Weed Biology AND Management

Weed Biology and Management **15**, 138–146 (2015)

RESEARCH PAPER

Impact of *Cuscuta australis* infection on the photosynthesis of the invasive host, *Mikania micrantha*, under drought condition

QUANG-VUONG LE[1,2], KUSHAN U. TENNAKOON[1]*, FAIZAH METALI[1], LINDA B.L. LIM[3] and JAY F. BOLIN[4]

[1]*Environmental and Life Sciences Program, Faculty of Science and Institute for Biodiversity and Environmental Research,* [3]*Chemistry Program, Faculty of Science, Universiti Brunei Darussalam, Gadong, Brunei Darussalam,* [2]*Faculty of Biology, Vinh University, Vinh, Vietnam and* [4]*Department of Biology, Catawba College, Salisbury, North Carolina, USA*

Cuscuta species (dodders) are widespread stem holoparasites that depend on host plants for their entire mineral and water and most carbohydrate requirements. Dodders negatively affect host photosynthesis but precise information on their impact on hosts in the presence of environmental stress factors (i.e. drought) is little known. In a pot experiment, the leaf traits, gas exchange and chlorophyll *a* fluorescence of the invasive climber, *Mikania micrantha*, parasitized by *Cuscuta australis*, were investigated in order to study variations of host photosynthesis in response to parasitism and drought. The results showed that the concomitant presence of *C. australis* infection and drought significantly impacted the leaf traits (i.e. increased leaf dry mass content), gas exchange (i.e. decreased stomatal conductance and transpiration rates and increased water-use efficiency) and quantum yield of chlorophyll *a* fluorescence of *M. micrantha*. The presence of a single stress factor (*C. australis* infection or drought), however, only significantly affected the leaf traits and gas exchange of *M. micrantha*. These results suggested that the combined additive effects of *C. australis* parasitism and drought significantly suppressed the photosynthesis of *M. micrantha* in relation to both stomatal and non-stomatal limitation of host photosynthesis. This study provides insights into *Cuscuta*–host interactions under drought conditions in the tropics.

Keywords: chlorophyll *a* fluorescence transient test, gas diffusional limitations, light-use efficiency, parasite–host interactions.

Cuscuta spp. (dodders) are widespread stem holoparasitic plants that belong to the Convolvulaceae (Parker & Riches 1993; Heide-Jørgensen 2008). They depend on host plants for their entire mineral and water and most of their carbohydrate requirements because they lack roots and leaves and possess a very low photosynthetic ability (Jeschke *et al.* 1994; Albert *et al.* 2008; Heide-Jørgensen 2008). *Cuscuta* spp. parasitize many different plants, induce negative impacts on the growth and yield of infected hosts and have significant effects on the structure and function of plant communities that are infested by these holoparasites (Yuncker 1932; Kuijt 1969; Marambe *et al.* 2002; Press & Phoenix 2005; Albert *et al.* 2008). In Guangdong province of China, *Cuscuta australis* was shown to produce higher infection rates on some invasive hosts (i.e. *Ipomoea cairica*, *Mikania micrantha* and *Wedelia trilobata*), thus benefiting the native species, such as *Dactyloctenium aegyptium*, *Paederia scandens* or *Pharbitis nil*, in a given community (Yu *et al.* 2011).

Communicated by T. Kobayashi.

**Correspondence to: Kushan U. Tennakoon, Environmental and Life Sciences Program, Faculty of Science, Universiti Brunei Darussalam, Tungku Link, Gadong BE 1410, Brunei Darussalam. Email: kushan.tennakoon@ubd.edu.bn*

The authors have no commercial interest in the findings presented.

Received 30 March 2015; accepted 26 June 2015

doi:10.1111/wbm.12077

Results from previous studies have revealed that *Cuscuta* parasitism suppressed host photosynthesis, captured host resources and consequently slowed host growth. For example, Shen *et al.* (2007, 2011) reported that *Cuscuta campestris* reduced the stomatal conductance, transpiration rates, chlorophyll (Chl) content and Rubisco concentration of an invasive host species, *M. micrantha*, leading to a decrease in the photosynthesis and growth of the infected host plants. The decreased Chl content in *M. micrantha* that is induced by parasitism might cause a decreased antenna size, thereby reducing the level of light capture, light-use efficiency and photosynthetic capacity of the host (Shen *et al.* 2007). Several parameters of the leaf Chl *a* fluorescence of the host, *Ambrosia trifida*, such as minimal and maximal fluorescences and electron transport rates, also were influenced by the parasitism of *C. campestris* (Vrbničanin *et al.* 2013). Thus, *C. campestris* infection appears to suppress host photosynthesis by limiting gas diffusion via stomatal and photosynthetic metabolic processes.

In contrast, studies by Jeschke and Hilpert (1997) and Jeschke *et al.* (1997) reported that *Cuscuta reflexa* infection increased stomatal conductance, transpiration rates and net photosynthesis in the leaves of the infected host plants, *Ricinus communis* and *Coleus blumei*, but suppressed their growth and dry matter accumulation. These impacts were attributed to sink-source effects, in which *C. reflexa* is a strong sink to redirect the flow of host resources to parasites and alters host physiology, such as photosynthesis and transpiration. A number of similar studies has shown the existence of sink-source effects in parasite–host plant systems that increase net photosynthesis in the host leaves (Clark & Bonga 1970; Cechin & Press 1993).

