

Fertilization benefits the facultative parasitic plant *Rhamphicarpa fistulosa* while gains by the infected host *Oryza sativa* are marginalized

Jonne Rodenburg^{1,*}, Ruben Dümmer^{2,3,†}, Yi-Han Ho^{1,4} and Lammert Bastiaans^{2,‡}

¹Natural Resources Institute, University of Greenwich, Central Avenue, Chatham Maritime, Kent ME4 4TB, UK, ²Centre for Crop Systems Analysis, Wageningen University & Research, P.O. Box 430, 6700 AK, Wageningen, the Netherlands, ³Van Hall Larenstein - University of Applied Sciences, Agora 1, 8934 CJ, Leeuwarden, the Netherlands and ⁴Taiwan Agricultural Research Institute, Council of Agriculture, 189 Zhongzheng Rd, Wufeng District, Taichung City 413008, Taiwan

[†]Present address: Van Hall Larenstein - University of Applied Sciences, Agora 1, 8934 CJ, Leeuwarden, the Netherlands

[‡]Present address: Taiwan Agricultural Research Institute, Council of Agriculture, 189 Zhongzheng Rd, Wufeng District, Taichung City 413008, Taiwan

*For correspondence. E-mail j.rodenburg@gre.ac.uk

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- **Background and Aims** *Rhamphicarpa fistulosa* (Hochst.) Benth. is an annual facultative parasitic plant adapted to hydromorphic soils. In sub-Saharan Africa it causes high crop losses as a weed in rainfed lowland rice (*Oryza sativa* L.). Fertilizers are often proposed as a control measure against hemiparasitic weeds, but an understanding of the nutrient effects on *R. fistulosa* is currently still elusive.
- **Methods** In two greenhouse pot experiments, conducted in 2016 in the Netherlands and in 2019 in the UK, host plants (*O. sativa*, cv IR64) and parasitic plants (*R. fistulosa*) were grown alone or combined and were subjected to different levels of nutrient availability. Biomass measurements were used to assess whether and how effects of nutrient availability are expressed in the host and parasite.
- **Key Results** Compared with parasite-free host plants, the biomass of parasite-infested plants was severely reduced, and nutrient effects on host plant biomass were less pronounced. Conversely, increased nutrient availability did not have an effect on parasitic plants when grown alone, but when grown with a host the parasitic plant biomass increased proportionally. Grown together, the combined biomass of host plant and parasite was substantially lower than that of the host plant grown alone. The ratio of biomass between host plant and parasite was unaffected by nutrient availability.
- **Conclusions** Fertilization benefits to rice plants are severely reduced but not completely nullified by *R. fistulosa* infection. The benefits to production and reproduction accrued by the parasite from increased nutrient availability are restricted to conditions in the presence of a host plant. Host presence and nutrient effects are thus observed to be synergetic; *R. fistulosa* plants parasitizing a suitable host respond strongly to increasing levels of nutrients. This is associated with an increased root biomass of the parasitic plant itself, but is more likely to result from exploitation of the nutrient uptake capacity of the host plant it parasitizes.

Key words: rice vampireweed, rice, *Rhamphicarpa fistulosa*, *Oryza sativa*, host–parasite interactions, hemiparasitism, parasitic weeds, fertilizer.

INTRODUCTION

Root parasitic plants parasitize other plants to fulfil (part of) their life cycle or to increase their fitness (Shen *et al.*, 2006). They attach to their host by means of a haustorium, a unique organ that forms a physiological bridge between the parasite and host vasculature (Joel, 2013). Parasitism has developed to varying degrees of dependence on the host plant (Westwood *et al.*, 2010). Achlorophyllous holoparasites have no leaf chlorophyll and are thus completely dependent on a host for their carbon acquisition. These parasites feed primarily on phloem. Chlorophyllous obligate hemiparasites depend fully on a host during the underground stages, but only in part during the above-ground stages, owing to the presence of leaf chlorophyll that enables them to assimilate part of their carbon needs themselves (Estabrook and Yoder, 1998). An even more independent

group of parasitic plants are the facultative hemiparasites. They can fulfil their life cycle independently, but whenever a suitable host plant grows in their vicinity they will parasitize it (Matthies, 1997). In comparison to independently growing counterparts, facultative parasitic plants that parasitize a host show more vigorous growth and seed production (Klaren and Janssen, 1978; Jiang *et al.*, 2003; Kabiri *et al.*, 2016). All hemiparasitic plants feed predominantly on xylem.

