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Host specific variation in photosynthesis of an obligate xylem-tapping mistletoe *Dendrophthoe curvata* in a Bornean heath forest

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Most mistletoe–host ecophysiological studies have paid attention to the influence of parasites on host performance. This paper explored the impact of varying hosts on the photosynthesis of a single mistletoe species. Here, we studied an obligate xylem-tapping tropical mistletoe (*Dendrophthoe curvata* (Blume) Miquel) parasitizing four different hosts (*Acacia auriculiformis* A. Cunn. Ex Benth, *Andira inermis* (W. Wright) DC., *Mangifera indica* L. and *Vitex pinnata* L.) in a homogeneous tropical heath forest patch in Brunei Darussalam. We compared photosynthetic capacity and photosynthesis-related characteristics of the mistletoe on four different hosts to evaluate the overall impact of hosts on the parasite. Results showed that the mistletoe–host patterns of CO_2 assimilation rates, transpiration rates and water use efficiency varied significantly based on the host. In the *D. curvata–Vitex pinnata* association, the mistletoe exhibited significantly lower CO_2 assimilation rates but showed no significant variations in transpiration rates and water use efficiency when compared to the host. In *D. curvata–Andira inermis* and *D. curvata–Mangifera indica* associations, the mistletoe showed significantly higher photosynthetic rates than the hosts, whereas in the *D. curvata–Acacia auriculiformis* association, there was no significant difference in photosynthetic rates between the counterparts. Host specificity also significantly influenced some mistletoe photosynthetic parameters such as light saturated photosynthesis, specific leaf area, leaf chlorophyll content, CO_2 assimilation rates, stomatal conductance, transpiration rates and water use efficiency. Different tree hosts intrinsically offer different resources to their obligate mistletoe parasites based on their physiology and environmental parameters. We argue that host-specific responses have driven these intra-specific variations in mistletoe physiology. This study provides background for future investigation on potential host-regulated mechanisms that drive functional changes in host-dependent mistletoes.

Mistletoes are parasitic flowering plants that attach to the stem of another plant (Mathiasen et al. 2008). Most of the ca 1400 mistletoe species belong to the families Loranthaceae, Viscaceae, Amphorogynaceae, Misodendraceae and Santalaceae within the order Santalales (Watson 2004, Nickrent et al. 2010). Mistletoes establish intimate haustorial connections with host vascular tissues via xylem system only or via both xylem and phloem systems. Xylem-tapping mistletoes can cover part of their carbon needs by photosynthesis (Marshall et al. 1994b, Tennakoon and Pate 1996, Popp and Richter 1998), therefore they only partially rely on host-derived carbon (Popp and Richter 1998) but entirely depend on hosts for water and mineral nutrients (Glatzel 1983, Popp and Richter 1998).

The extensive literature on the gas exchange and nutritional relationships of many mistletoe species have allowed the development of a relatively detailed picture of parasite–host relationships (Bell and Adams 2011). Many aspects of parasite–host physiology have been evaluated including carbon, water and mineral relationships (Lamont and Southall 1982, Glatzel 1983, Schulze et al. 1984, Ehleringer et al. 1986, Küppers 1992, Tennakoon and Pate 1996, Těšitel et al. 2010, Türe et al. 2010, Tennakoon et al. 2011, 2014, Chen et al. 2013, Scalon et al. 2013, Westwood 2013), photosynthesis, stomatal conductance and transpiration (Hollinger 1983, Ullmann et al. 1985, El-Sharkawy et al. 1986, Goldstein et al. 1989, Küppers et al. 1992, Flanagan et al. 1993, Johnson and Choinski 1993, Marshall et al. 1994a, Strong et al. 2000, Urban et al. 2012). It was reported that most mistletoes have generally higher stomatal conductance and transpiration rates but lower CO_2 assimilation rates and water use efficiency than hosts. Higher stomatal conductance and transpiration rates in the mistletoe may facilitate the movement of water and nutrients from hosts to mistletoes (Schulze and Ehleringer 1984, Goldstein et al. 1989) or be evidenced by phloem mobile mineral trapping (such as potassium) in mistletoe leaves due to the lack of phloem connectivity between hemiparasitic mistletoes

and hosts (Glatzel 1983, Glatzel and Geils 2008). Lower photosynthetic capacity and water use efficiency in the mistletoe can be explained by the fact that most mistletoes receive some heterotrophic carbon from the host xylem sap (Küppers et al. 1992, Těšitel et al. 2010).

However, there are some exceptional reports on the general physiology of mistletoe–host relationships. For example, Marshall et al. (1994a) and Lüttge et al. (1998) did not find any significant differences in photosynthetic rates of mistletoes and their hosts in many parasite–host pairs. In contrast, Küppers (1992) and Chen et al. (2013) reported that the mistletoes had lower transpiration rates than associated hosts. These patterns are attributed to the impacts of environmental conditions (i.e. water and nitrogen availability) in reducing the transpiration rate of mistletoes (Küppers 1992, Chen et al. 2013) or the varying levels of heterotrophic carbon acquisition of mistletoes from their hosts (Küppers et al. 1992, Marshall et al. 1994a, Lüttge et al. 1998). This demonstrated that parasite–host relationships are complicated and can vary in different associations (Lüttge et al. 1998, Glatzel and Geils 2008), and the debate continues as to whether mistletoe physiology is driven primarily by the hosts that they associate with or environmental factors or a combination of both.