These contrasting findings indicate that the exact mode of the impact of *Cuscuta* infection on host photosynthesis is still unknown and might be context-dependent. In fact, parasite–host interactions vary with the environmental conditions, where the negative effects that are induced by parasites on the infected hosts might be negligible when the resources are abundant (Shen *et al.* 2006, 2013). The co-occurrence of other environmental stressors under typical conditions might result in a modulation of the effects on target plants (Osmond *et al.* 1987). It is therefore necessary to consider the impacts of *Cuscuta* infection on host photosynthesis in the context of other environmental factors.

Drought is one of the primary environmental factors that suppress the photosynthesis of terrestrial plants by reducing stomatal conductance (e.g. Ashraf & Harris 2013; Brestic & Zivcak 2013). More severe or prolonged drought results in the impairment of the plant's photosynthetic apparatus (Ashraf & Harris 2013). These impacts resemble the effects of *Cuscuta* parasitism on infected host plants, as described above. It has been reported that drought is an important driver of the distribution and structure of plant communities in Borneo (Tyree *et al.* 1998). However, the impacts of the co-occurrence of *Cuscuta* infection and drought on the infected host plants in tropical Borneo are still unclear. It was presumed that *Cuscuta*-infected host plants might experience more severe photosynthetic decline under drought conditions. In order to test this hypothesis, the leaf traits, gas exchange parameters and Chl *a* fluorescence transient (OJIP) of the climber, *M. micrantha* H.B.K. (Asteraceae), growing under the combined conditions of *C. australis* R. Brown (Convolvulaceae) infection and a water deficit regime, were analyzed. The O, J, I and P steps of the OJIP transient correspond to the redox states of photosystem (PS) II and PS I and to the electron transfer efficiencies through electron acceptors (Strasser *et al.* 2000, 2004). *Mikania micrantha* was listed among the 10-worst exotic species in South-East and South Asia (Tripathi *et al.* 2011). Thus, the findings of this study could provide insights into the impact of *C. australis* infection on the photosynthesis of one of its preferred hosts under drought conditions and compare the relative strengths of two stressors; namely, parasitism and drought.

MATERIALS AND METHODS

Plant culture and design

This study was carried out from January to October 2014 in the plant nursery of the Universiti Brunei Darussalam (4°58′494″N, 114°53′775″E) under the following environmental conditions: maximal photosynthetically active radiation (PAR) of 750 μmol(quantum) m^{-2} s^{-1}, mean temperature of 30°C and mean air relative humidity of 70%. The host plant that was used to investigate the impact of *C. australis* parasitism was *M. micrantha*.

Uniformly sized shoot cuttings of *M. micrantha* were excised from the mother plants (comprising two nodes, 30–35 cm) and were established in pots (20 cm height × 20 cm upper diameter × 15 cm lower diameter, one cutting per pot) that contained a mixture of top soil, sand and farmyard manure at the ratio of 2:2:1 by volume, respectively. The pots with *M. micrantha* were well watered daily with 500 mL of tap water at 06.00 hours and 100 mL of half-strength modified Hoagland solution with a composition as follows (Hershey 1994): the macro-elements were characterized by 1 mol L^{-1} KNO_3, 1 mol L^{-1} $Ca(NO_3)_2 \times 4H_2O$, 0.5 mol L^{-1} NH_4NO_3, 500 mg L^{-1} Fe-EDTA, 1 mol L^{-1}

$MgSO_4 \times 7H_2O$ and 0.5 mol L^{-1} KH_2PO_4, while the micro-elements were composed of 0.43 g H_3BO_3, 0.91 g $MnCl_2 \times 4H_2O$, 0.11 g $ZnSO_4 \times 4H_2O$, 0.026 g $CuSO_4$ and 0.05 g $H_3MoO_4 \times H_2O$ in 1 L of distilled water. One month after planting, the *M. micrantha* individuals that showed a uniform height and stem diameter (n = 80) were selected for *C. australis* inoculation.

Half of the selected *M. micrantha* pots (n = 40) were inoculated with *C. australis*. In order to ensure concurrent and successful haustorial attachment, the excised *C. australis* stems (~30 cm-long segment per host) were twined around the lowest internodes of the *M. micrantha* hosts. The other half of the *M. micrantha* pots were not inoculated with *C. australis*.

All of the *C. australis*-infected and uninfected treatments were watered twice (06.00 hours and 18.00 hours) every day with 500 mL of tap water for 5 weeks. At the end of this 5 week period, both the *C. australis*-infected and uninfected *M. micrantha* pots were subjected to drought-stress treatments for 1 week, either withholding water (drought) or watering daily (non-drought). Thus, a combination of non-drought (6 weeks well-watered) and drought (5 weeks well-watered and the sixth week water withheld) with non-parasite (*C. australis*-uninfected) and parasite (6 weeks *C. australis*-infected) resulted in four combinations of treatments: (i) no drought with no *C. australis* infection (n = 20); (ii) no drought with *C. australis* infection (n = 20); (iii) drought with no *C. australis* infection (n = 20); and (iv) drought with *C. australis* infection (n = 20).

After 1 week of drought treatment (i.e. at the beginning of the seventh week of the treatment period), the *M. micrantha* leaves were measured for gas exchange and Chl *a* fluorescence and were harvested in order to determine the Chl content and dry mass.

