

RESEARCH ARTICLE

Slavery in plants: how the facultative hemi-parasitic plant *Rhamphicarpa fistulosa* can completely dominate its host

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Keywords

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Abstract

The rain-fed lowland rice weed *Rhamphicarpa fistulosa* (Rice Vampireweed) is a facultative root parasitic plant. Growth and reproduction of *R. fistulosa* benefit considerably from parasitism, but how this affects the host plant is not well established. We determined accumulation and partitioning of rice–parasite biomass in two pot experiments. First, rice (cv. IR64) was grown under eight *R. fistulosa* densities (15–1000 seeds per pot) with two sampling times. Next, 2 parasite densities (6 and 13 plants per pot) were combined with 9 destructive samplings. Infection increased host root: shoot ratios and decreased host plant height, leaf area and tiller number. Reductions in light interception were followed by reductions in light use efficiency, causing 22–71% losses in host plant biomass and 78–100% losses in host kernel production. Parasitism eventually caused a complete standstill of host plant growth, while the parasite managed to gradually increase its share in total host plant–parasite biomass up to 50–82%. This implies that ultimately the host plant was producing solely for the sake of the parasite. Due to its facultative nature, *R. fistulosa* may incorrectly be perceived as relatively harmless. Upon infection this Rice Vampireweed, however, turns into a genuine slave master, whereby it completely dominates its host.

Introduction

Rhamphicarpa fistulosa (Hochst.) Benth. (Rice Vampireweed), is an annual root hemi-parasitic weed that has recently developed into an important pest of rain-fed lowland rice. The species is native and widely distributed in sub-Saharan Africa and increasingly encountered in agro-ecosystems, in particular in marginal rain-fed lowland areas (Rodenburg *et al.*, 2015; Kabiri *et al.*, 2015). The hemi-parasitic nature of *R. fistulosa* implies that apart from extracting assimilates from its host through a developed attachment organ known as haustorium, the parasitic plant produces assimilates independently through photosynthesis (Parker & Riches, 1993; Ouédraogo *et al.*, 1999). More characteristic is that the species is facultative in nature, meaning that it does not depend on a host plant for completion of its life cycle. However, when attached to a host, it benefits considerably in terms of growth and reproduction (Ouédraogo *et al.*, 1999; Kabiri *et al.*, 2016).

The effect of *R. fistulosa* infection on host plant performance is not well established. Some of the Orobanchaceae are known to reduce the photosynthetic rate of their host, thereby hampering host plant biomass accumulation (Press & Stewart, 1987; Cechin & Press, 1993). In return the host, which is often taller, can cast shade on the parasite reducing its photosynthesis and thus limiting parasite growth (Mardoian & Borowicz, 2016). Most members of the Orobanchaceae family manipulate their hosts to allocate more carbon to the roots which, in turn, is partly withdrawn by the parasite (Hibberd *et al.*, 1998; Watling & Press, 2001). This withdrawal of assimilates and nutrients by the parasite is at the cost of host plant growth. The infestation level of the parasite is an important determinant of the eventual host plant damage. The infestation level affects the infection levels, and also the time of first attachment which in turn determines the ultimate level of reduction in host plant biomass accumulation, as was demonstrated by van Ast *et al.* (2000)

for *Striga hermonthica* infested sorghum plants. The interaction between host plant and parasite ultimately determines the reduction in biomass accumulation and kernel production of the host plant.

In this study, the focus was on the interaction between rice and *R. fistulosa*. Two greenhouse pot experiments were conducted to obtain a better insight in the consequences of parasite attachment for host plant biomass accumulation. In both experiments, a single rice plant was used per pot. In the first experiment, meant to understand the influence of infestation level on host plant performance, rice was grown under a wide range of *R. fistulosa* infestation levels with destructive host plant and parasite samplings at just two moments in time. In the second experiment, the number of destructive samplings was increased to investigate the chronological effects of this host–parasite interaction. The purpose was to determine the moment in time when the parasite most likely attaches to the host and which host parameters are first affected. This could give an indication of the parasite's propensity to virulence on its host and the level of susceptibility of the host to the parasite. In this experiment, the number of parasite infestation levels was limited to two. Pure stands of *R. fistulosa* were included to obtain a more complete comprehension of the host plant–parasite interaction.

Materials and methods

In 2011 and 2014, greenhouse pot experiments were conducted at Wageningen University, the Netherlands whereby rice, cultivar IR64, was used as the host plant species. In the greenhouse, screens were used to create a day length of 12 h (from 7.00 a.m. to 7.00 p.m.). Supplemental lighting was provided by lamps (SON-T Agro, 400 W, Philips Lighting B.V., The Netherlands) that automatically switched on when photosynthetically active radiation inside the greenhouse dropped below $910 \mu\text{E m}^{-2} \text{s}^{-1}$.

Experiment 1

In Experiment 1, conducted from 13 July to 25 November 2011, the response of rice cultivar IR64 to eight *R. fistulosa* seed infestation levels – 0, 15, 31, 62, 125, 250, 500, 1000 seeds per pot – was investigated. Seeds of *R. fistulosa* were collected in 2009 from an infested rice field in Kyela, Tanzania. The germination percentage of these seeds was 60%. Growing conditions were set to 26°C/23°C for day/night temperature, but during warm summer days daytime temperature was regularly higher with a maximum of 33°C. Relative humidity varied between 50% and 70%.

The experiment followed a randomised complete block design with 6 replicates and 2 sampling times, at 60 DAS (days after sowing of both host and parasite) and at maturity (110 DAS), resulting in a total of 96 pots. Imperforated pots with 5 L capacity were filled with 6 kg of a mixture of coarse sand and arable soil (1:1 v/v). The pots were watered daily to maintain saturated conditions. Rice seeds were pregerminated for 48 h in an incubator at 33°C, after which one pregerminated seed was planted in the centre of each pot at a depth of 1–2 cm. On the same day, *R. fistulosa* seeds were mixed with about 9 g of dry sand and evenly sprinkled on top of the soil surface. Fertilisers were applied in two split applications, one at 18 DAS and another at 56 DAS (maximum tillering). At each application, 624 mg of Nitrogen Phosphorous Potassium (NPK) (12:10:18) fertiliser per pot was provided, equivalent to a total of 50 kg N ha⁻¹.