Across the range of parasitism types, root parasitic plants can have a large impact on the performance of their host. Worldwide, ~50 root parasitic plant species attack crops, and less than one-third of these are known as weeds causing notable socio-economic impact (Parker, 2013). A prime example of an impactful parasitic weed is *Rhamphicarpa fistulosa* (Hochst.) Benth (Ouédraogo *et al.*, 1999). This facultative parasitic plant has a broad host range but is most often observed on cereal

crops, particularly rice, as suggested by its vernacular name, rice vampireweed (Rodenburg et al., 2015). The species is being observed increasingly as a weed in rainfed lowland rice production systems in sub-Saharan Africa, where it causes high agronomic and economic losses (Rodenburg et al., 2016b). Rice plants infected by *R. fistulosa* show strongly reduced above-ground biomass production, light interception and light use efficiency (Kabiri et al., 2017), resulting in 24–73 % yield losses at the crop level, depending on the resistance and tolerance level of the cultivar (Rodenburg et al., 2016a). A range of other facultative parasitic plants are known to be weedy, including *Aeginetia indica* Roxb., *Buchnera hispida* Buch.-Ham. ex. D.Don, *Odontites verna* (Bell.) Dum. (syn. *Bartisia odontites*), *Melampyrum arvense* L., *Rhinantus minor* L. and *Rhinantus angustifolius* C. Gmelin (syn. *Rhinantus serotinus*), but none of them is known to threaten food security at a comparable scale and increment to *R. fistulosa* (Parker, 2013).

Early work on *R. fistulosa* suggested that this parasitic weed is an indicator for poor soil fertility (Porteres, 1948). Reports from rice-growing environments in West Africa confirmed that *R. fistulosa* is primarily a problem of poorly fertile soils (N'Cho et al., 2014) and that the weed could be suppressed by application of fertilizer (Rodenburg et al., 2011). This would corroborate observed associations of other facultative parasitic plants with low soil nutrient availability, such as *Odontites verna* (Bell.) Dum. in wheat (Moss et al., 2004) and *Rhinanthus minor* L. in pastures (Smith and Cox, 2014). Applications of fertilizer (in particular, nitrogen) have been shown to control *Striga hermonthica* (Del.) Benth. and *Striga asiatica* (L.) Kuntze (e.g. Farina et al., 1985; Raju et al., 1990; Jamil et al., 2012), related but obligate hemiparasitic weeds in tropical cereal production systems. Recently, however, it was shown that *R. fistulosa* has an affinity with high soil potassium contents (Houngbedji et al., 2020) and available phosphorus and organic matter contents, and that fertilizer application had a stimulating rather than suppressive effect on this facultative parasitic weed in rice in the field (Tippe et al., 2020).

To enhance our understanding of the effects of nutrient availability on *R. fistulosa* and the interaction with its host, we conducted experiments in semi-controlled conditions. The effect of increased nutrient rates on pure stands of host plant and parasite and on the host plant–parasite association were studied. The specific objectives of this study were as follows: (1) to investigate whether negative effects of parasitism on host biomass accumulation can be compensated by increased nutrient levels;

and (2) ascertain whether biomass and reproduction of the facultative parasite *R. fistulosa* are suppressed or stimulated by increased nutrient availability.

MATERIALS AND METHODS

Locations, germplasm and treatments

Two independent greenhouse pot experiments were conducted. The first pot experiment (experiment 1) was conducted in 2016 at Wageningen University (WUR) in Wageningen, the Netherlands, and the second (experiment 2) in 2019 at the Natural Resources Institute (NRI), University of Greenwich in Chatham, UK, both between April and late August. In both experiments, rice (*Oryza sativa* L., subspecies *indica*, variety IR64) was used as the host plant species and rice vampireweed (*Rhaphicarpa fistulosa* [Hochst.] Benth., ecotype from Kyela, Tanzania, collected in 2010) as the parasitic plant species. Rice vampireweed is an annual facultative parasitic plant, distributed in tropical Australia and sub-Saharan Africa, with average seed sizes of 0.2 mm × 0.55 mm (Rodenburg et al., 2015). The time from germination to the production of the first mature seeds is ~100 days (Kabiri et al., 2016). Seeds of both plant species were sourced from Tanzania through the Africa Rice Center.