Gas exchange measurements

Nine fully expanded mature *M. micrantha* leaves from the fifth to the 10th nodes from the base were randomly selected from each treatment group (one leaf per host individual, n = 9) for gas exchange measurements using a portable gas exchange system (LI-6400XT; Li-Cor®, Lincoln, NE, USA). The measurements were taken according to the methods described by Johnson and Murchie (2011) and Le *et al.* (2014). The flow rate of gas into the chamber, leaf temperature and relative humidity inside the chamber were maintained at 500 μmol s^{-1}, 25°C and 50–60%, respectively. The photosynthetic light response curves of the leaves were developed under a range of PAR: 1800, 1500, 1000, 500, 250, 120, 60, 40 and 10 μmol(quantum) m^{-2} s^{-1}.

The instantaneous gas exchange parameters, measured at a PAR of 1500 μmol(quantum) m^{-2} s^{-1} (CO_2 assimilation rate [μmol(CO_2) m^{-2} s^{-1}] [A], stomatal conductance [mol(H_2O) m^{-2} s^{-1}] [g_s], transpiration rate [mmol(H_2O) m^{-2} s^{-1}] [E] and ratio of intercellular to chamber air CO_2 concentration [%] [C_i/C_a]) of the *M. micrantha* leaves, were recorded directly or calculated from the output readings of the portable gas exchange system, as described by Le *et al.* (2014). The water-use efficiency (WUE) [μmol(CO_2) mmol$(H_2O)^{-1}$] was calculated by the A/E ratio (see Farquhar & Richards 1984).

Leaf dry mass content and chlorophyll content measurements

Twenty fully expanded mature *M. micrantha* leaves from the fifth to the 10th nodes from the base were randomly collected from each treatment group (one leaf per host individual) and then randomly pooled into five replicates (n = 5) for the determination of the leaf dry mass content (LDMC). A similar sampling procedure was applied for the determination of the Chl content (n = 5). The LDMC (%) and leaf-extractable Chl content (mg cm^{-2}) were determined according to Hiscox and Israelstam (1979), Jayasinghe *et al.* (2004) and Le *et al.* (2014).

Chlorophyll *a* fluorescence transient measurements

The OJIP transient kinetics of the Ch *a* fluorescence of the *M. micrantha* leaves were measured with a portable chlorophyll fluorometer (OS-30P+; Opti-science, Inc., Hudson, New Hampshire, USA) during the night (22.00 hours–02.00 hours) to ensure appropriate dark adaptation, following Redillas *et al.* (2011). Twenty fully expanded mature *M. micrantha* leaves from the fifth to the 10th nodes from the base were randomly selected from each treatment group (one leaf per host individual, n = 20) for the OJIP tests. Chlorophyll fluorescence was induced by red light of 3500 μmol m^{-2} s^{-1}, with the saturation light width at 1.0 s (Strasser *et al.* 2000).

The Chl *a* fluorescence intensities at different time points after the light excitation of each OJIP transient observation were read directly from a fluorometer. The quantum yields of Chl *a* at time zero were interpreted as the: (i) maximum quantum yield of primary photochemistry (φ_{Po}); (ii) probability that a trapped exciton moves an electron into the electron transport beyond the primary quinone acceptor of PS II (ψ_0); (iii) quantum yield of electron transport (φ_{Eo}); and (iv) quantum yield of energy dissipation (φ_{Do}) (as described by Strasser *et al.* [2000, 2004]).

Statistical analysis

A two-way ANOVA and post-hoc Tukey's Honestly Significant Difference test for a full factorial 2 × 2 design (drought and parasite) were conducted with R version 3.1.2. (R Core Team 2014).

RESULTS

Leaf traits and gas exchange parameters of *Mikania micrantha*

The patterns of the photosynthetic light response curves showed that *M. micrantha* photosynthesis was negatively affected by *C. australis* infection and drought (Fig. 1). The *C. australis*-infected *M. micrantha* plants under drought conditions exhibited lower CO_2 assimilation rates than in the other treatments as the PAR levels increased.

Cuscuta australis infection increased the LDMC and reduced the Chl content, A, g_s and E of *M. micrantha* under both non-drought and drought conditions (Table 1, $P < 0.05$). However, *C. australis* infection induced higher effects on the LDMC, g_s and E of *M. micrantha* plants under drought conditions than on the host plants that were not subjected to drought stress (35% *vs* 18%, 56% *vs* 24% and 55% *vs* 26%, respectively; $P < 0.05$). For the Chl content and A, the magnitudes of the effects that were induced by the parasite were still higher, but not significantly, on the *M. micrantha* plants under drought conditions than on the host plants that were not subjected to drought stress (33% *vs* 25% and 37% *vs* 27%, respectively; $P > 0.05$). *Cuscuta australis* infection reduced the C_i/C_a ratio and increased the WUE of the *M. micrantha* plants only when the host plants were subjected to drought conditions (Table 1, $P < 0.05$).

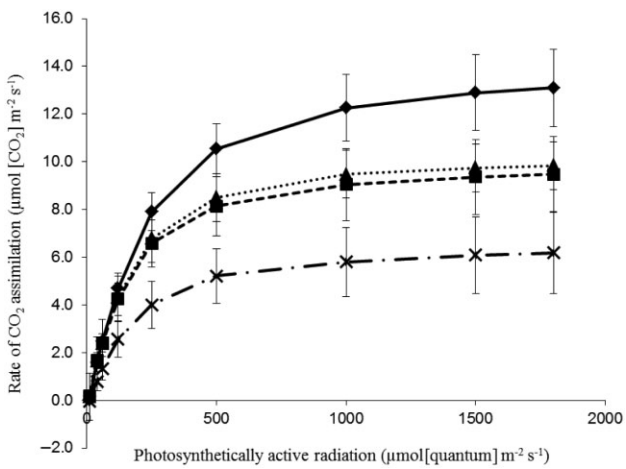

Fig. 1. Photosynthetic light response curves of *Mikania micrantha* that was grown under a 6 week treatment period of drought and *Cuscuta australis* infection. Non-drought, 6 weeks well-watered; drought, 5 weeks well-watered and the sixth week water withheld; uninfected, *C. australis*-uninfected; infected, 6 weeks *C. australis*-infected. The data are expressed as the mean ± standard deviation ($n = 9$). (◆), Non-drought and uninfected; (■), non-drought and infected; (▲), drought and uninfected; (×), drought and infected.