Experiment 2

Experiment 2 ran from 22 April to 9 July 2014 in a greenhouse with growing conditions set to 26°C/23°C for day/night temperature. Actual temperatures in the experimental period varied between 20.5°C and 29.9°C, while relative humidity varied between 57% and 100%. Seeds of *R. fistulosa* used were collected in 2010, from an infested rice field in Kyela, Tanzania. The germination percentage was 48%. The treatments consisted of pure stand rice and rice grown in association with a low and a high number of *R. fistulosa* plants, whereby the low number was set at 6 and the high number at 13 plants per pot. There were also pure stands of *R. fistulosa* plants grown without a host at identical infestation levels. The experiment was set up as a randomised complete block design, with four replicates. The growth and development of rice plants and *R. fistulosa* plants was followed at 9 sampling dates around 35, 42, 50, 57, 64, 71, 78, 99 and 111 DAS. The final harvest (111 DAS) comprised 3 pots per replicate for each treatment, resulting in a total of 180 pots for the entire experiment.

Pots were sealed at the bottom, and had a capacity of 6 L. Pots were filled by approximately 7.1 kg of a mixture of dry arable soil and sand. Sowing of rice and *R. fistulosa* seeds was done on the same day, using the methodology described for Experiment 1. In treatments including parasites, 150 seeds of *R. fistulosa* were sown per pot. At 28–31 DAS, 14 days after first emergence of the parasite, *R. fistulosa* seedlings were thinned back to 6 and 13 seedlings to establish a low and high density treatment. After that, removing of newly emerged seedlings was done on a weekly basis. Fertiliser was applied in 3 split applications, at 57, 66 and 71 DAS. At each application 167 mg of NPK fertiliser (12:10:18) per pot was provided, equivalent to a total of 20 kg N ha⁻¹.

Table 1 The effect of *R. fistulosa* seed infestation level on actual *R. fistulosa* plant numbers and height, leaf area, tiller production, panicle production and kernel weight of rice (Experiment 1) at 60 and 110 days after sowing (DAS)^a

<i>R. fistulosa</i> seeds/pot	60 DAS				110 DAS		
	Actual <i>R. fistulosa</i> no.	Rice height (cm)	Rice leaf area (cm ²)	Rice tiller no.	Actual <i>R. fistulosa</i> no.	Rice panicle no.	Rice kernel weight (g)
0	0	28.4 ^a	1231 ^a	7.3 ^{ab}	0.0	6.7 ^a	15.74 ^a
15	3.2 ^d	25.4 ^b	1150 ^a	8.0 ^a	5.6 ^c	3.7 ^b	3.43 ^b
31	7.8 ^d	25.2 ^b	1122 ^a	6.2 ^{ab}	8.0 ^c	2.5 ^b	1.72 ^b
62	21.8 ^d	24.4 ^{bc}	637 ^b	5.8 ^{bc}	15.8 ^c	0.8 ^c	0.51 ^b
125	25.5 ^d	23.4 ^c	564 ^{bc}	4.2 ^{cd}	24.6 ^{bc}	0.7 ^c	2.00 ^b
250	70.8 ^c	22.2 ^d	546 ^{bc}	4.3 ^{cd}	37.2 ^b	0.2 ^c	0.0 ^b
500	113 ^b	21.1 ^e	332 ^{bc}	4.1 ^d	35.5 ^b	0.0 ^c	0.0 ^b
1000	183.5 ^a	20.8 ^e	271 ^c	3.5 ^d	56.5 ^a	0.0 ^c	0.0 ^b
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
SED	9.90	0.695	145.6	0.923	7.09	1.10	1.812
LSD	20.22	1.382	295.8	1.877	14.44	2.30	3.683

SED, standard error of differences of means; LSD, least significant difference at the 95% confidence level.

^aValues (means) followed by a different letter are significantly different ($P < 0.05$).

Destructive samplings

At the sampling dates outlined above, rice tillers were counted and rice plant height was measured from the base of the stem to the youngest ligule of the main shoot. After that, rice plants were separated into leaves, stems and roots. At final harvest, panicles were included. Rice plant leaf area was measured with a desk-top leaf area meter, model LI-3100C (LI-COR, Lincoln, NE, USA). Roots were cleaned by carefully washing with water. In pots containing rice and *R. fistulosa* plants, the mixed root system was put in a plastic bag after washing, and frozen overnight at -20°C . The freezing process turned the roots of *R. fistulosa* into a dark purple colour, facilitating separation from host plant roots. All above-ground biomass of all parasites were also harvested to account for the parasite biomass in each pot. All plant parts of both host and parasite were dried in an oven at 70°C for 48 h to determine dry weight.

Weekly data on total rice plant dry weight and leaf area that were obtained between 35 and 78 DAS in Experiment 2, were used to determine light interception and light use efficiency (LUE) between control and *Rhamphicarpa* infested plants. Linear interpolation between sampling dates resulted in daily estimates of leaf area per plant. Accordingly, green leaf area duration (LAD) was defined as the summation of daily values of per plant leaf area from 35 DAS and onwards. Next, total plant dry weight was plotted against LAD for both control and *Rhamphicarpa*-infested treatments. The slope of the line that evolved between total plant dry weight and LAD was used as a proxy for how efficiently light was turned into rice dry matter (LUE). Differences between treatments were interpreted in terms of differences in light interception and LUE.

Statistical analysis

Data were subjected to analysis of variance (ANOVA) using statistical software GenStat for Windows 17th Edition (GenStat, 2013). Prior to ANOVA, data distributions and variances were checked for normality and homoscedasticity as described by Sokal & Rohlf (1995). The analysis was then followed by comparison of means by least significant difference at the 95% confidence level ($P < 0.05$).