Each experiment included three plant treatment levels: (1) rice grown alone (one rice plant per pot); (2) *R. fistulosa* grown alone (ten *R. fistulosa* plants per pot); and (3) rice grown together with *R. fistulosa* (one rice plant, ten *R. fistulosa* plants). Experiments involved a nutrient application treatment, with the equivalent of 0, 25, 50 or 100 % of the recommended fertilizer application rate for lowland rice as provided by Wopereis et al. (1999). Experiment 1 had two additional fertilizer treatment levels, at 150 and 200 %. The quantity and composition of the 100 % nutrient treatment, expressed as the weight (in grams) of essential plant nutrients per pot, is provided in Table 1.

Experiment 1 (WUR)

A first experiment, using a randomized complete block design in five blocks (replicates), with three plant levels and six fertilizer application levels as treatments (total of 90 pots), was conducted in a greenhouse of Wageningen University

TABLE 1. Nutrient application of the 100 % treatment in each experiment (experiment 1 at WUR, experiment 2 at NRI) in grams per pot (macronutrients) or milligrams per pot (micronutrients), using an equivalent of 120 kg N ha⁻¹ as benchmark (grey). Other nutrients varied between experiments, depending on fertilizer composition.

Macronutrient	Experiment 1	Experiment 2	Micronutrient	Experiment 1	Experiment 2
NO ₃	0.392	0.230	Mn	0.992	0.823
NH ₄	0.038	0.169	B	0.488	0.206
N (urea)	–	0.082	Cu	0.072	0.206
Sum of N	0.430	0.481	Mo	0.108	0.021
P (P ₂ O ₅)	0.070	0.206	Zn	0.739	0.206
K (K ₂ O)	0.636	0.206	Fe	4.412	2.469
Mg	0.100	0.041	–	–	–

(51°59'4.40"N, 5°39'56.77"E). Screens were used to create 12 h day length (08:00–20:00 h). Air temperature was on average 27 °C during the day and 23 °C during the night. Humidity varied from 60 to 80 %. When daytime light intensity outside the greenhouse dropped below 910 $\mu\text{mol m}^{-2} \text{s}^{-1}$, high-pressure sodium lamps (SON-T Agro, 400 W, Philips) switched on automatically for complementary lighting. Plants were grown in 6 L pots, filled up to 80 % (2–3 cm below the rim) with a mixture of 50 % sand and 50 % arable soil, from the experimental farm Droevendaal (BIO-5), with a total nitrogen content of 1.17 g per pot (~0.018 %).

Rice seeds were pregerminated on a moistened filter paper in Petri dishes for 48 h. Seeds were then planted in the middle of the pots. At rice planting, 100 seeds of *R. fistulosa* (germination rate: 41 %) were mixed with sand, then spread out evenly over the soil surface of each pot that was given this treatment. *Rhamphicarpa fistulosa* seedlings were later thinned to attain ten plants per pot. To ensure that *R. fistulosa* seedlings already infecting the host were not uprooted, thinning was done systematically and selectively. Uprooting started with plants growing furthest away from the host plant, gradually moving closer to the host, and prioritized smaller over larger plants. From experience, parasites growing closest to the host plant are the first to infect the host, and parasitizing plants can be distinguished from autotropic plants based on clear size differences.

Plants were watered on a daily basis to keep the potted soils at or near saturated conditions. A nutrient solution (see composition in Table 1) was supplied once every week to arrive at the nutrient treatment levels mentioned above.

Experiment 2 (NRI)

A second experiment, using a split-plot design in six replicates, with a fertilizer treatment at four steps on the plot level and a plant treatment at three steps on the sub-plot level (total of 72 pots), was conducted in a greenhouse at the Medway campus of the University of Greenwich, Chatham Maritime, UK (51°23'50.7"N, 0°32'28.0"E). The average air temperature was 30 °C, and humidity ranged from 45 to 50 %. Supplementary lighting was provided daily (06:00–18:00 h), at 550 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Plants were grown in 6 L pots (radius: 10.4 cm; height: 18 cm), filled up to 80 % (2–3 cm below the rim) with a mixture of 50 % sand (Horticultural Sharp Sand, Melcourt, UK) and 50 % arable soil (Meadowmat Low Fertility Soil, Harrowden Turf Limited, UK), with a total nitrogen content of 1.08 g per pot (~0.023 %). Pots were sealed at the bottom to avoid drainage.