Drought conditions significantly affected the LDMC, g_s and E of the *C. australis*-infected *M. micrantha* plants, compared to the *C. australis*-uninfected ones. The magnitudes of the effects were 100% *vs* 75%, 58% *vs* 28% and 54% *vs* 25%, respectively. *Cuscuta australis* infection and drought had synergistic interactions on the LDMC, g_s, C_i/C_a, E and WUE, but exhibited additive effects on the Chl content and A ($P < 0.05$).

Chlorophyll *a* fluorescence transient test

The *C. australis*-infected and uninfected *M. micrantha* plants that were grown without any drought stress conditions and the *C. australis*-uninfected *M. micrantha* plants that were grown under drought stress conditions exhibited similar OJIP patterns (Fig. 2). However, the *C. australis*-infected *M. micrantha* plants that were grown under drought conditions showed an OJIP pattern that was distinct from the other treatments because a lower maximal fluorescence ($F_M = P$) was expressed.

Cuscuta australis infection had different effects on the Chl *a* fluorescence parameters of the *M. micrantha* plants that were grown under both water treatments; namely, normal watering and drought-induced regimes (Fig. 3). The *C. australis*-infected *M. micrantha* plants that were grown under the induced-drought conditions showed a lower F_M, φ_{Po}, φ_{Eo} and Ψ_0, but a higher φ_{Do}, than the *M. micrantha* plants that were subjected to the other treatments ($P < 0.05$).

DISCUSSION

Gas exchange characteristics are good indicators of plant stress due to their direct link to net photosynthesis, whereas a Chl *a* fluorescence analysis is a useful tool to assess the behavior of the photosynthetic apparatus in response to environmental changes (Von Caemmerer & Farquhar 1981; Schreiber *et al.* 1995; Strasser *et al.* 2000, 2004; Long & Bernacchi 2003). The results of this study showed that 6 weeks of *C. australis* infection reduced the A, g_s, E and Chl content but increased the LDMC of its

Table 1. Leaf traits and gas exchange performance of *Mikania micrantha* that was grown under a 6 week treatment period of drought and *Cuscuta australis* infection†,‡

Leaf traits and gas exchange parameters of *Mikania micrantha*	Treatment§				*F*-values from the ANOVA		
	Non-drought		Drought		Drought	Parasite	Drought × parasite
	Uninfected	Infected	Uninfected	Infected			
Leaf traits (n = 5)							
Leaf dry mass content (%)	7.8 ± 0.3a	9.2 ± 0.6b	13.7 ± 0.3c	18.5 ± 1.2d	620.04***	102.22***	32.04***
Chlorophyll content (mg cm^{-2})	0.028 ± 0.003c	0.021 ± 0.003b	0.018 ± 0.001b	0.012 ± 0.002a	72.28***	36.09***	0.17NS
Instantaneous gas exchange parameters measured at 1500 μmol(quantum) m^{-2} s^{-1} (n = 9)							
CO_2 assimilation rate (μmol CO_2 m^{-2} s^{-1})	12.9 ± 1.6c	9.4 ± 1.8b	9.7 ± 1.6b	6.1 ± 1.6a	35.19***	43.65***	0.01NS
Stomatal conductance (mol H_2O m^{-2} s^{-1})	0.152 ± 0.012c	0.115 ± 0.009b	0.110 ± 0.009b	0.048 ± 0.012a	244.76***	199.42***	12.40**
Intercellular to chamber air CO_2 concentration ratio (%)	58.4 ± 5.6b	61.5 ± 5.9b	58.2 ± 7.0b	44.3 ± 7.6a	16.01***	6.05*	15.20**
Transpiration rate (mmol H_2O m^{-2} s^{-1})	1.90 ± 0.18c	1.40 ± 0.14b	1.42 ± 0.15b	0.64 ± 0.17a	125.75***	134.89***	5.77*
Water-use efficiency (μmol CO_2 $mmol^{-1}$ H_2O)	6.8 ± 1.0a	6.7 ± 1.3a	6.9 ± 1.3a	9.6 ± 1.2b	13.37***	10.16**	11.61**

† Data were measured at the beginning of the seventh week of the treatment period and are expressed as the mean ± standard deviation; ‡ the tests for differences among treatments were conducted by using a two-way ANOVA and post-hoc Tukey's Honestly Significant Difference test at a 95% confidence interval (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ and NS, not significant; different letters indicate differences of the means at the 5% significance level); § non-drought, 6 weeks well-watered; drought, 5 weeks well-watered and the sixth week water withheld; uninfected, *Cuscuta australis*-uninfected; infected, 6 weeks *Cuscuta australis*-infected.