Result

Influence of parasite density (Experiment 1)

First emergence of *R. fistulosa* seeds was observed at 6 DAS, and by 60 DAS the number of established *R. fistulosa* plants was about 18–35% of the number of seeds per pot (Table 1). From that time onwards (60 DAS) the amount of parasite biomass increased with increasing parasite densities, while the amount of host biomass decreased. At maturity of the host, this density effect was still reflected in host plant biomass, but there was no longer a significant density effect observed on parasite biomass. Moreover, with the exception of some of the lower densities, there was no further increase in host plant biomass beyond 60 DAS.

At 60 DAS the biomass accumulated by the parasite, steadily increased with density (Fig. 1a; $F_{6,29} = 28.15$; $P < 0.001$). Also, the fraction that *R. fistulosa* accumulated from the total biomass (host + parasite) increased with density level (Fig. 1a; $F_{6,35} = 23.31$; $P < 0.001$) such that at high infestation levels the parasite had accumulated more than half of the total biomass. While the biomass of the parasite increased with density, there was a decline

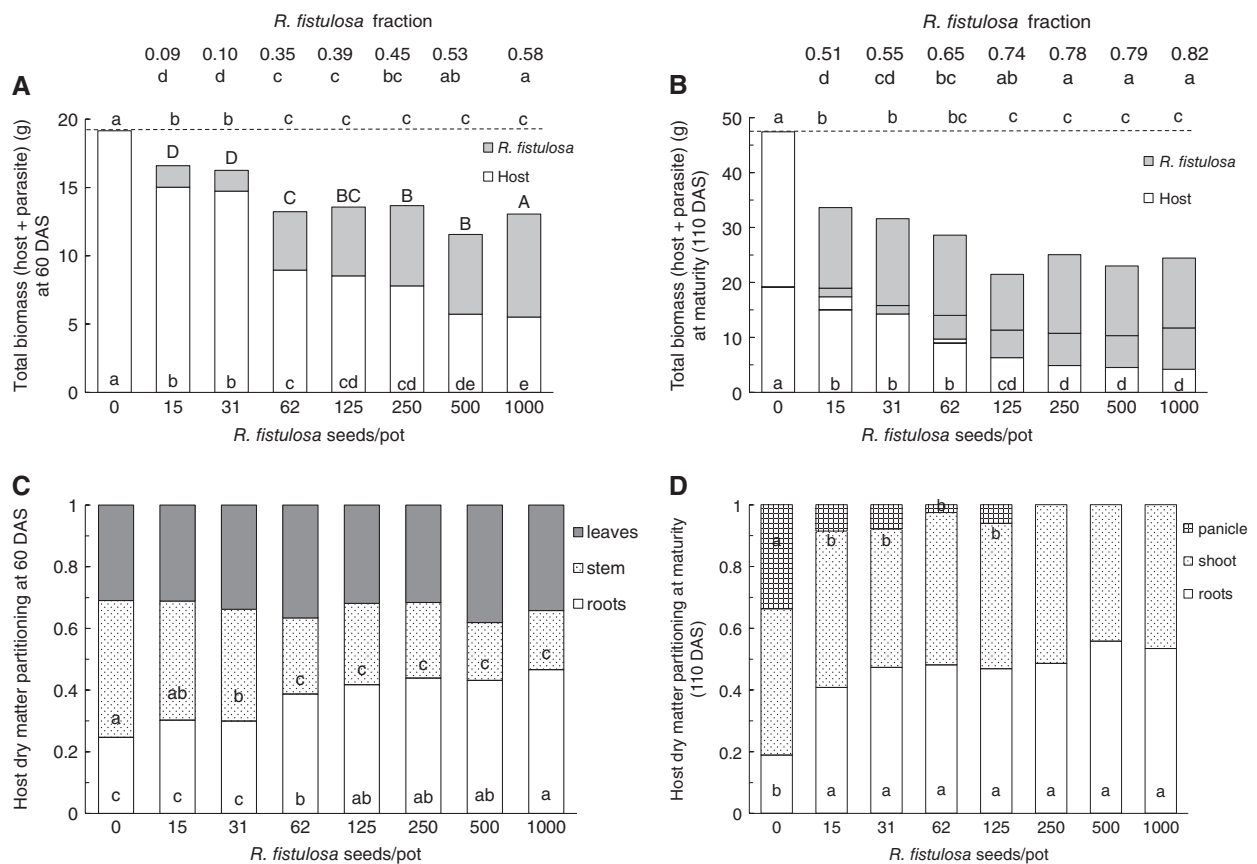


Figure 1 Total biomass of rice and *R. fistulosa* (g pot⁻¹) at 60 (a) and 110 DAS (days after sowing) (b) and the fraction of biomass partitioned to host plant parts at 60 (c) and 110 DAS (d) as a function of *R. fistulosa* infestation level (*R. fistulosa* seeds/pot). The dotted lines above (a) and (b) indicate the average dry weight of *R. fistulosa*-free rice plants. Division marks between the bars in (c) indicate the amount of biomass accrued by either host or parasite between 60 and 110 DAS. Above (a) and (b) the fraction of *R. fistulosa* dry weight to the total biomass (host and parasite) is presented. Bars indicated with different letters are significantly different ($P < 0.05$).

in the biomass of infested rice plants with increasing parasite density (Fig. 1a; $F_{7,35} = 25.15$; $P < 0.001$). At all densities, the total biomass at 60 DAS was significantly lower than the sole biomass of non-infested rice plants (Fig. 1a; $F_{5,35} = 5.53$; $P < 0.001$).