In most studies, the impact of the mistletoe on the host have been investigated (Logan et al. 2002, Bickford et al. 2005, Reblin et al. 2006, Logan et al. 2012), but surprisingly little is known about how the host can regulate mistletoe physiology, except for its gas exchange (Marshall et al. 1994b) and growth (Bickford et al. 2005). Different host species may respond differently to the same environmental conditions (Bazzaz 1996) and this raises the question of whether a particular mistletoe host can have an impact on mistletoe performance.

In this study, we address the variability of physiological relationships exhibited by four co-occurring tropical mistletoe–host associations in which the same mistletoe species [*Dendrophthoe curvata* (Blume) Miquel (Loranthaceae)] is parasitizing four different host species [*Acacia auriculiformis* A. Cunn. Ex Benth (Fabaceae), *Andira inermis* (W. Wright) DC. (Fabaceae), *Mangifera indica* L. (Anacardiaceae), and *Vitex pinnata* L. (Verbenaceae)]. We investigated photosynthetic light responses of these mistletoe–host associations to evaluate their photosynthetic parameters (light saturated photosynthesis, apparent quantum yield and light compensation point) and instantaneous gas exchange parameters (CO_2 assimilation rates, stomatal conductance, intercellular to ambient CO_2 concentration, transpiration rates and water use efficiency). Parallel observations of leaf traits (specific leaf area and leaf dry matter content) and leaf chlorophyll profiles (chlorophyll content and chlorophyll a to b ratio) were made for comparison to the physiological variations shown by the mistletoe *D. curvata* when parasitizing four different hosts.

Material and methods

Study site, species and sampling

This study was conducted on four *Dendrophthoe curvata* (Blume) Miquel (Loranthaceae)-host associations viz: (1) *D. curvata–A. auriculiformis* A. Cunn. Ex Benth (Fabaceae), (2) *D. curvata–A. inermis* (W. Wright) DC. (Fabaceae), (3) *D. curvata–M. indica* L. (Anacardiaceae) and (4) *D. curvata–V. pinnata* L. (Verbenaceae). These co-occurring associations were studied at one homogeneous tropical heath forest patch (04°58′N, 114°58′E) in Brunei Darussalam during sunny days (9 am to 11 am) from June 2012 to June 2013. Nine host individuals with parasitizing mistletoes were selected randomly for each of the four associations. Fully expanded and healthy leaves of mistletoe and host were sampled at the top of the stunted tree canopy (2–3 m) with the assumption that the leaves have equal exposure to light, humidity and temperature during their growth.

Specific leaf area (SLA) and leaf dry matter content (LDMC)

Three leaves of mistletoe and host from each of nine host individuals with parasitizing mistletoe were sampled. Leaves of mistletoe or host from the same association were mixed and then divided randomly into three replicates.

The area of fresh leaves was measured using a leaf area meter. These leaves were then weighed immediately for fresh mass and dried in oven at 70°C until a constant dry weight was reached. Specific leaf area (SLA: $cm^2\ g^{-1}$) was calculated by ratio of leaf area to leaf dry mass. Leaf dry matter content (LDMC: %) was determined by ratio of leaf dry mass to leaf fresh mass (Marambe et al. 2002).

Chlorophyll (Chl) content

Three leaves of mistletoe and host from each of nine host individuals with parasitizing mistletoe were sampled. Leaves of mistletoe or host from the same association were mixed and then divided randomly into five replicates.

Leaf extractable Chl concentration was determined as described by Hiscox and Israelstam (1979). Leaf discs ($2\ cm^2$) were punched from seven positions in each fresh leaf using a cork borer and then the Chl was extracted in glass tubes using 7 ml pre-heated dimethyl sulphoxide (DMSO) at 65°C for 30 min. Each extract was topped up to 10 ml with DMSO and 3 ml of each final extract was measured for absorbance at 645 and 663 nm using a spectrophotometer. Chlorophyll (Chl) concentration was calculated as follows (Arnon 1949):

$$\text{Chl a } (\text{mg l}^{-1}) = 12.7 \times OD_{663} - 2.69 \times OD_{645}$$
$$\text{Chl b } (\text{mg l}^{-1}) = 22.9 \times OD_{645} - 4.68 \times OD_{663}$$
$$\text{Total Chl } (\text{mg l}^{-1}) = 20.2 \times OD_{645} + 8.02 \times OD_{663}$$

Chl concentration ($mg\ l^{-1}$) was then converted to Chl content per leaf area ($mg\ cm^{-2}$).

Gas exchange measurements

One leaf of mistletoe and host from each of nine host individuals with parasitizing mistletoe were sampled. Gas exchange was measured on detached leaves as described by Yan and Chuan-Kuan (2011). Leaf gas exchange was measured using a portable gas exchange system. We used a 2×3 cm chamber and a LED 6400-02B lamp as the light source. All measurements were made under at 50–60% humidity

inside the chamber. Gas flow rate into the chamber, leaf temperature and chamber CO_2 concentrations were maintained at 500 µmol s^{-1}, 25°C and 400 ppm, respectively during the measurement process. Before each measurement, a leaf was clamped into the chamber and left for stabilization to the measuring conditions for 15–30 min until CO_2 assimilation rates and stomatal conductance values were steady. Light response gas exchanges of leaves were developed under a range of photosynthetic active radiation (PAR) of: 1800, 1500, 1000, 500, 250, 120, 60, 40 and 10 µmol quantum m^{-2} s^{-1}.

