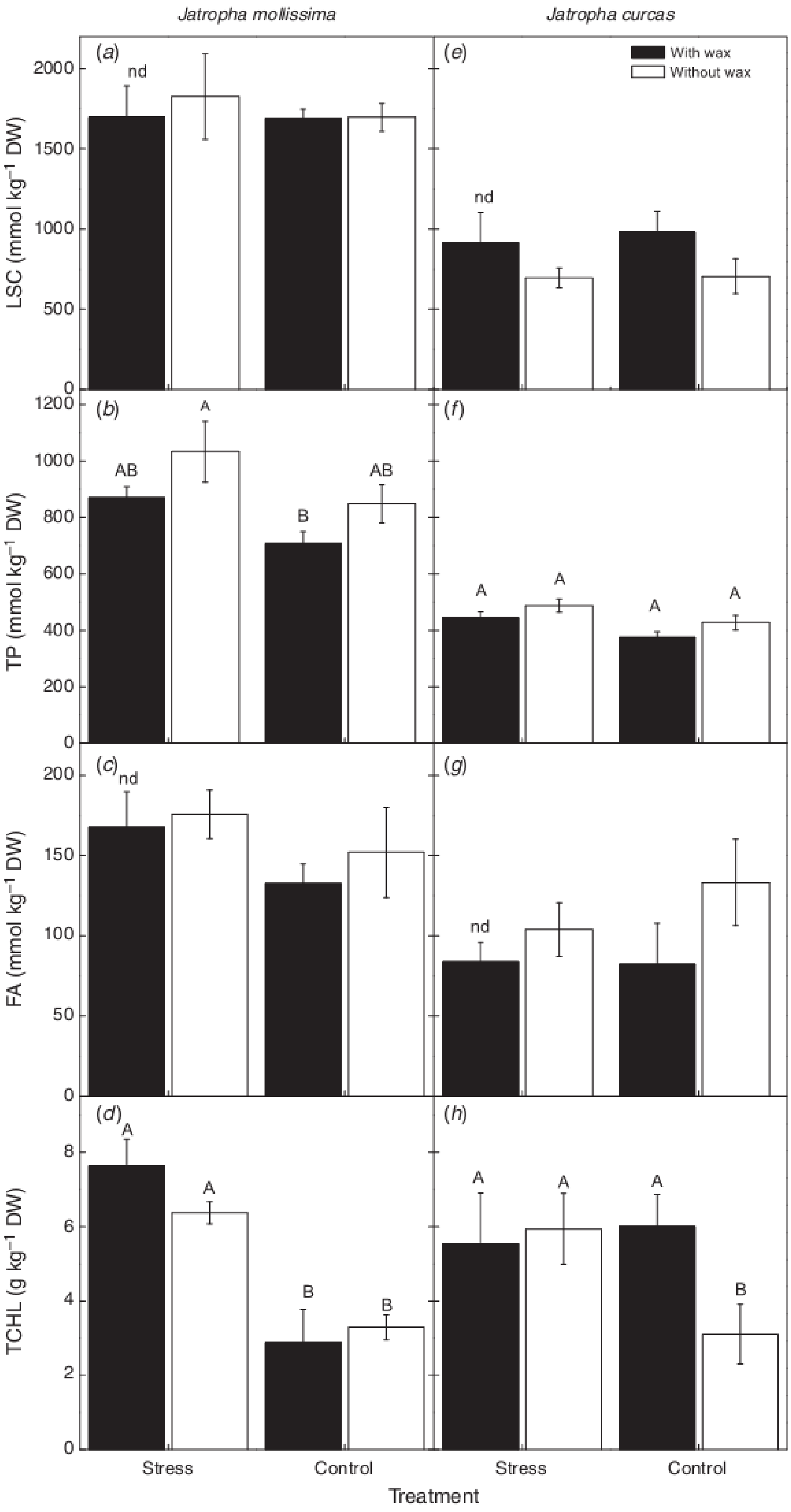

Jatropha mollissima
Jatropha curcas
With wax
Without wax
(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
nd
AB
A
B
LSC (mmol kg⁻¹ DW)
TP (mmol kg⁻¹ DW)
FA (mmol kg⁻¹ DW)
TCHL (g kg⁻¹ DW)
0
500
1000
1500
2000
200
400
600
800
1200
50
100
150
2
4
6
8
Stress
Control
Treatment