Compared to the *R. fistulosa*-free rice plants, a reduction in dry matter allocated to the stems was observed at densities of 31 seeds per pot and higher (Fig. 1c; $F_{7,35} = 13.51$; $P < 0.001$), whereas an increased fraction dry matter allocated to the roots started at 62 seeds per pot (Fig. 1c; $F_{7,35} = 13.51$; $P < 0.001$). Dry matter allocation to the leaves was not affected by *R. fistulosa* but rice plant height was. From the lowest density of 15 seeds per pot onwards, infested plants were stunted. At 60 DAS, height reductions compared to *R. fistulosa*-free rice plants ranged from 3 cm, at a density of 15 seeds per pot, to nearly 8 cm at 1000 seeds per pot (Table 1). At the same time, rice leaf area was reduced at densities ≥ 62 seeds per pot, while

tiller production was reduced at densities ≥ 125 seeds per pot (Table 1).

At 110 DAS, at densities ≥ 250 seeds per pot, the number of *R. fistulosa* plants was markedly lower than that at 60 DAS (Table 1). This decrease was not observed at lower *R. fistulosa* infestation levels, suggesting that the decrease at higher infestation levels resulted from density dependent mortality. Between 60 and 110 DAS the parasite biomass at all densities increased considerably. At 110 DAS, there were no significant differences between the absolute biomass of *R. fistulosa* plants across parasite densities (Fig. 1b; $F_{7,27} = 1.87$; $P = 0.122$), but the fraction of total biomass (host and parasite) accumulated by the parasite increased with density (Fig. 1b; $F_{7,27} = 28.15$; $P < 0.001$). At the highest parasite infestation level this fraction was even higher than 80%. Between 60 and 110 DAS, there was a 2.5-fold increase in the biomass of non-infested rice plants (Fig. 1b). In contrast, biomass of infested rice plants increased only marginally (at the lowest infestation

levels) or not at all (at the highest infestation levels). The significant differences in rice biomass between infestation levels thus remained (Fig. 1b; $F_{5,34} = 28.15$; $P < 0.001$). At all *R. fistulosa* infestation levels, the total biomass of host and parasite at 110 DAS was significantly lower than the biomass of non-infested rice plants (Fig. 1b; $F_{5,34} = 8.32$; $P < 0.001$).

The fraction dry matter allocated to the roots remained significantly higher in infested than in parasite-free host plants, with no differences among infestation levels (Fig. 1d; $F_{7,34} = 1.35$; $P < 0.001$). Dry matter distribution to the vegetative shoot parts (leaf and stem) was not affected by parasite infestation level (Fig. 1d; $F_{7,34} = 0.96$; $P < 0.473$). Compared to that of *R. fistulosa*-free rice plants the fraction dry matter allocated to the panicles (Fig. 1d; $F_{7,34} = 15.55$; $P < 0.001$) and the final kernel dry weight (Table 1; $F_{7,34} = 17.35$; $P < 0.001$) were significantly reduced in infested plants. Reduction in kernel dry weight varied from 78% at the lowest infestation level to 100% at the three highest infestation levels. The number of panicles produced by infested rice plants was reduced from 6.7 panicles per plant for parasite-free plants to 3.7 panicles at an *R. fistulosa*-infestation level of 15 seeds per pot, gradually dropping to zero at infestation levels of 500 and 1000 seeds per pot (Table 1; $F_{7,34} = 9.55$; $P < 0.001$).

Time course of parasitism (Experiment 2)

No interaction effect between host plant presence and parasite density on parasite biomass was observed on any of the sampling dates. Host plant presence resulted in a significantly higher parasite biomass, except during the early stages, at 35 and 50 DAS (Fig. 2). At harvest, parasite biomass in presence of a host was about twice the biomass in the absence of a host. Differences between the two densities were still significant, but compared to the earlier sampling dates these differences decreased.

Parasite plant density (either 6 or 13 plant per pot) had a significant positive effect on parasite biomass (except at 50 and 57 DAS). At 57 DAS the biomass accumulated by *R. fistulosa* (as a fraction of the combined host plant–parasite biomass) differed significantly between infestation levels (Fig. 3; $F_{1,6} = 12.95$; $P = 0.001$). However, it was not until 71 DAS that a significant reduction in the total host plant–parasite biomass was observed at plants infested with 13 *R. fistulosa* plants per pot (Fig. 3; $F_{1,6} = 7.38$; $P = 0.024$). Later, at 99 DAS ($F_{1,6} = 28.81$; $P < 0.0010$) and 111 DAS ($F_{1,6} = 60.57$; $P < 0.001$), the total biomass at both infestation levels was significantly lower than that of *R. fistulosa*-free rice plants.

Steady parasite-inflicted host plant biomass reductions were also first observed at 71 DAS, and increased thereafter (Fig. 3). These reductions started at the density

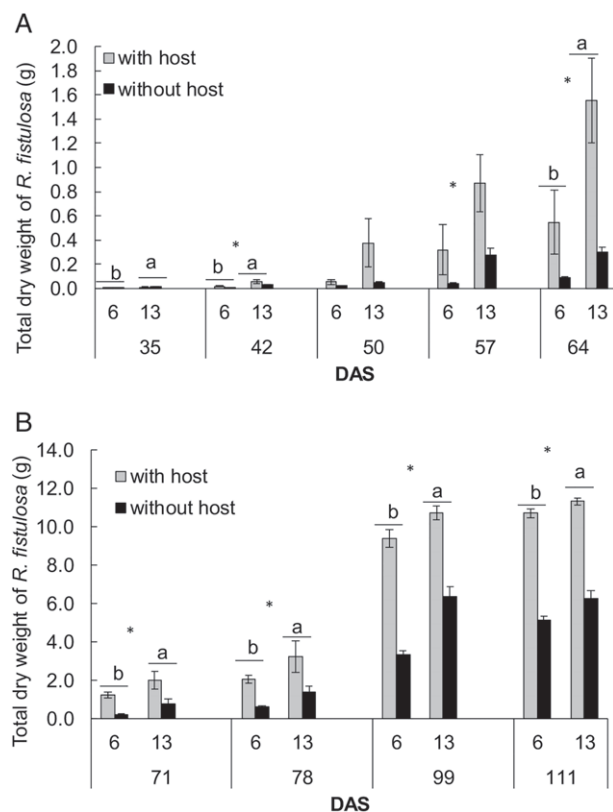


Figure 2 Development of *R. fistulosa* biomass (g pot^{-1}) in association with a host (grey bars) or without a host (black bars) at 35, 42, 50, 57 and 64 DAS (days after sowing) (a) and at 71, 78, 99 and 111 DAS (b). Bars with different letters show significant differences ($P < 0.05$) between infestation of 6 and 13 *R. fistulosa* per pot for the corresponding sampling date. An asterisk (*) above the bars indicate that there was a significant ($P < 0.05$) host effect on parasite biomass.