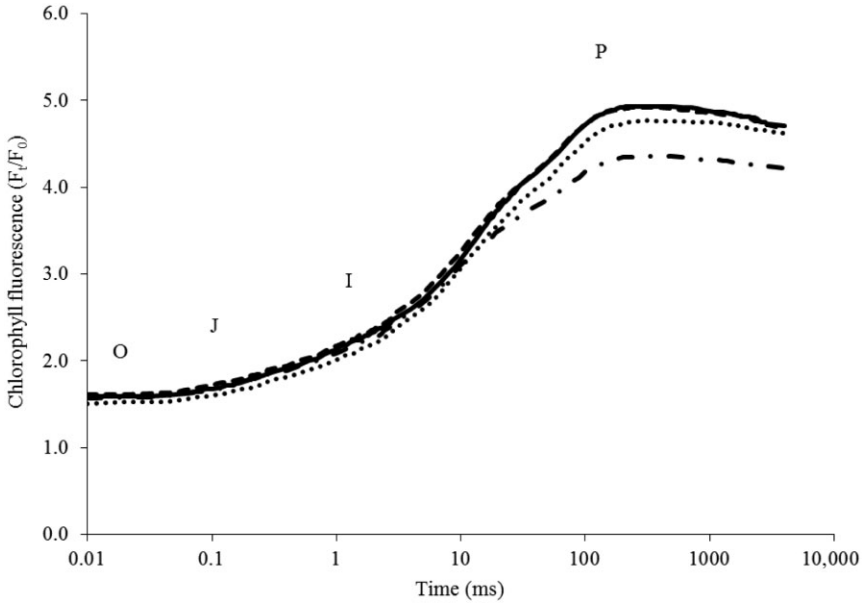

Fig. 2. Chlorophyll *a* fluorescence transient curves of the darkness-adapted leaves of *Mikania micrantha* that was grown under a 6 week treatment period of drought and *Cuscuta australis* infection. Non-drought, 6 weeks well-watered; drought, 5 weeks well-watered and the sixth week water withheld; uninfected, *C. australis*-uninfected; infected, 6 weeks *C. australis*-infected; F_0, O, J and I, fluorescence intensities at 0 s, 20 μs, 3 ms and 30 ms, respectively; F_t, fluorescence intensities at time *t*; P, maximal fluorescence. The data are expressed as the mean (n = 20). (—), Non-drought and uninfected; (- - -), non-drought and infected; (·····), drought and uninfected; (━ ·), drought and infected.

host, *M. micrantha*, under both well-watered and drought conditions. Here, this can be interpreted as that *C. australis* acted as a suppressor, rather than as a stimulator, of host photosynthesis, which is in agreement with previous studies that have been conducted to evaluate the influence of *C. campestris* on *M. micrantha* (Shen *et al.* 2007, 2011, 2013). However, the current results contrast with those of the investigations by Jeschke and Hilpert (1997) and Jeschke *et al.* (1997), where *C. reflexa* infections caused an increased g_s, E and A of the hosts, *R. communis* and *C. blumei*. The current study also found that *C. australis* infection had no effect on the shape of the OJIP transient and that all Chl *a* fluorescence parameters were extracted from the JIP tests of the host, *M. micrantha*, under well-watered conditions. These findings were different from the results of Vrbničanin *et al.* (2013). They reported that *C. campestris* infection negatively affected several parameters of chlorophyll fluorescence of its host, *A. trifida*, in the absence of drought stress. Two possible explanations could be that *C. campestris* had stronger negative impacts on its host (Fathoulla & Duhoky 2008) than *C. australis* used in this study or that *A. trifida* might be more tolerant to *Cuscuta* infection than *M. micrantha* (Farah & Al-Abdulsalam 2004; Barath & Csiky 2012). Two studies have indicated that non-native hosts might have a lower level of tolerance to parasitism when parasitized by alien parasites (Prider *et al.* 2009; Li *et al.* 2012). The root hemiparasite, *Rhinanthus minor*, also has been reported to reduce the φ_{Po} of its host, *Phleum berolinii*, but not of *Plantago lanceolata* (Cameron *et al.* 2008). Many other parasitic angiosperms also affect host photosynthesis through their influence on stomatal behavior, rather than by affecting their photosynthetic metabolism (Frost *et al.* 1997; Watling & Press 2001). Regardless, the results of this study demonstrated that the host, *M. micrantha*, under ample water conditions, was inhibited in its photosynthesis in response to *C. australis* infection by partial stomatal closure before any impairment of the photosynthetic apparatus occurred.

Drought stress can inhibit leaf photosynthesis via both stomatal and non-stomatal limitations, but the level of water deficit that can damage the structure and behavior of the photosynthetic apparatus is still debatable (Ashraf & Harris 2013; Brestic & Zivcak 2013). However, there is consensus that mild drought induces stomatal closure and increases WUE, whereas severe drought inhibits the metabolic process of photosynthesis and reduces the WUE, resulting in a photosynthetic decline (Flexas & Medrano 2002; Ashraf & Harris 2013; Snider *et al.*