Photosynthetic light response curves were fitted into the Mitscherlich model (Potvin et al. 1990, Peek et al. 2002) using R software as follows:

$$Y = \alpha\,[1 - e^{-\beta(X - \delta)}]$$

where Y = CO_2 assimilation rate (A: µmol CO_2 m^{-2} s^{-1}), X = photosynthetic active radiation (PAR: µmol quantum m^{-2} s^{-1}), α = light-saturated photosynthesis (A_{sat}: µmol CO_2 m^{-2} s^{-1}), β = apparent quantum yield (A_{qe}: µmol CO_2 μmol^{-1} quantum), δ = light compensation point (LCP: µmol quantum m^{-2} s^{-1}).

Statistical analysis

All statistical analyses, including student t-test, analysis of variance (ANOVA) and post-hoc Tukey test (Tukey HSD), were conducted using R ver. 3.0.1.

Results

Photosynthetic capacity and related parameters of *D. curvata*–host associations

We first measured CO_2 assimilation rates (A) of the mistletoe *D. curvata* and its associated hosts (*A. auriculiformis*, *A. inermis*, *M. indica* and *V. pinnata*) in response to different levels of PAR (Fig. 1). The pattern of photosynthetic light response curves suggested that photosynthetic capacity of *D. curvata* was generally higher than that of *A. inermis* and *M. indica* but lower than of *A. auriculiformis* and *V. pinnata*. To further confirm this, we fitted these light response photosynthesis curves into Mitscherlich model to determine photosynthetic light response parameters (A_{sat}, A_{qe} and LCP) (Table 1). There was no significant difference between *D. curvata* and associated hosts in overall A_{sat} (t-test: $p = 0.181$) and overall LCP (t-test: $p = 0.118$). Overall A_{qe} of *D. curvata* was significantly lower than associated hosts (t-test: $p < 0.001$).

However, patterns of those physiological attributes varied when each mistletoe–host association was considered separately (Table 1). For example, A_{sat} of *D. curvata* was higher, but not significantly higher than that of the host *A. auriculiformis* (t-test: $p = 0.234$). In the *D. curvata*–*A. inermis* association, the mistletoe A_{sat} was significantly higher than its host (t-test: $p < 0.001$). In the *D. curvata*–*M. indica* association, the mistletoe A_{sat} was also significantly higher than for the host (t-test: $p < 0.001$). However, in the *D. curvata*–*V. pinnata* association the mistletoe A_{sat} was significantly lower than for the host (t-test: $p < 0.01$).

Noticeably, *D. curvata* parasitizing *M. indica* had the lowest A_{sat} compared with *D. curvata* parasitizing other hosts (*A. auriculiformis*, *A. inermis* and *V. pinnata*), while *M. indica* also had the lowest A_{sat} compared with other hosts (Table 1).

Foliar traits and chlorophyll profiles of *D. curvata*–host associations

We obtained parallel data pertaining foliar traits (SLA and LDMC) and chlorophyll profiles (Chl content and Chl a/b in leaves) of the mistletoe *D. curvata* and its associated hosts (Table 1). Overall SLA, LDMC and leaf Chl a/b of the mistle-

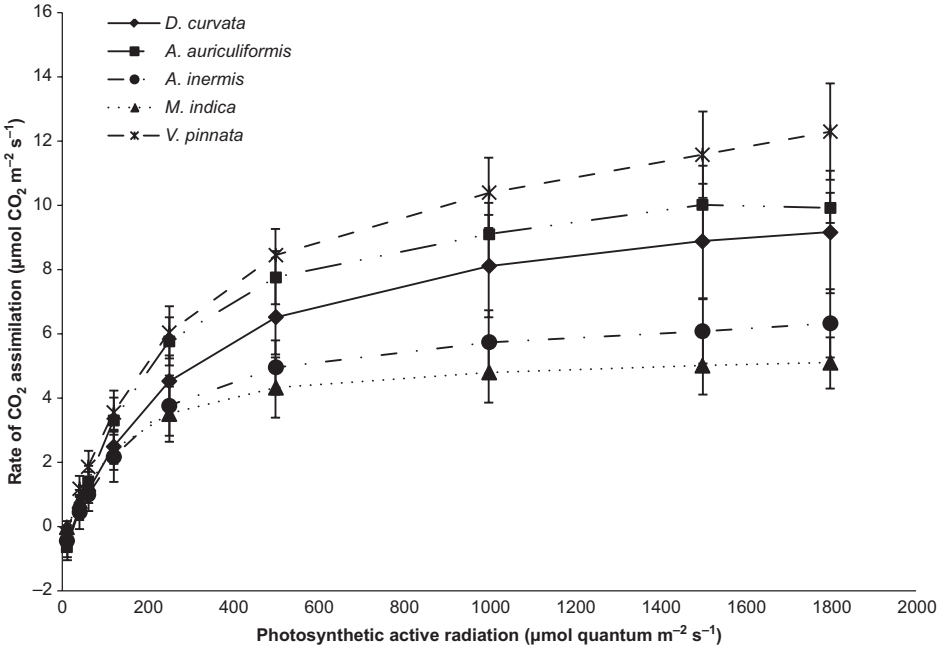

Figure 1. Photosynthetic light response curves of the mistletoe *Dendrophthoe curvata* and its associated hosts (*Acacia auriculiformis*, *Andira inermis*, *Mangifera indica* and *Vitex pinnata*). The data are expressed as means ± standard deviation (n = 36 for mistletoe, n = 9 for host).