of 13 parasite plants ($F_{2,6} = 14.18$; $P = 0.005$). At 99 and 111 DAS, both infestation levels resulted in significantly reduced host plant biomass compared to the non-infested plants (99 DAS; $F_{2,6} = 163.7$; $P < 0.001$ and 111 DAS; $F_{2,6} = 98.24$; $P < 0.001$). The host plant biomass at the two infestation densities remained significantly different from one another (Fig. 3b). Biomass accumulation of the rice plants in the second half of the growing season was again minimal, at both 6 and 13 *R. fistulosa* plants per pot, resulting in significant reductions in kernel yield. *Rhamphicarpa fistulosa*-free rice plants produced 11.3 g grains (data not shown) while infested plants produced only 0.88 g at 6 and 0.22 g at 13 *R. fistulosa* plants per pot ($F_{3,6} = 80.62$; $P < 0.001$).

Starting at 42 DAS, infested rice plants distributed relatively more dry matter to the roots at the expense of the stems, compared to parasite-free plants (Fig. 4; $F_{2,6} = 16.41$; $P = 0.004$). At 78 DAS there was also a clearer difference between the two densities ($F_{2,6} = 25.77$;

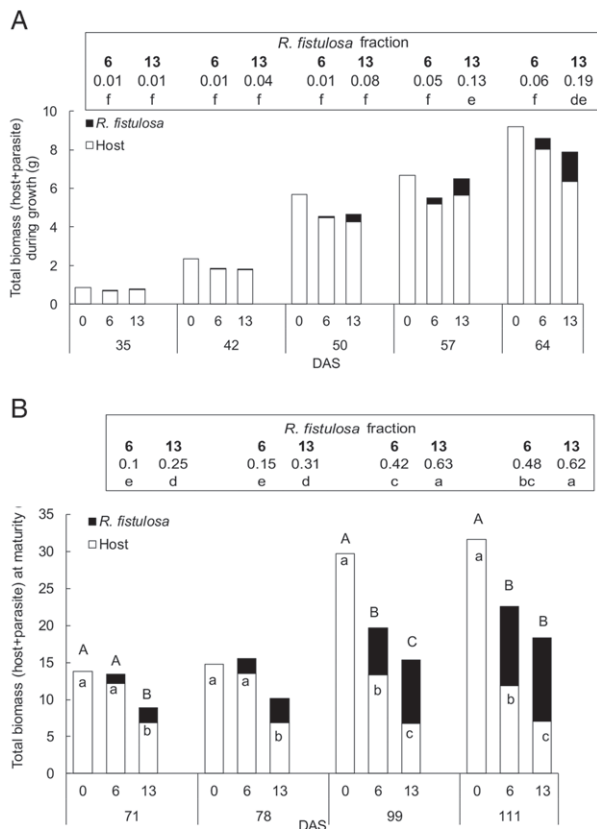


Figure 3 Development of total biomass (g pot^{-1}) of rice (white bars) and *R. fistulosa* (black bars) at different sampling dates in response to infestations of 0, 6 and 13 *R. fistulosa* plants per pot. For each sampling date, bars indicated with different lower-case letters indicate significant differences ($P < 0.05$) in host biomass, whereas bars indicated with different upper-case letters specify significant differences ($P < 0.05$) in total biomass (host and parasite). Above each figure, the fraction of *R. fistulosa* dry weight to the total biomass is shown.

$P = 0.001$). At maturity (111 DAS), while in *R. fistulosa*-free rice plants about 5% of total dry matter was present in the leaves, in the parasite-infested plants this was 17% ($F_{3,6} = 131.66$; $P < 0.001$). More prominently, whereas 34% of the total dry matter of non-infested rice plants was found in the roots, 50% and 59% of the dry matter of infested plants was found in this part ($F_{2,6} = 60.57$; $P < 0.001$). In the infested plants only 11% (at 6 parasites per pot) and 5% (at 13 parasites) of total dry matter was found in the panicles, compared to 33% of total dry matter of the uninfested plants (Fig. 4; $F_{2,6} = 111.05$; $P < 0.001$).

Significant parasite-inflicted height reductions were first observed at 64 DAS at both parasite densities. Height differences between these densities occurred only at 99 and 111 DAS (Table 2). Steady parasite-inflicted leaf area reductions were observed from 71 DAS onwards at an

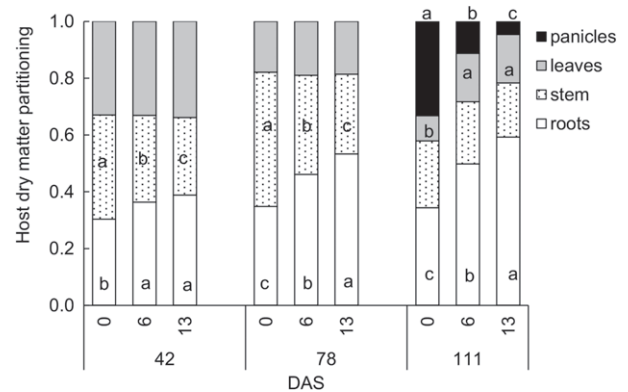


Figure 4 The fraction biomass partitioned to panicles, leaves, stems and roots in response to infestations of 0, 6 and 13 *R. fistulosa* plants per pot at 42, 78 and 111 days after sowing (DAS). Bars with different letters are significantly different ($P < 0.05$) at the corresponding sampling date.

infestation of 13 *R. fistulosa* plants per pot. At the lower infestation level leaf area reductions were only significant at 99 and 111 DAS. Steady reductions in tiller number were observed from 71 DAS onwards at an infestation of 13 *R. fistulosa* plants per pot. At 6 parasites per pot, tiller reductions compared to the parasite-free rice plants were only significant at 99 and 111 DAS.