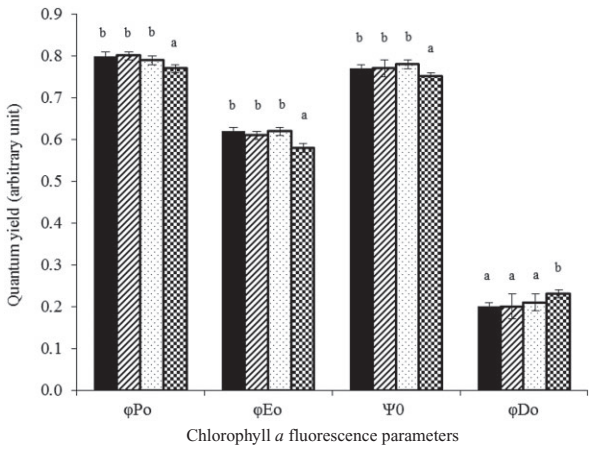

Fig. 3. Quantum yield of chlorophyll *a* fluorescence of *Mikania micrantha* that was grown under a 6 week treatment period of drought and *Cuscuta australis* infection. φ_{Po}, Maximum quantum yield of primary photochemistry; φ_{Eo}, quantum yield of electron transport; Ψ_0, probability that a trapped exciton moves an electron into the electron transport beyond the primary quinone acceptor of photosystem II; φ_{Do}, quantum yield of energy dissipation; non-drought, 6 weeks well-watered; drought, 5 weeks well-watered and the sixth week water withheld; uninfected, *C. australis*-uninfected; infected, 6 weeks *C. australis*-infected. The data are expressed as the mean ± standard deviation (n = 20). Different letters indicate differences of the means among treatments at the 5% significance level. (■), Non-drought and uninfected; (▨), non-drought and infected; (□), drought and uninfected; (▩), drought and infected.

2014). One week of induced drought that was caused by not watering the *C. australis*-infected and uninfected *M micrantha* plants at the end of the 6 week experimental period significantly affected the LDMC, Chl content, A, g_s and E of *M. micrantha*. However, the drought stress treatment did not induce significant changes in the C_i/C_a, WUE and Chl *a* fluorescence parameters of the *C. australis*-uninfected *M. micrantha* plants. This suggests that the water deficit regimes in this study (1 week of water withholding) only imposed mild drought stress on *M. micrantha*.

Similar responses in the gas exchange and Chl *a* fluorescence parameters of *M. micrantha* to a single stress factor (either induced drought or *C. australis* infection) in this study indicated that *C. australis* parasitism and drought stress might negatively affect the host, *M. micrantha*, similarly. It is possible that holoparasites maintain lower water potentials relative to their hosts, thus inducing a water deficit on their associated hosts (Westwood 2013), resulting in the close resemblance of the *M. micrantha* responses when the host plants were exposed to either drought or *C. australis* infection. A single induced stressor in this study (either induced drought or *C. australis* infection) did not result in significant changes in the C_i/C_a and WUE of *M. micrantha*. This suggests that the invasive plant, *M. micrantha*, is still capable of sustaining a C_i and WUE at optimal levels in response to stresses that are induced by either *C. australis* parasitism or drought alone, at least on a short time scale.

Notably, the results of this study revealed the deleterious combined effects of *C. australis* infection and drought on *M. micrantha* photosynthesis. The concomitant presence of a water deficit regime and *C. australis* parasitism induced additive effects on the Chl content and A and synergetic impacts on the LDMC, g_s, C_i/C_a, E, WUE, F_M, φ_{Po}, φ_{Eo}, φ_{Do} and ψ_0 of *M. micrantha*. Many photosynthetic parameters (e.g. the electron transport rate, carboxylation efficiency and WUE) of C_3 plants strongly correlate with the g_s and steady-state Chl fluorescence greatly depends on the g_s (Medrano *et al.* 2002). Specifically, both stomatal and non-stomatal limitations of terrestrial plant photosynthesis occur under severe stress conditions when the g_s is lower than 0.1 mol H_2O m^{-2} s^{-1} (Medrano *et al.* 2002; Ennahli & Earl 2005). In this study, the combination of *C. australis* infection and drought reduced the g_s to 0.048 mol H_2O m^{-2} s^{-1} and increased non-photochemical quenching (with a decrease in the φ_{Po}, φ_{Eo} and ψ_0 and an increase in the φ_{Do}) of the host, *M. micrantha*. Therefore, the decline in *M. micrantha* photosynthesis that was caused by *C. australis* infection under induced-drought conditions can be attributed to both gas diffusional limitations (partial stomatal closure) and non-stomatal limitations (metabolic impairment). Shen *et al.* (2007) suggested the possibility of the reduced light-use efficiency of *M. micrantha*, induced by *C. campestris* infection, but they did not demonstrate evidence related to the impairment of the photosynthetic apparatus. The Chl *a* fluorescence data in this study revealed that *M. micrantha* reduced its light-use efficiency when it was subjected to the presence of both *C. australis* infection and drought. Hence, it is argued that the decline in photosynthesis of *M. micrantha* that was induced by *C. australis* parasitism is further additive under drought stress and that this addition is related to both stress-induced stomatal and non-stomatal inhibitions on host photosynthesis.

In this study, it was reported for the first time the combined impacts of *C. australis* on the photosynthesis of the invasive plant, *M. micrantha*, under drought conditions. It was found that the inhibitory effects on the CO_2 assimilation of *M. micrantha*, caused by the infection

of the holoparasite, *C. australis*, became more severe because of the additive negative impacts of drought stress on host stomatal behavior and the PS II quantum yield. This study provides insights into the varying magnitudes of the impacts of *C. australis* on *M. micrantha* with changes in the abiotic environments of the tropics.

ACKNOWLEDGMENTS

This work was supported by funding provided by the Brunei Research Council (UBD-Science and Technology Research Grant No. 8) and Graduate Research Scholarship Programme, Universiti Brunei Darussalam. Logistic and laboratory facilities provided by the Faculty of Science and Institute for Biodiversity and Environmental Research, Universiti Brunei Darussalam, are gratefully acknowledged.