Table 1. Photosynthetic parameters (A_{sat}: light saturated photosynthesis; A_{qe}: apparent quantum yield; LCP: light compensation point), foliar traits (SLA: specific leaf area; LDMC: leaf dry matter content) and chlorophyll profiles (Chl content: chlorophyll content; Chl a/b: ratio of chlorophyll a to b) of the mistletoe *Dendrophthoe curvata* and its associated hosts (*Acacia auriculiformis*, *Andira inermis*, *Mangifera indica* and *Vitex pinnata*).

Species Mistletoe parasitizing host	A_{sat} (µmol CO_2 m^{-2} s^{-1}) (n = 9)	A_{qe} (µmol CO_2 µmol^{-1} quantum) (n = 9)	LCP (µmol quantum m^{-2} s^{-1}) (n = 9)	SLA (cm^2 g^{-1}) (n = 3)	LDMC (%) (n = 3)	Chl content (mg cm^{-2}) (n = 5)	Chl a/b (n = 5)
D. curvata parasitizing *A. auriculiformis*	10.5 ± 1.4^{A}	0.0027 ± 0.0006^{A}	22 ± 11^{A}	0.64 ± 0.12^{A}	35.7 ± 3.7^{A}	0.043 ± 0.010^{A}	2.34 ± 0.11^{A}
D. curvata parasitizing *A. inermis*	9.0 ± 1.3^{A}	0.0029 ± 0.0006^{A}	9 ± 9^{A}	0.76 ± 0.10^{A}	36.7 ± 5.3^{A}	0.037 ± 0.003^{AC}	2.15 ± 0.67^{A}
D. curvata parasitizing *M. indica*	6.7 ± 1.3^{B}	0.0033 ± 0.0004^{A}	21 ± 13^{A}	0.62 ± 0.08^{A}	37.4 ± 3.8^{A}	0.016 ± 0.002^{B}	1.45 ± 0.17^{A}
D. curvata parasitizing *V. pinnata*	9.8 ± 1.4^{A}	0.0031 ± 0.0006^{A}	23 ± 12^{A}	1.01 ± 0.03^{B}	30.3 ± 1.0^{A}	0.029 ± 0.002^{BC}	2.05 ± 0.09^{A}
ANOVA							
F-value	12.73	1.52	2.91	12.54	2.12	14.64	3.47
p-value	< 0.001	0.23	0.05	< 0.01	0.18	< 0.01	0.07
Host	(n = 9)	(n = 9)	(n = 9)	(n = 3)	(n = 3)	(n = 5)	(n = 5)
A. auriculiformis	$9.8 \pm 1.0_{ns}{}^{a}$	$0.0040 \pm 0.0014^{*a}$	$22 \pm 7_{ns}{}^{a}$	$0.94 \pm 0.21_{ns}{}^{a}$	$35.4 \pm 2.6_{ns}{}^{a}$	$0.033 \pm 0.008_{ns}{}^{a}$	$3.19 \pm 0.52^{*abc}$
A. inermis	$6.1 \pm 1.0^{***b}$	$0.0043 \pm 0.0013^{*ac}$	$22 \pm 15_{ns}{}^{a}$	$1.38 \pm 0.18^{**bc}$	$43.9 \pm 3.2_{ns}{}^{b}$	$0.011 \pm 0.003^{***b}$	$4.86 \pm 0.89^{*b}$
M. indica	$4.9 \pm 0.8^{**b}$	$0.0058 \pm 0.0011^{***bc}$	$7 \pm 6^{*b}$	$0.93 \pm 0.04^{**a}$	$50.4 \pm 2.1^{**c}$	$0.013 \pm 0.004_{ns}{}^{b}$	$2.36 \pm 0.54^{*c}$
V. pinnata	$11.9 \pm 1.5^{**c}$	$0.0028 \pm 0.0008_{ns}{}^{a}$	$7 \pm 7^{**b}$	$1.25 \pm 0.03^{***ac}$	$44.3 \pm 1.0^{***b}$	$0.036 \pm 0.006_{ns}{}^{a}$	$3.47 \pm 0.85^{*abc}$
ANOVA							
F-value	71.15	9.43	8.01	7.63	20.12	16.91	6.27
p-value	< 0.001	< 0.001	< 0.001	< 0.01	< 0.001	< 0.001	0.02
Overall	(n = 4)	(n = 4)	(n = 4)	(n = 4)	(n = 4)	(n = 4)	(n = 4)
Mistletoe	9.0 ± 1.9	0.0030 ± 0.0006	19 ± 12	0.76 ± 0.18	35.0 ± 4.4	0.031 ± 0.011	2.00 ± 0.46
Host	$8.2 \pm 3.0_{ns}$	$0.0042 \pm 0.0016^{***}$	$14 \pm 12_{ns}$	$1.13 \pm 0.24^{***}$	$43.5 \pm 5.9^{***}$	$0.023 \pm 0.013_{ns}$	$3.47 \pm 1.12^{***}$

The data are expressed as means ± standard deviation.
Mistletoe intra-specific and host inter-specific differences using one-way analysis of variance (ANOVA) and post-hoc TukeyHSD test (Different letters indicate differences of means at 5% significant levels, $p < 0.05$).
Mistletoe and specific host comparisons, and general mistletoe host comparisons using Student t-test ($_{ns}$: Not significant, the degree of significance is indicated as follows: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$).

toe *D. curvata* were significantly lower than those of associated hosts in all four association investigated (t-test: $p < 0.001$). There was no significant difference in overall leaf Chl content among the mistletoe *D. curvata* and hosts (t-test: $p = 0.126$).