Fig. 4

RESUMO

O objetivo do trabalho foi avaliar o efeito da restrição hídrica e da remoção da cera epicuticular no mecanismo de trocas gasosas em *Jatropha curcas* L. e *Jatropha mollissima* (Pohl) Baill., em casa de vegetação. O experimento obedeceu ao delineamento em blocos ao acaso, com 2 espécies, 2 tratamentos (controle e estressado - 80% e 25% da capacidade do pote, respectivamente), 2 tipos de folha (com e sem cera) e 7 repetições. Foram analisados conteúdo hídrico relativo (CHR), condutância estomática (g_s), assimilação (P_N), transpiração (E), temperatura foliar (T_f), teor e composição da cera e os *n*-alcanos cuticulares. Além disso, carboidratos solúveis totais (CST), aminoácidos livres totais (ALT), proteínas solúveis totais (PST) e o conteúdo de clorofila também foram avaliados. Ambas as espécies mantiveram o CHR elevado mesmo sob restrição hídrica (*J. cucas*: 70-80%; *J. mollissima*: 80-90%) e apresentaram eficiente controle estomático que refletiu em reduções nas taxas de assimilação e transpiração nas plantas sob estresse hídrico. As folhas dos indivíduos que tiveram a cera epicuticular removida apresentaram taxas reduzidas de g_s, P_N e E apenas nos primeiros dias do estresse, atingindo valores semelhantes aos seus grupos de referência a partir do quinto dia do estresse. Ainda, as duas espécies apresentaram T_f superior a temperatura ambiente e um aumento foi observado em plantas sob restrição hídrica. A T_f também foi afetada pela remoção da cera, com as folhas desse tratamento apresentando de 1,5 °C a 2,5 °C a mais que sua folhas de referência. Foi observado também, aumento de aproximadamente 70% no teor de cera epicuticular nos indivíduos sob restrição hídrica, entretanto, uma análise qualitativa do extrato da cera epicuticular não mostrou diferenças na composição química de acordo com o tratamento hídrico, sendo composta predominantemente por ácidos graxos/triterpenos ácidos, triterpenos alcoólicos/alcoóis primários e *n*-alcanos. Mas a análise quantitativa mostrou diferença na abundância relativa dessa classe, com predomínio do C31 (cerca de 45%) em *J. mollissima* e do C29 (cerca de 30%) em *J. curcas*. Apesar das alterações nas trocas gasosas não foi observado diferença quanto ao metabolismo de CST, ALT, PT e clorofila. Os resultados sugerem que plantas jovens de *J. mollissima* e *J.curcas* são capazes de tolerar déficit hídrico, mantendo seu metabolismo e que a integridade da cera é fundamental para manutenção das trocas gasosas.

Palavras-chaves: Euphorbiaceae, cutícula, déficit hídrico, trocas gasosas

ABSTRACT

The purpose of this study was to evaluate the effect of water stress and removal of the epicuticular wax in gas exchange mechanism in *Jatropha curcas* L. and *Jatropha mollissima* (Pohl) Baill. growing in greenhouse. We used the experimental design of randomized blocks with treatments arranged in a factorial 2 x 2 x 2 x 7: two species, two treatments (control and stress - 80% and 25% of the capacity of the pot, respectively), two leaf types (with and without wax) and 7 determinations. Were analyzed the relative water content (RWC), stomatal conductance (g_s), assimilation (P_N), transpiration (E), leaf temperature (T_l), wax content and composition, and *n*-alkanes profiles. In addition, total soluble carbohydrates (LCS), total free amino acids (FAA), total soluble protein (TP) and chlorophyll content were also evaluated. The two species maintained RWC high even under water restriction (*J. curcas*: 70-80%; *J. mollissima*: 80-90%) and showed an efficient stomatal control which resulted decreases in CO_2 assimilation and transpiration rates in the plants under water stress. The leaves of plants that had epicuticular wax removed showed decreases rates of *gs*, *PN* and *E* only in the first days of stress, reaching similar values from the fifth day. The two species showed T_l above environment temperature and an increase was observed in plants under water stress. The T_l was also affected by the removal of wax, increasing from 1.5 °C to 2.5 °C in relations to control group (leaves with wax). It was observed an increase in the epicuticular wax content (approximately 70%) in the plants under water deficit. A qualitative analysis of epicuticular wax composition not showed differences in relation to the chemical types of compounds between different treatments. On the other hand, a quantitative analysis of *n*-alkanes profiles showed differences, with a predominance of untriacontane in *J. mollissima* (approximately 45%) and nonacosane (C29) in *J. curcas* (approximately 30%). Although change was observed in gas exchange, it was not observed difference in the of CST, ALT, PT and chlorophyll content. The results suggest that young plants of *J. curcas* and *J. mollissima* are able to tolerate drought and the integrity of the epicuticular wax layer is crucial for waterproofing and maintenance of gas exchange.

Keywords: Euphorbiaceae, cuticle, water deficit, gas exchange

ANEXO

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09-Oct-2012

Dear Dr Santos:

Thank you for sending the revised version of this paper (Epicuticular wax removal influences gas exchange and water relations in the leaves of an exotic and native species from a Brazilian semi-arid region under induced drought stress) and for dealing so thoroughly with the referees' comments. I have now had the opportunity to examine your revised manuscript and I am pleased to accept it for publication in Australian Journal of Botany.