REFERENCES

Albert M., Belastegui-Macadam X., Bleischwitz M. and Kaldenhoff R. 2008. *Cuscuta* spp: "Parasitic plants in the spotlight of plant physiology, economy and ecology". In: *Progress in Botany*, vol. 69 (ed. by Lüttge U., Beyschlag W. and Murata J.). Springer, Heidelberg, 267–277.

Ashraf M. and Harris P.J.C. 2013. Photosynthesis under stressful environments: an overview. *Photosynthetica* **51**, 163–190.

Barath K. and Csiky J. 2012. Host range and host choice of *Cuscuta* species in Hungary. *Acta Bot. Croat.* **71**, 215–227.

Brestic M. and Zivcak M. 2013. PSII fluorescence techniques for measurement of drought and high temperature stress signal in crop plants: protocols and applications. In: *Molecular Stress Physiology of Plants* (ed. by Rout G.R. and Das A.B.). Springer, Dordrecht, 87–131.

Cameron D.D., Geniez J.-M., Seel W.E. and Irving L.J. 2008. Suppression of host photosynthesis by the parasitic plant *Rhinanthus minor*. *Ann. Bot. (Lond.)* **101**, 573–578.

Cechin I. and Press M.C. 1993. Nitrogen relations of the sorghum *Striga hermonthica* host–parasite association: growth and photosynthesis. *Plant Cell Environ.* **16**, 237–247.

Clark J. and Bonga J.M. 1970. Photosynthesis and respiration in black spruce (*Picea mariana*) parasitized by eastern dwarf mistletoe (*Arceuthobium pusillum*). *Can. J. Bot.* **48**, 2029–2031.

Ennahli S. and Earl H.J. 2005. Physiological limitations to photosynthetic carbon assimilation in cotton under water stress. *Crop Sci.* **45**, 2374–2382.

Farah A.F. and Al-Abdulsalam M.A. 2004. Effect of field dodder (*Cuscuta campestris* Yuncker) on some legume crops. *Sci. J. King Faisal Univ.* **5**, 103–113.

Farquhar G.D. and Richards R.A. 1984. Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes. *Funct. Plant Biol.* **11**, 539–552.

Fathoulla C.N. and Duhoky M.M.S. 2008. Biological and anatomical study of different *Cuscuta* species. *J. Dohuk Univ.* **11**, 22–39.

Flexas J. and Medrano H. 2002. Drought-inhibition of photosynthesis in C_3 plants: stomatal and non-stomatal limitations revisited. *Ann. Bot. (Lond.)* **89**, 183–189.

Frost D.L., Gurney A.L., Press M.C. and Scholes J.D. 1997. *Striga hermonthica* reduces photosynthesis in sorghum: the importance of stomatal limitations and a potential role for ABA? *Plant Cell Environ.* **20**, 483–492.

Heide-Jørgensen H.S. 2008. *Parasitic Flowering Plants*. Brill, Leiden, Netherlands.

Hershey D.R. 1994. Solution culture hydroponics: history & inexpensive equipment. *Am. Biol. Teach.* **56**, 111–118.

Hiscox J.D. and Israelstam G.F. 1979. A method for the extraction of chlorophyll from leaf tissue without maceration. *Can. J. Bot.* **57**, 1332–1334.

Jayasinghe C., Wijesundara D.S.A., Tennakoon K.U. and Marambe B. 2004. *Cuscuta* species in the lowlands of Sri Lanka, their host range and host–parasite association. *Trop. Agric. Res.* **16**, 223–241.

Jeschke W.D. and Hilpert A. 1997. Sink-stimulated photosynthesis and sink-dependent increase in nitrate uptake: nitrogen and carbon relations of the parasitic association *Cuscuta reflexa–Ricinus communis*. *Plant Cell Environ.* **20**, 47–56.

Jeschke W.D., Baig A. and Hilpert A. 1997. Sink-stimulated photosynthesis, increased transpiration and increased demand-dependent stimulation of nitrate uptake: nitrogen and carbon relations in the parasitic association *Cuscuta reflexa–Coleus blumei*. *J. Exp. Bot.* **48**, 915–925.

Jeschke W.D., Bäumel P., Räth N., Czygan F.-C. and Proksch P. 1994. Modelling of the flows and partitioning of carbon and nitrogen in the holoparasite *Cuscuta reflexa* Roxb. and its host *Lupinus albus* L. II. Flows between host and parasite and within the parasitized host. *J. Exp. Bot.* **45**, 801–812.

Johnson G. and Murchie E. 2011. Gas exchange measurements for the determination of photosynthetic efficiency in Arabidopsis leaves. In: *Chloroplast Research in Arabidopsis* (ed. by Jarvis R.P.). Humana Press, New York, 311–326.

Kuijt J. 1969. *The Biology of Parasitic Flowering Plants*. University of California Press, Berkeley, CA.

Le Q-V., Tennakoon K.U., Metali F., Lim L.B.L. and Bolin J.F. 2014. Host specific variation in photosynthesis of an obligate xylem-tapping mistletoe *Dendrophthoe curvata* in a Bornean heath forest. *Nord. J. Bot.* doi: 10.1111/njb.00628.

Li J., Jin Z. and Song W. 2012. Do native parasitic plants cause more damage to exotic invasive hosts than native non-invasive hosts? An implication for biocontrol. *PLoS ONE* **7**, 1–8.

Long S.P. and Bernacchi C.J. 2003. Gas exchange measurements, what can they tell us about the underlying limitations to photosynthesis? Procedures and sources of error. *J. Exp. Bot.* **54**, 2393–2401.