A linear relationship was found between total per plant dry weight and green LAD of control plants. From 35 to 78 DAS, LAD increased to around $14.000 \text{ cm}^2\text{d}$ and total rice plant dry weight increase per unit LAD – hence the conversion of intercepted radiation in rice plant dry weight, that is, the LUE – was about $1.0 \text{ g per } 1000 \text{ cm}^2\text{d}$ (Fig. 5a and Fig. 5b). At the high infestation level (Fig. 5b), total LAD was reduced to about $10.000 \text{ cm}^2\text{d}$, implying that infested rice plants only captured about two thirds of the light of a control plant. This reduction in light interception was obvious from 57 DAS onwards. From this sampling time on, also the LUE dropped dramatically and reached a value of around $0.2 \text{ g per } 1000 \text{ cm}^2\text{d}$. At the low infestation level (Fig. 5b) between 35 and 78 DAS, only a reduction in light interception was observed while no clear reduction in LUE was noticed. Light interception of parasite-infested plants lagged behind from 57 DAS onwards and was about $1250 \text{ cm}^2\text{d}$ lower than the control at 78 DAS. This corresponds to a reduction of about 1.25 g in rice plant dry weight. At the low parasite density, the accumulated rice plant dry weight at 99 and 111 DAS was comparable to the dry weight observed at 78 DAS (Fig. 3). Dry matter accumulation during these last growth stages had come to a standstill.

At 64 DAS, the rice plants at the highest infestation level (13 plants per pot) reached a total plant dry weight of around 6.5 g . From then on, hardly any further increase in plant dry weight was observed, whereas green leaf area

Table 2 The effect of *R. fistulosa* infestation level (0, 6 and 13 plants per pot) on rice plant height (cm), rice tiller number and rice leaf area (cm² plant⁻¹) at 35, 42, 50, 57, 64, 71, 78, 99 and 111 days after sowing (Experiment 2)^a

DAS	Infestation level (<i>R. fistulosa</i> plants/pot)	Sampling time (DAS)								
		35	42	50	57	64	71	78	99	111
Height (cm)	0	9.8	16.2	16.6	17.2	20.0 ^a	22.3 ^a	28.3 ^a	49.8 ^a	50.8 ^a
	6	10.9	14.8	16.8	16.0	16.8 ^b	17.6 ^b	19.9 ^b	34.8 ^b	29.9 ^b
	13	11.0	12.8	16.4	15.9	16.2 ^b	16.1 ^b	16.3 ^b	21.7 ^c	24.3 ^c
	<i>F</i> _{2,6}	0.76	5.24	0.46	2.13	17.16	14.18	29.46	33.82	125.53
	<i>P</i>	0.506	0.048	0.653	0.200	0.003	0.005	<0.001	<0.001	<0.001
Tiller numbers	0	3.25	8.50 ^a	10.50 ^a	11.25	10.25	10.50 ^a	9.25 ^a	8.50 ^a	8.08 ^a
	6	2.75	6.75 ^{ab}	10.00 ^a	8.50	10.00	11.50 ^a	10.75 ^a	6.75 ^a	4.92 ^b
	13	2.75	5.25 ^b	7.50 ^b	8.75	8.75	8.25 ^b	7.50 ^b	4.25 ^b	3.92 ^b
	<i>F</i> _{2,6}	0.12	6.66	5.47	4.83	1.43	12.87	6.25	11.53	34.07
	<i>P</i>	0.889	0.030	0.044	0.056	0.310	0.007	0.034	0.009	<0.001
Leaf area (cm ²)	0	89.8	217.0	270.0	409.0 ^a	389.0	382.0 ^a	463.0 ^a	230.0 ^a	135.1 ^a
	6	90.5	179.0	292.0	274.0 ^b	479.0	364.0 ^a	431.0 ^a	73.0 ^b	13.2 ^b
	13	90.88	164.0	278.0	276.0 ^b	285.0	209.0 ^b	158.0 ^b	20.0 ^b	3.3 ^b
	<i>F</i> _{2,6}	0.00	2.42	0.12	5.34	3.12	10.45	16.62	20.90	293.14
	<i>P</i>	0.999	0.170	0.888	0.047	0.118	0.011	0.004	0.002	<0.001

DAS, days after sowing.

^aValues (means) followed by a different letter are significantly different ($P < 0.05$). Bold figures show *P* values. The letters x and y, associated to the *F* values, indicate degrees of freedom of treatments (x) and degrees of freedom of residuals (y).

was still present showing that the production of assimilates only benefits the parasite. This is clearly illustrated in Fig. 5a: whereas LAD is still progressing, total plant dry weight has come to a standstill, indicating a LUE close to nil. For the low infestation level (6 plants per pot) the reduction in biomass up to 78 DAS could still be explained by a reduced light interception, implying that till that moment LUE was hardly affected (Fig. 5).

Discussion

The second experiment carried out in this study included pots with independently growing *R. fistulosa* parasites adjacent to parasites growing with a host. This set-up enabled a comparison between the biomass accumulation of parasites grown with and without a host plant, showing the net-gain of parasitism and sustaining the hypothesis that the parasite benefits from assimilate withdrawal from its host.