We continued to compare these attributes of the mistletoe and the host for each mistletoe–host pair. In all four mistletoe–host associations (*D. curvata–A. auriculiformis*, *D. curvata–A. inermis*, *D. curvata–M. indica* and *D. curvata–V. pinnata*), Chl a/b of *D. curvata* was significantly lower than those of its associated hosts (t-test: $p < 0.05$, Table 1). For SLA, in three mistletoe–host associations (*D. curvata–A. inermis, D. curvata–M. indica* and *D. curvata–V. pinnata*), SLA of *D. curvata* was significantly lower than those of the associated hosts (t-test: $p < 0.01$, $p < 0.01$ and $p < 0.001$, respectively). In the remaining association (*D. curvata–A. auriculiformis*), SLA of the mistletoe was still lower, but not significantly, than that of the host (t-test: $p = 0.092$). Leaf dry matter content (LDMC) of *D. curvata* was significantly lower than its hosts in two mistletoe–host associations (*D. curvata–M. indica* and *D. curvata–V. pinnata*) (t-test: $p < 0.01$ and $p < 0.001$, respectively), whereas in other two mistletoe–host associations (*D. curvata–A. auriculiformis* and *D. curvata–A. inermis*), there was no significant difference in LDMC between the mistletoe and its hosts (t-test: $p = 0.912$ and $p = 0.113$, respectively). For Chl content, there was no significant difference between the mistletoe and associated hosts in three associations (*D. curvata–A. auriculiformis*, *D. curvata–M. indica* and *D. curvata–V. pinnata*) (t-test: $p = 0.259$, $p = 0.282$ and $p = 0.090$, respectively). However, in the remaining association (*D. curvata–A. inermis*), the mistletoe showed a significantly higher Chl content than the host (t-test: $p < 0.001$).

Noticeably, *D. curvata* parasitizing *M. indica* also had the lowest SLA and Chl a/b (in addition to the lowest A_{sat}) and the highest LDMC compared with *D. curvata* parasitizing other hosts (*A. auriculiformis*, *A. inermis* and *V. pinnata*), although these variations were not significant. In concomitance with this pattern, *M. indica* showed the lowest SLA and Chl a/b (in addition to the lowest A_{sat}) and the highest LDMC among the four host species investigated (Table 1).

Instantaneous gas exchange parameters of *D. curvata*–host associations

We evaluated the instantaneous gas exchange performance (in relation to A, g_s, C_i/C_a, E and WUE) of *D. curvata* and its associated hosts at PAR of 1500 µmol quantum m^{-2} s^{-1} (equivalent to full sunlight conditions experienced in tropical heath forests where these associations naturally co-inhabit) (Table 2). Results showed that, similar to A_{sat}, there was no significant difference in overall A between *D. curvata* and its associated hosts (t-test: $p = 0.212$). However, overall g_s, C_i/C_a, and E of *D. curvata* were significantly higher than those of associated hosts (t-test: $p < 0.001$), while overall WUE of *D. curvata* was significantly lower than that of associated hosts (t-test: $p < 0.001$).

When the comparisons of these parameters were considered for each single mistletoe–host association, the mistletoe had significantly higher A than the respective hosts

Table 2. Instantaneous gas exchange performance (A: CO_2 assimilation rate; g_s: stomatal conductance; C_i/C_a: intercellular to ambient CO_2 concentration ratio; E: transpiration rate; WUE: water use efficiency) of the mistletoe *Dendrophthoe curvata* and its associated hosts (*Acacia auriculiformis*, *Andira inermis*, *Mangifera indica* and *Vitex pinnata*) on fully sunny days (measured at 1500 µmol quantum m^{-2} s^{-1}) in a tropical heath forest of Brunei Darussalam.