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- **Accessory publications**
- **General presentation**
- **Submission and preparation of manuscripts**
- **Title**
- **Summary text for the Table of Contents**
- **Abstract**
- **Text**
- **References**
- **Use of referencing software**
- **Units**
- **Mathematical formulae**
- **Gene sequences**
- **Enzyme nomenclature**
- **Chemical nomenclature**
- **Tables**
- **Illustrations**
- **Photographs**
- **Line drawings**

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Return to Index

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Return to Index

Journal policy and notes

Papers of relevance to Southern Hemisphere ecosystems will be considered for publication in the Journal in the fields of ecology and ecophysiology; conservation biology and biodiversity; forest biology and management; cell biology and tissue culture; paleobotany; reproductive biology and genetics; mycology and pathology; and structure and development. Review articles will also be considered. Authors interested in publishing a review article are invited to contact the Editor or an appropriate member of the Editorial Board. **Australian Journal of Botany** does not publish the results of biological surveys if the main body of the results is only the results of a biological survey. However, the Journal will publish papers that present survey data in comparative and ecological contexts. All papers are refereed. Please be aware that the acceptance rate of papers is about 33%.

Submission of a paper implies that the results have not been published and are not being considered for publication elsewhere. It also implies that all coauthors of the paper have consented to its submission. Authors of multi-authored papers may wish to assign relative values to their contributions, or to indicate that two or more authors contributed equally to a paper. This can be done in a note at the end of the address field on the paper. The Journal will use its best endeavours to ensure that work published is that of the named authors except where acknowledged and, through its reviewing procedures, that any published results and conclusions are consistent with the primary data. It takes no responsibility for fraud or innaccuracy on the part of the contributors. **There are no page charges.**

Return to Index

Distribution information and lodging specimens

In cooperation with other botanical institutions, distributional data for rare and threatened plant taxa will be masked. Precise distributional data are not published for species that are listed as known only from the type locality, or whose conservation status is given as E or V by Briggs and Leigh (1988). Where specimen citations for rare taxa have been deliberately abbreviated to achieve less precision in order to protect the taxa, this should be stated. *The Wildlife Protection (Regulation) of Exports and Imports Act* of 1982 (amended in 1986) states that all type material and unicates collected in Australia must be lodged in an appropriate national or state institution. Consequently, Journal policy requires that type material of all of the taxa collected in Australia after 1982, and discussed by the author of a manuscript, be lodged in a suitable Australian herbarium.

For all papers, whether presenting morphological, cytological or molecular data, voucher specimens must be cited, along with the herbarium where lodged. For population studies where large series of specimens of one taxon are examined, citation of exemplar specimens is acceptable. In tables that list material examined, the voucher specimen (collection) and the herbarium where lodged are to be listed for each entry. If there are one or few herbaria involved, this can be indicated instead of in the materials and methods, or in the table caption.

Return to Index

Online publication

Papers are published online immediately after final corrections are completed. No corrections are allowed after this time.

Return to Index

Turner Review Series

The Journal continues to publish the series of papers known as the Turner Reviews, which aims to provide critical, state-of-the-art evaluations that advance our knowledge in current, key areas of botanical research. This series will be commissioned by invitation, and is numbered in sequence. We would greatly appreciate advice and suggestions on prospective reviews for this important series.

The Journal reserves the right to fast-track excellent papers, including those of the Turner Review Series.

Return to Index

Accessory publications

Supplementary material of a detailed nature that may be useful to other workers, but which is not essential to the printed paper, may be lodged with the Managing Editor, provided that it is submitted with the manuscript for inspection by the referees. Such material will be made available on request and a note to this effect should be included in the paper. Where practicable and appropriate this material will also be made available online.

Return to Index

General presentation

The works should be presented concisely and clearly in English. Introductory material, including a review of the literature, should not exceed what is necessary to indicate the reason for the work and the essential background. All pages of the manuscript should contain LINE NUMBERING to aid the referees in their task. Authors are advised to note the typographical conventions and the layout of headings, tables, and illustrations exemplified in recent issues of the Journal. Observance of these and the following requirements will shorten the interval between submission and publication.

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Return to Index

Submission and preparation of manuscripts
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Return to Index

Title

This should be concise and informative and should contain all keywords necessary to facilitate retrieval by modern searching techniques. Titles including generic or specific names should also contain the name of taxa at higher rank, e.g. Division, Class, Order or Family. Nomenclatural authorities should be omitted from the title. An abridged title that does not exceed 50 characters should also be supplied for use as a running head.