The ultimate effects of hemi-parasites may be difficult to predict, because even closely related species can have very different effects on the same host species as was observed by Matthies (1997). The present results, for the first time demonstrate that a facultative parasitic plant can completely enslave its host. Towards maturity of both host and parasite, the biomass of the infected host plant appeared to remain constant while the green leaf area still continued to be present and the biomass of the parasite kept on increasing. This indicated that any

assimilates produced by the host were being taken up by the parasite. The host seemed to be led to produce assimilates solely for the benefit of the parasite. This is a kind of parasite-induced manipulation that has not been described before with parasitic plants.

Hemi-parasitic plants have been shown to manipulate the host plant in such way that the advantages from parasitism are higher than the disadvantages from host competition. Studies with other facultative parasites (i.e. *Castilleja integra*, *C. miniata* and *C. chromosa*) have shown a reduction of total host and parasite biomass but the biomass accumulated by the hemi-parasitic plants was lower than the reduction in biomass of the host plants. This is in conformity with our results. Whereas *R. fistulosa* accumulated most of the biomass of the total host-parasite association, the combined sum of host and parasite biomass was always lower than that of *R. fistulosa*-free rice plants alone. A similar dominance of the parasite in the total (host + parasite) biomass was observed with the facultative hemi-parasitic plant *Rhinanthus minor* on the grass species *Phleum bertolonii* (Cameron et al., 2008) and also with *C. integra*, *C. miniata* and *C. chromosa*, and the annual hemi-parasite *Orthocarpus purpurascens* on *Lolium perenne* (Matthies, 1997). Matthies (1997) also found that facultative parasites had a lower efficiency of resource utilisation than the host. While this implies that the parasite will do better when environmental resource availability increases, Fibich et al. (2010)

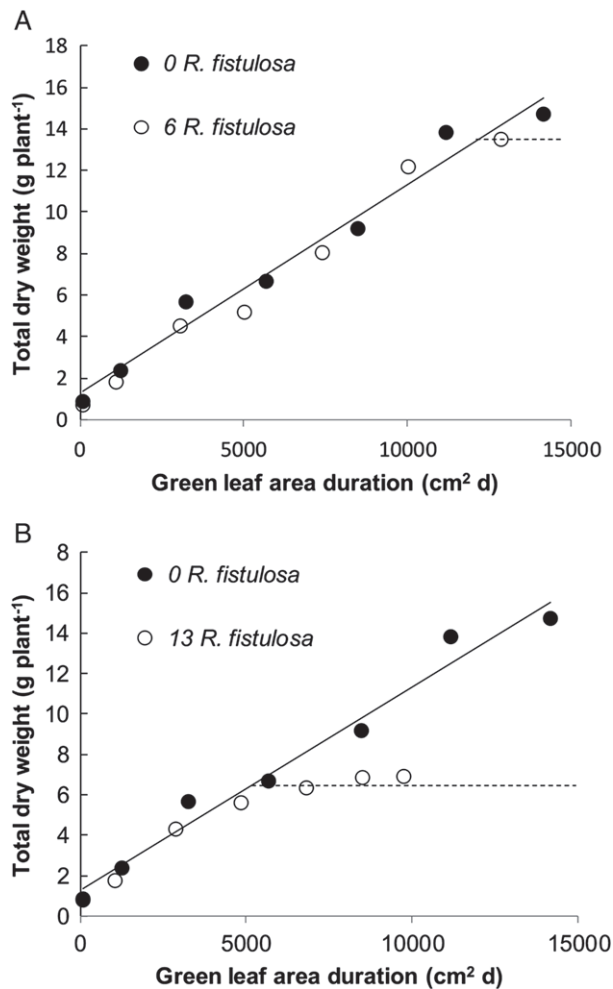


Figure 5 Relationship between rice plant dry weight (g/plant) and green leaf area duration (cm²d) for uninfested rice plants and for rice plants infested with 6 *R. fistulosa* (a) and 13 *R. fistulosa* plants per pot (b). The solid line represents the result of linear regression ($y = 0.001x + 1.292$; $R^2 = 0.97$) on data obtained with the control plants. The horizontal dotted line represents the average plant dry weight of infected host plants at 99 and 111 DAS (days after sowing).

also demonstrated that, because hosts are better competitors for light, concomitant increases in host performance will outcompete hemi-parasites once the resource availability reaches a certain level. Yet, in this study, the observed reduction of the above-ground host biomass at the expense of the host root biomass implies that *R. fistulosa* is indeed quite successful in manipulating the host to optimise the balance between resource acquisition and competition. In further studies with *R. fistulosa*, the trade-off between parasitism and competition with its host should be investigated.

A reduction of productivity in the presence of hemi-parasites may be a common occurrence, indicating

that the negative impacts some facultative parasites have on their hosts is attributed to withdrawal of assimilates. For example, *R. minor* on barley, trafficked up to 20% of host solutes from the xylem sap, reducing host growth by a similar magnitude of 22% (Jiang *et al.*, 2003). Seel & Press (1996) reported that infection of the perennial grass host *Poa alpina* by *R. minor* affected flower bud formation and biomass partitioning. The present results show that even relatively low infestation levels of *R. fistulosa* already resulted in reductions in kernel weight of the host plant of up to 79–92%. *Rhamphicarpa*-induced suppression of host biomass resulted in the parasite accumulating more than 50–80% of total biomass (host and parasite), depending on the level of infection. Yet, the degree of *Rhamphicarpa*-induced suppression of host biomass could not be fully accounted for by the biomass obtained by the parasite as uninfested rice plants had more biomass than the combined sum of the biomass of the parasite and the infected host. As the loss in host biomass cannot be fully explained by a gain in parasite biomass, the relation between the host and parasite cannot be purely characterised as a source-sink relation but entails a pathological component. Such a pathological effect can be caused by (a) poor resource use efficiency by the parasite such that the gain in the parasite is less than the reduction in host biomass (Matthies, 1995, 1996, 1997). It has been shown in many studies that the resource efficiency of hemi-parasites is generally lower than that of their hosts (Bloom *et al.*, 1985; Aflakpui *et al.*, 1998; Shen *et al.*, 2005; Hibberd *et al.*, 1998; Matthies, 1995, 1997). A pathological effect can also be caused by (b) disruption of host water and nutrient balances (Watling & Press, 2001), (c) alteration of host plant growth regulators causing changes in host plant allometry, (d) negative feedback effects of disrupted host water, nutrient balances and host plant regulators on host plant photosynthesis through self-shading, drought stress or malnutrition effects (Parker & Riches, 1993). For instance, *Cuscuta* (dodder) species have been observed to create stronger sinks for assimilates than any of the host's organs (Parker & Riches, 1993). With *Striga*, a pathological effect caused by an imbalance in growth regulators in the host has been reported. The growth regulator imbalance includes an increase in abscisic acid levels and a reduction in the levels of cytokinins and gibberellic acid that in turn negatively affect host photosynthesis and host growth (Gurney *et al.*, 1995; Taylor *et al.*, 1996; Watling & Press, 2001).