Species Mistletoe parasitizing host	A (µmol CO_2 m^{-2} s^{-1}) (n = 9)	g_s (mol H_2O m^{-2} s^{-1}) (n = 9)	Ci/Ca (%) (n = 9)	E (mmol H_2O m^{-2} s^{-1}) (n = 9)	WUE (µmol CO_2 $mmol^{-1}$ H_2O) (n = 9)
D. curvata parasitizing *A. auriculiformis*	10.3 ± 1.3^{A}	0.132 ± 0.040^{AB}	62.8 ± 8.3^{A}	1.78 ± 0.52^{AB}	6.1 ± 1.4^{A}
D. curvata parasitizing *A. inermis*	8.8 ± 1.2^{A}	0.191 ± 0.080^{A}	75.1 ± 7.6^{B}	2.60 ± 0.93^{A}	3.7 ± 1.1^{B}
D. curvata parasitizing *M. indica*	6.8 ± 1.3^{B}	0.166 ± 0.073^{AB}	76.3 ± 11.7^{B}	2.29 ± 0.97^{AB}	3.6 ± 2.1^{B}
D. curvata parasitizing *V. pinnata*	9.6 ± 1.3^{A}	0.114 ± 0.020^{B}	61.6 ± 3.6^{A}	1.61 ± 0.25^{B}	6.0 ± 0.6^{A}
ANOVA					
F-value	13.16	3.10	7.92	3.53	8.92
p-value	< 0.001	< 0.05	< 0.001	< 0.05	< 0.001
Host	(n = 9)	(n = 9)	(n = 9)		(n = 9)
A. auriculiformis	$9.9 \pm 1.3_{ns}{}^{a}$	$0.068 \pm 0.008^{***a}$	$36.7 \pm 6.5^{***a}$	$0.99 \pm 0.14^{***a}$	$10.2 \pm 1.2^{***a}$
A. inermis	$6.1 \pm 1.0^{***b}$	$0.045 \pm 0.012^{***a}$	$40.4 \pm 9.0^{***a}$	$0.66 \pm 0.17^{***a}$	$9.5 \pm 1.7^{***a}$
M. indica	$5.0 \pm 0.9^{**b}$	$0.038 \pm 0.013^{***a}$	$40.3 \pm 13.0^{***b}$	$0.53 \pm 0.19^{***a}$	$10.0 \pm 2.3^{***a}$
V. pinnata	$11.6 \pm 1.3^{**c}$	$0.140 \pm 0.053_{ns}{}^{b}$	$58.2 \pm 14.0_{ns}{}^{b}$	$1.98 \pm 0.68_{ns}{}^{b}$	$6.4 \pm 1.9_{ns}{}^{b}$
ANOVA					
F-value	66.41	25.17	6.93	28.29	8.60
p-value	< 0.001	< 0.001	< 0.01	< 0.001	< 0.001
Overall	(n = 4)	(n = 4)	(n = 4)	(n = 4)	(n = 4)
Mistletoe	8.9 ± 1.8	0.151 ± 0.064	68.9 ± 10.5	2.07 ± 0.81	4.9 ± 1.8
Host	$8.2 \pm 3.0_{ns}$	$0.073 \pm 0.049^{***}$	$43.9 \pm 13.6^{***}$	$1.04 \pm 0.67^{***}$	$9.0 \pm 2.3^{***}$

The data are expressed as means ± standard deviation.
Mistletoe intra-specific and host inter-specific differences using one-way analysis of variance (ANOVA) and post-hoc Tukey HSD test (Different letters indicate differences of means at 5% significant levels, $p < 0.05$).
Mistletoe and specific host comparisons, and general mistletoe host comparisons using student t-test ($_{ns}$: Not significant, the degree of significance is indicated as follows: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

in two associations (*D. curvata–A. inermis* and *D. curvata–M. indica*) (t-test: $p < 0.001$ and $p < 0.01$, respectively, Table 2). In *D. curvata–A. auriculiformis* association, mistletoe still showed higher A, but not significantly, than the associated host (t-test: $p = 0.615$), while in *D. curvata–V. pinnata* association, mistletoe showed significantly lower A than the associated host (t-test: $p < 0.01$). For g_s, C_i/C_a, E and WUE, in three associations (*D. curvata–A. auriculiformis*, *D. curvata–A. inermis* and *D. curvata–M. indica*), the mistletoe exhibited a significantly higher g_s (t-test: $p < 0.001$), significantly higher C_i/C_a (t-test: $p < 0.001$), significantly higher E (t-test: $p < 0.001$) and significantly lower WUE (t-test: $p < 0.001$) than hosts. Noticeably, we did not find any significant differences in g_s, C_i/C_a, E and WUE between the mistletoe and associated host in the remaining association (*D. curvata–V. pinnata*) (t-test: $p = 0.189$, $p = 0.497$, $p = 0.147$ and $p = 0.612$, respectively).

Variation in foliar traits, chlorophyll profiles, photosynthetic parameters and instantaneous gas exchange parameters

We explored the influence of varying hosts on photosynthesis and photosynthesis-related attributes of *D. curvata*. We generated photosynthetic light-response curves for *D. cuvata* parasitizing four different hosts (*A. auriculiformis*, *A. inermis*, *M. indica* and *V. pinnata*) (Fig. 2). We applied analysis of variance (ANOVA) on all measured attributes (A_{sat}, A_{qe}, LCP, SLA, LDMC, Chl content, Chl a/b, A, g_s, C_i/C_a, E and WUE) of *D. curvata* parasitizing different host species (*A. auriculiformis*, *A. inermis*, *M. indica* and *V. pinnata*) to evaluate host-specific variation exhibited in *D. curvata* photosynthesis.

There were no significant differences in A_{qe}, LCP, LDMC and Chl a/b (ANOVA: $p = 0.229$, $p = 0.05$, $p = 0.176$ and $p = 0.071$, respectively) among *D. curvata* parasitizing four different host species (Table 1). Other attributes (A_{sat}, SLA, Chl content, A, g_s, C_i/C_a, E and WUE) showed significant differences among *D. curvata* parasitizing different hosts (ANOVA: $p < 0.001$, $p < 0.01$, $p < 0.01$, $p < 0.001$, $p < 0.05$, $p < 0.001$, $p < 0.05$ and $p < 0.001$, respectively) (Table 1–2).

Discussion

The primary objective of this study was to assess the impacts of different hosts on the photosynthesis related physiological performance of mistletoes. Here, we used a mistletoe–host system in which one mistletoe species (*D. curvata*) parasitizes four different hosts (*A. auriculiformis*, *A. inermis*, *M. indica* and *V. pinnata*) and both parasite and hosts co-inhabit one site under uniform microhabitat conditions. This paper examined a number of interrelated physiological features which might be considered relevant to the complex interactions of mistletoe–host associations and explores mistletoe performance on different hosts under similar microhabitat conditions.