Return to Index

Summary text for the Table of Contents

This is a three-sentence paragraph of 50 to 80 words written for interested non-experts, such as journalists, teachers, government workers, etc. The text should be free from scientific jargon, and written at the level of an article in a science magazine. Your first sentence should engage the reader, convincing them that this is an important area. The second sentence should introduce the problem addressed in the paper, and state your main discovery. The final sentence should describe how the results fit into the bigger picture (i.e. implications or impact of the discovery).

Return to Index

Abstract

This should state concisely, preferably in fewer than 200 words, the scope of the work and the principal findings, and should be suitable for use by abstracting services. Species names mentioned in the abstract should include nomenclatural authorities. Acronyms and references should be avoided.

Return to Index

Text

This should normally be divided into sections, e.g. Introduction, Materials and Methods, Results, Discussion, Acknowledgments, References. All main headings should be in upper and lower case bold type, aligned at the left. Minor headings should be in light italics. The folllowing also should be adhered to: spell out numbers lower than 10 unless accompanied by a unit, e.g. 2 mm, 15 mm, two plants, 15 plants, but 5 out of 15 plants; leave a space between a numeral and its unit; use the ´ise´ construction, not ´ize´; indicate approximate positions of figures and tables on the manuscript.

Synonymies should be indicated by a smaller font size and the first line of each synonymous species should be indented. Where presented, synonymy should immediately follow taxon headings.

Latin diagnoses for new species should be given in English to allow checking by the referees.

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Return to Index

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- *Journal article*

Lucas EJ, Harris SA, Mazine FF, Bellsham SR, Lughadha EMN, Telford A, Gasson PE, Chase MW (2007) Suprageneric phylogenetics of Myrteae, the generically richest tribe in Myrtaceae (Myrtales). *Taxon* **56**, 1105-1128.

- *Book*

Hesse M, Halbritter H, Zetter R, Weber M, Buchner R, Frosch-Radivo A, Ulrich S (2009) ´Pollen terminology: an illustrated handbook.´ (Springer: New York)

- *Book chapter*

Walton TJ (1990) Waxes, cutin and suberin. In ´Methods in plant biochemistry. Vol. 4: lipids, membranes and aspects of photobiology´. (Eds PM Dey, JB Harbone) pp. 105-108. (Academic Press: London)

- *Online reference*

Radcliffe J, Catley M, Fischer T, Perrett K, Sheridan K (2003) ´Review of plant research biosecurity protocols.´ (Department of Agriculture, Fisheries and Forestry: Canberra) Available at http://www.daff.gov.au/__data/assets/pdf_file/0006/146913/review.pdf [Verified 1 April 2012]

Return to Index

Use of referencing software

To obtain the style file for this journal, please go to the following websites.

If using ´Reference Manager´, visit http://www.refman.com/support/rmoutputstyles.asp.

If using ´ProCite´, visit http://www.procite.com/support/pcoutputstyles.asp.

If using ´EndNote*´ software, visit http://www.endnote.com/support/enstyles.asp.

*You will find the style file under the ´Botany´ category, listed as **Australian Journal of Botany**.

Return to Index

Units

The International System of Units (Système International d´Unités, SI units) should be used for exact measurement of physical qualities and as far as practical elsewhere. Measurements of radiation should be given as irradiance or photon flux density, or both, and the waveband of the radiation should be specified. Luminous flux density units (e.g. lux) should not be used. Do not use the double solidus in complex groupings of units, e.g.mmol/m^2/s; use the negative index system instead, i.e. mmol m^{-2} s^{-1}.

Return to Index

Mathematical formulae

Correctly align and adequately space all symbols. Avoid two-line mathematical expressions wherever possible especially in the running text. Display each long formula on a separate line with at least two lines of space above and below it.

Return to Index

Gene sequences

All sequences used as data must be deposited in one of the international nucleotide sequence databases, preferably GenBank, National Center for Biotechnology Information, 8600 Rockville Pike, Bethesda, MD 20894, USA. Email: gb-sub@ncbi.nlm.nih.gov. Request information at gsdb@gsdb.ncgr.org. Post-review final manuscript will not be accepted until sequence database accession numbers are included.

Return to Index

Enzyme nomenclature

Names should conform to Recommendations (1992) of the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology on the Nomenclature and Classification of Enzymes as published in ´Enzyme Nomenclature 1992´ (Academic Press: San Diego). If you wish to use a name other than the recommended name, at the first mention of the alternative name identify it by giving the recommended name and its EC number.

Return to Index

Chemical nomenclature

The recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature should be followed when naming compounds such as amino acids, carbohydrates, lipids, steroids, vitamins, etc. Refer to other biologically active compounds, such as metabolic inhibitors, plant growth regulators, buffers (in accordance with IUPAC Rules of Chemical Nomenclature), once and then by their most widely accepted common name.

Return to Index

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Return to Index

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Return to Index

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Return to Index

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Return to Index

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