Marambe B., Wijesundara S., Tennakoon K., Pindeniya D. and Jayasinghe C. 2002. Growth and development of *Cuscuta chinensis* Lam. and its impact on selected crops. *Weed Biol. Manag.* **2**, 79–83.

Medrano H., Escalona J.M., Bota J., Gulias J. and Flexas J. 2002. Regulation of photosynthesis of C_3 plants in response to progressive drought: stomatal conductance as a reference parameter. *Ann. Bot. (Lond.)* **89**, 895–905.

Osmond C.B., Austin M.P., Berry J.A., Billings W.D., Boyer J.S., Dacey J.W.H. *et al.* 1987. Stress physiology and the distribution of plants. *Bioscience* **37**, 38–48.

Parker C. and Riches C.R. 1993. *Parasitic Weeds of the World: Biology and Control*. CAB International, Wallingford, UK, 111–163.

Press M.C. and Phoenix G.K. 2005. Impacts of parasitic plants on natural communities. *New Phytol.* **166**, 737–751.

Prider J., Watling J. and Facelli J.M. 2009. Impacts of a native parasitic plant on an introduced and a native host species: implications for the control of an invasive weed. *Ann. Bot.* **103**, 107–115.

R Core Team. 2014. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna.

Redillas M.C.F.R., Strasser R.J., Jeong J.S., Kim Y.S. and Kim J-K. 2011. The use of JIP test to evaluate drought-tolerance of transgenic rice overexpressing *OsNAC10*. *Plant Biotechnol. Rep.* **5**, 169–175.

Schreiber U., Bilger W. and Neubauer C. 1995. Chlorophyll fluorescence as a nonintrusive indicator for rapid assessment of in vivo photosynthesis. In: *Ecophysiology of Photosynthesis* (ed. by Schulze E.D. and Caldwell M.M.). Springer, Heidelberg, Germany, 49–70.

Shen H., Hong L., Chen H., Ye W-H., Cao H-L. and Wang Z-M. 2011. The response of the invasive weed *Mikania micrantha* to infection density of the obligate parasite *Cuscuta campestris* and its implications for biological control of *M. micrantha*. *Bot. Stud.* **52**, 89–97.

Shen H., Hong L., Ye W., Cao H. and Wang Z. 2007. The influence of the holoparasitic plant *Cuscuta campestris* on the growth and photosynthesis of its host *Mikania micrantha*. *J. Exp. Bot.* **58**, 2929–2937.

Shen H., Xu S-J., Hong L., Wang Z-M. and Ye W-H. 2013. Growth but not photosynthesis response of a host plant to infection by a holoparasitic plant depends on nitrogen supply. *PLoS ONE* **8**, 1–12.

Shen H., Ye W., Hong L., Huang H., Wang Z., Deng X. *et al.* 2006. Progress in parasitic plant biology: host selection and nutrient transfer. *Plant Biol.* **8**, 175–185.

Snider J.L., Collins G.D., Whitaker J., Perry C.D. and Chastain D.R. 2014. Electron transport through photosystem II is not limited by a wide range of water deficit conditions in field-grown *Gossypium hirsutum*. *J. Agron. Crop Sci.* **200**, 77–82.

Strasser R.J., Srivastava A. and Tsimilli-Michael M. 2000. The fluorescence transient as a tool to characterize and screen photosynthetic samples. In: *Probing Photosynthesis: Mechanisms, Regulation and Adaptation* (ed. by Yunus M., Pathre U. and Mohanty P.). Taylor and Francis, London, 445–483.

Strasser R.J., Tsimilli-Michael M. and Srivastava A. 2004. Analysis of the chlorophyll *a* fluorescence transient, advances in photosynthesis and respiration. In: *Chlorophyll Fluorescence: A Signature of Photosynthesis* (ed. by Papageorgiou G.C. and Govindjee). Springer, Dordrecht, Netherlands, 321–362.

Tripathi R.S., Khan M.L. and Yadav A.S. 2011. Biology of *Mikania micrantha* H.B.K.: a review. In: *Invasive Alien Plants: An Ecological Appraisal for the Indian Subcontinent* (ed. by Bhatt J.R., Singh J.S., Singh S.P., Tripathi R.S. and Kohli R.K.). CAB International, Wallingford, UK, 99–107.

Tyree M.T., Patiño S. and Becker P. 1998. Vulnerability to drought-induced embolism of Bornean heath and dipterocarp forest trees. *Tree Physiol.* **18**, 583–588.

Von Caemmerer S. and Farquhar G.D. 1981. Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. *Planta* **153**, 376–387.

Vrbničanin S., Sarić-Krsmanović M. and Božić D. 2013. The effect of field dodder (*Cuscuta campestris* Yunck.) on morphological and fluorescence parameters of giant ragweed (*Ambrosia trifida* L.). *Pestic. Phytomed. (Belgrade)* **28**, 57–62.

Watling J.R. and Press M.C. 2001. Impacts of infection by parasitic angiosperms on host photosynthesis. *Plant Biol.* **3**, 244–250.

Westwood J.H. 2013. The physiology of the established parasite–host association. In: *Parasitic Orobanchaceae* (ed. by Joel D.M., Gressel J. and Musselman L.J.). Springer, Heidelberg, Germany, 87–114.

Yu H., Liu J., He W-M., Miao S-L. and Dong M. 2011. *Cuscuta australis* restrains three exotic invasive plants and benefits native species. *Biol. Invasions* **13**, 747–756.

Yuncker T.G. 1932. The genus *Cuscuta*. *Mem. Torrey Bot. Club* **18**, 113–331.