Obligate xylem-tapping mistletoes usually exhibit lower CO_2 assimilation rates, higher transpiration rates and lower water use efficiency than the respective hosts (Hollinger 1983, Goldstein et al. 1989, Flanagan et al. 1993, Johnson and Choinski 1993). In our study, the instantaneous transpiration (E) of the mistletoe showed significantly higher rates and the WUE were lower than the respective hosts in three associations (*D. curvata–A. auriculiformis*, *D. curvata–*

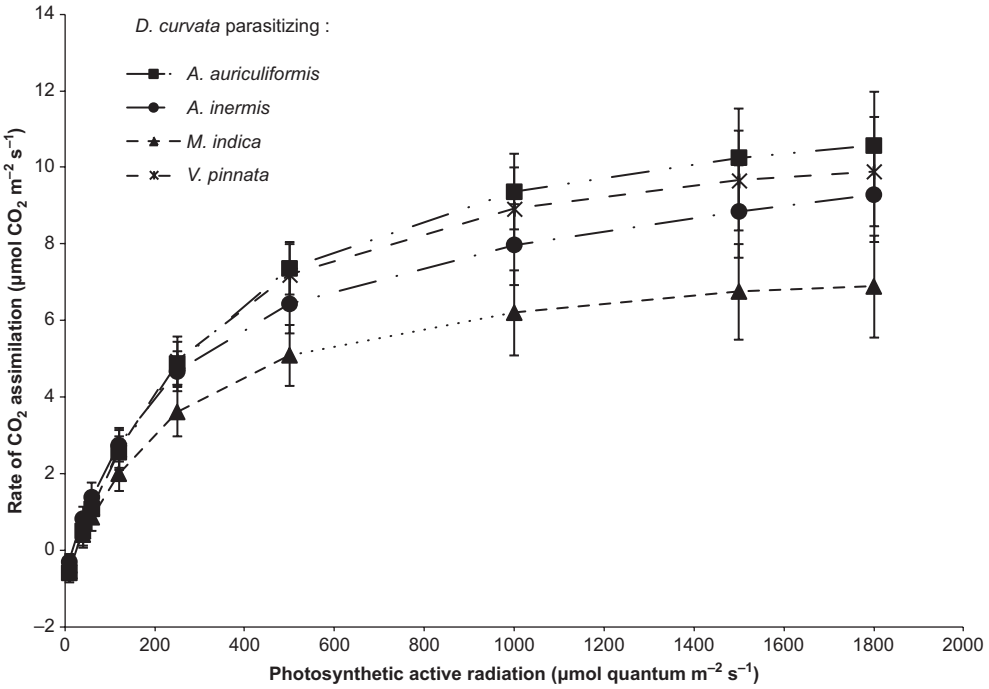

Figure 2. Photosynthetic light response curves of mistletoe *Dendrophthoe curvata* parasitizing four host species (*Acacia auriculiformis*, *Andira inermis*, *Mangifera indica* and *Vitex pinnata*). The data are expressed as means ± standard deviation (n = 9).

A. inermis and *D. curvata–M. indica*) in agreement with most previous studies (Hollinger 1983, Goldstein et al. 1989, Flanagan et al. 1993, Johnson and Choinski 1993). In all these instances, one would suppose that mistletoes directly extract xylem-derived nutrients from the hosts through the transpiratory stream along the lumen-to-lumen haustorial pathways that commonly exist in the mistletoe–host haustorial interface (Tennakoon and Pate 1996).

However, in *D. curvata–V. pinnata* association, the mistletoe did not show any significant differences in E and WUE compared to the host. Some previous studies have also reported less common patterns of lower transpiration rates in the mistletoe compared to the host (Hellmuth 1971, Küppers et al. 1992, Chen et al. 2013). Hellmuth (1971) have attributed this pattern to water availability. In contrast, Küppers et al. (1992) demonstrated that under permanently low plant water status, mistletoes maintain a lower transpiration rate due to the high organic carbon concentration in the host xylem. Thus, mistletoes can afford to be partially carbon heterotrophic at low transpiration rates to avoid the excess accumulation of other inorganic compounds, such as potentially harmful heavy metals. Similarly, Chen et al. (2013) have explained that under salt stress with nitrogen sufficient conditions, the mistletoe reduces its transpiration rates to avoid salt accumulation without violating its demand of nitrogen. These studies attributed the modulation in transpiration process of the mistletoe but not of the host.

When photosynthetic capacities of the mistletoe and the host were compared, our study showed that the mistletoe exhibited lower A_{sat} and A than the host in only one association (*D. curvata–V. pinnata*) which is in agreement with most previous studies (Hollinger 1983, Goldstein et al. 1989, Flanagan et al. 1993, Johnson and Choinski 1993). In contrast, the mistletoe showed significantly higher A_{sat} and A than the hosts in two associations (*D. curvata–A. inermis* and *D. curvata–M. indica*). In the remaining association (*D. curvata–A. auriculiformis*), the mistletoe also exhibited higher A_{sat} and A, but not significantly, than the host. Some other studies also reported the similarity of photosynthetic rates between the mistletoe and the host but did not specify the role of the mistletoe or the host responsible for this less common pattern (Marshall et al. 1994a, Lüttge et al. 1998).