The missing biomass in the rice–*R. fistulosa* interaction could also be a result of a more direct host photosynthetic repression by *R. fistulosa*. Negative effects on host leaf photosynthetic rate are commonly observed in a variety of interactions with obligate parasitic plant species (Cechin & Press, 1993; Watling & Press, 2001; Rodenburg *et al.*, 2008;

Rodenburg *et al.*, 2017) and with other facultative parasitic plants (Hwangbo *et al.*, 2003; Cameron *et al.*, 2008). Specifically in a study with *R. minor*, Hwangbo *et al.* (2003) observed that the parasite reduced host photosynthesis, leaf area and biomass which is in conformity with our findings. However, despite the negative effects of *R. minor* on its host, the total biomass of the association was not less than that of the uninfected host. This contradicts with our findings, pointing to a parasite species-specific, a host-species-specific effect or a combination of these. It could be that *R. minor* is more 'autotrophic' than *R. fistulosa* implying that the latter, has a higher degree of host dependence. Hwangbo *et al.* (2003) also showed that a parasite-inflicted reduction in host-plant leaf area will result in reduced light interception, followed by a reduced production of biomass. It is this feedback mechanism that also contributed to the gap between the biomass accumulated by healthy and parasite-infected rice plants in this study.

Parasite induced manipulation of host plants has been commonly observed in host–parasite interactions (Hibberd *et al.*, 1998; Watling & Press, 2001; Jiang *et al.*, 2003; Matthies, 1997). This is supported by the present results that demonstrated that *Rhamphicarpa*-infected host plants distributed more biomass to the roots at the expense of the stems. The relative increase in biomass distributed to the roots by infected plants was previously hypothesized to make carbon available for parasite withdrawal, which is usually followed by an increase in parasite biomass (Hibberd *et al.*, 1998; Watling & Press, 2001). The current research supports this hypothesis, as, at 42 DAS, the first notion of a significant change in biomass partitioning of the host, coincided with a doubling of parasite biomass grown in presence of a host compared to host-less parasites. Since at this stage, biomass of the parasites is relatively small compared to that of the host, already small uptakes of host plant assimilates will be reflected in considerable increases in parasite biomass, as was observed here. Moreover, it is evident that these small withdrawals may not directly result in significant changes in host plant biomass. Gradually, the parasite may obtain a stronger influence on the physiology of its host plant. This was reflected in the present results showing significant changes in plant height at 64 DAS, followed by significant and consistent changes in biomass, leaf area and tiller number, noticed at 71 DAS.

In this study we clearly demonstrate that an observed parasite induced reduction of host plant biomass (22–71%) and host plant kernel production (78–100%) is originating first from reductions in host-plant light interception, followed by reductions in host-plant LUE. Reductions in light interception are caused by reduced

green leaf area and this is probably the result of the common source–sink relations previously observed in host–parasite interactions, whereby the parasitic plant is the external sink that draws assimilates, minerals, and water from the host, and whereby the parasite consumes more resources than any organ of the host itself (Shen *et al.*, 2006). The reductions in LUE can stem from reduced functionality of the photosynthetic apparatus of the host plant which in turn can have several causes, as previously mentioned. In further studies with *R. fistulosa*, the assimilate flow from host to parasite and the pathological effects should be investigated, both quantitatively – to see how important resource extraction is – as well as qualitatively – to understand which nutrients and growth regulators are primarily involved.

The considerable parasite-inflicted damage depicted by rice as a host showed that rice cultivar, IR64 is considerably susceptible to *R. fistulosa* infection. Yet, *R. fistulosa* had a high propensity to virulence on its host. By definition, parasite virulence is the rate at which a host becomes infected and the level of damage inflicted to the host by the parasite (Gandon *et al.*, 2002). A recent study by Rodenburg *et al.* (2016) confirmed varietal differences of rice cultivars in infection level and yields in fields infested by *R. fistulosa*. A number of useful rice varieties were identified. It was also suggested that varietal differences in *R. fistulosa* biomass could be used to select for resistance, while low *R. fistulosa*-inflicted yield loss could be the basis for selection of tolerant lines. *Striga* tolerance is based on reduced negative impacts on host photosynthesis in some host (like rice) varieties. Whether this is also true for *R. fistulosa* could be subject to further studies. The use of resistant and tolerant cultivars could be an effective and inexpensive component of an integrated *R. fistulosa* management strategy. The only problem sometimes encountered is that both farmers and consumers usually have their varietal preferences regarding rice grain characteristics like taste and size.

In conclusion, our results show that even relatively low infestation levels of *R. fistulosa* severely reduce the biomass of the rice host. Once the parasite succeeds to infect the host, it eventually manipulates its host in such a way that the rice plant starts to fully produce to the benefit of the parasite. Preventing the establishment of a connection between host and parasite thus seems a key for avoiding yield reduction. The good news is that the facultative nature of the parasite ensures that emergence is preceding infection. As our research shows that it takes around 6 weeks before the first symptoms of parasite infection are noticed, there seems to be a sufficiently long time gap at the start of the rice growing season to control the parasite and to prevent severe damage to the crop.

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