In this study, we suggest that the variations of mistletoe–host physiological patterns, especially in relation to A_{sat}, A, E and WUE, can be attributed to varying performances of both counterparts. The higher photosynthetic rates of the mistletoe compared to hosts in three associations (*D. curvata–A. auriculiformis*, *D. curvata–A. inermis* and *D. curvata–M. indica*) can be attributed to the possibility of hosts diminishing their CO_2 assimilation rates in response to unfavorable environmental conditions. Photosynthesis and transpiration processes are inter-related and co-regulated by stomatal conductance to optimize water-use (Farquhar and Sharkey 1982). Under water deficit but optimum sunlight conditions, the host reduces its CO_2 assimilation and transpiration rates as a consequence of stomatal closure, while the mistletoe still maintains stomatal opening and normal photosynthetic intensity in the same microhabitat conditions (Ullmann et al. 1985, El-Sharkawy et al. 1986, Glatzel and Geils 2008). This argument can be used to explain the patterns that we have obtained in the mistletoe–host associations that we investigated. In three associations (*D. curvata–A. auriculiformis*, *D. curvata–A. inermis* and *D. curvata–M. indica*), the mistletoe showed significantly higher g_s and E, and significantly lower WUE than the respective hosts. In contrast, in the remaining association (*D. curvata–V. pinnata*), it is likely that *V. pinnata* did not reduce its stomatal conductance (it showed the highest g_s among four hosts investigated) to control water loss like in other three hosts, thus it still maintained the highest photosynthetic and transpiration rates among the investigated hosts. Consequently, in *D. curvata–V. pinnata* association, the host exhibited higher A_{sat} and A, and also no significant differences in E and WUE, compared to the mistletoe.

It is possible that although all four investigated associations were growing in the same habitat, different host species may have different water status levels due to species specific adaptations (e.g. deeper roots), different growth strategies, age, composition and even the position of plant where mistletoes have attached. For example, it was reported that *V. pinnata* adapts better towards a low resource growth strategy than some common secondary forest species (e.g. *Glochidion obscurum*, *Lagerstroemia speciosa*) in Peninsular Malaysia (Hashim and Hughes 2010). From this observation, we hypothesize that *D. curvata–V. pinnata* association did not experience a temporary water deficit like the other three associations (*D. curvata–A. auriculiformis*, *D. curvata–A. inermis* and *D. curvata–M. indica*). Thus, under sufficient water supply conditions, the host had a similar level of water use but higher photosynthesis compared to mistletoe because the host did not diminish its CO_2 assimilation rate (by partially closing its stomata) to control water loss. However, in this study, we could not gather evidence to explain why *V. pinnata* did not experience temporary water stress as compared to other three hosts (*A. auriculiformis*, *A. inermis* and *M. indica*), even though the hosts were co-occurring in an apparently homogenous tropical heath forest vegetation. Moreover, we cannot rule out local host race formation (Norton and Carpenter 1998) of *D. curvata* on the host plants, though we consider this unlikely because *D. curvata* and investigated hosts were from a single homogeneous patch of heath forest and both mistletoes and hosts were spatially mixed within the plant community.

Possessing a parasitic lifestyle, mistletoes fully depend on hosts for xylem-derived minerals and water (Ehleringer et al. 1985, Glatzel and Geils 2008). Thus, it is plausible to hypothesize that parasitized hosts can influence biology and physiology of mistletoes. A number of investigations have provided evidence to link the mistletoe performance (A, WUE or shoot growth) with the changes of host solute (nitrogen or water and carbon) uptake levels (Marshall et al. 1994b, Bickford et al. 2005). Scalon et al. (2013) have reported that the same mistletoe had different mineral profiles when parasitizing aluminum and non-aluminum accumulating hosts. In our study, *D. curvata* parasitizing different hosts demonstrated statistically significant differences for A_{sat}, SLA, Chl content, A, g_s, C_i/C_a, E and WUE. The impacts of host nature on a mistletoe can be explained by the fact that the xylem-tapping obligate hemiparasitic mistletoe completely depend on the host derived xylem sap

for water and nutrients (Shen et al. 2006, Glatzel and Geils 2008). Ultimately, these varying dependency levels regulate CO_2 assimilation rates and photosynthetic-related traits of the mistletoe.

In this study, *D. cuvata* parasitizing on *M. indica* showed different physiological patterns when compared to *D. curvata* parasitizing other hosts. In this association (*D. curvata–M. indica*), the mistletoe showed the lowest A_{sat}, A, SLA, LDMC and Chl a/b when compared to other associations. Similarly, the *M. indica* also had the lowest A_{sat}, *A*, SLA, LDMC and Chl a/b among four host species investigated. This concomitance suggests that the synchronization of the physiology of *D. curvata* is dependent on its host *M. indica*.

Our present comparison of physiological parameters between a single mistletoe species parasitizing four different hosts provide some new perspectives on the role of hosts in these parasite–host associations. However, the significance of these findings cannot be properly evaluated yet until more comparisons with other mistletoe–host associations are undertaken in varying habitat conditions and under different nutritional regimes. Hence, our findings should be viewed as an extended evidence of increased host dependency by tropical mistletoes, as suggested from a number of interrelated biological and physiological features which might be considered relevant to the complex interactions of the mistletoe *D. curvata* and its commonly exploited hosts in tropical heath forests of Brunei Darussalam.

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