Rice seeds were pregerminated and planted as explained for experiment 1 (on 26 April). Ten days before rice sowing, *R. fistulosa* seeds (germination rate: 26 %) were sown as explained for experiment 1, but at a higher rate (150 seeds per pot) to compensate for the lower germination rate. Thinning to ten *R. fistulosa* plants per pot was done as described for experiment 1. Plants were watered every 2 days, to keep the potted soils around saturated conditions. A nutrient solution (Hard Water 211, ICL Specialty Fertilizers, UK; for composition, see Table 1) was supplied three times, providing 40 % of the total fertilizer dose at 12 days after rice sowing (DAS) and 40 DAS, and 20 % at 60 DAS, to arrive at the fertilizer treatment levels mentioned above.

Observations and measurements

Rice plant tiller numbers were counted at 95 DAS (experiment 1) and 94 DAS (experiment 2). *Rhamphicarpa fistulosa* plant height was measured at 109 DAS (experiment 1) and 108 DAS (experiment 2) on all ten plants and averaged. *Rhamphicarpa fistulosa* seed capsules were removed upon ripening, on a daily basis, from all ten plants growing in a pot. Removed capsules were air-dried for 3 weeks and weighed. Upon maturity, opening and drying, seed capsules harvested in experiment 2 were also sieved using analytical sieves (mesh sizes: 500 μm to 1 mm) to extract and weigh seeds. All plants were harvested destructively at 122 DAS, when the majority of host and parasitic plants reached physiological maturity. Above-ground biomass dry weights were obtained for both the host and the parasite (again, all ten plants growing in a pot), whether growing alone or in combination. Plants were cut off at the soil surface level. For rice, the stem and leaves were separated before drying. In experiment 1, biomass dry weights of roots of *R. fistulosa* (all plants growing in a pot) and rice were assessed. These were obtained after carefully washing the root systems with water until all non-root materials were removed. In pots where rice and *R. fistulosa* were grown together, the roots of both species were separated following methods explained by Kabiri et al. (2017).

All biomass dry weights were assessed after drying the plant materials at 70 °C for 48 h in a plant and soil drying oven and weighing on a digital laboratory bench-top weighing scale. The fraction of plant root biomass out of the total plant biomass (henceforward referred to as root fraction) was calculated as the below-ground host or parasite biomass dry weight divided by the sum of the below- and above-ground host or parasite biomass dry weight, obtained from all plants growing in a pot.

Statistical analyses

Data analyses were done in R v.3.5.1 for Mac (Urbanek et al., 2016). Data analyses were performed for each experiment separately, considering differences in environmental conditions and nutrient compositions of the fertilizer treatments. Host plant and parasite height and biomass (both above-ground and roots) were analysed using a linear analysis of covariance (ANCOVA) model, with treatment (host alone; parasite alone; host and parasite) as the three-level categorical factor and nutrient dose as a covariate. The ANCOVA was done using R script `ancovas.R`, to compare the adjusted means of parameters of the host plant grown with or without the parasite and parameters of the parasite grown with or without a host plant (e.g. McDonald, 2014; Mangiafico, 2015). Host–plant tiller numbers were analysed with a generalized linear model (GLM), which had a log link and quasi-binomial errors, to allow for overdispersion. The GLM had a categorical factor with two levels (host alone; host and parasite) and nutrient level as a covariate. The relationship between total biomass (host and parasite) and parasite biomass used a linear regression model with no intercept and was based on mean values. Pearson correlation analyses were conducted between all parasite (*R. fistulosa*) and host (*O. sativa*) parameters.

RESULTS

Plant nutrition effects on the host with and without a parasite

Host plant stem, leaf and total shoot biomass was significantly affected by nutrient and parasite treatments in both experiments (Table 2). Both in the absence and presence of the parasite, above-ground host biomass increased with nutrient level, and at any nutrient level the host biomass was significantly higher

in the absence than in the presence of the parasite (Figs 1 and 2). Significant nutrient by parasite-presence interaction effects on stem, leaf and total host biomass were observed (Table 2). The difference between biomass dry weights of parasite-free and parasite-infested host plants diverged with increasing nutrient rates. Even at the highest nutrient rate, parasite-infested hosts did not produce biomass levels comparable to parasite-free hosts in the absence of plant nutrition (Figs 1 and 2). The combined dry weight of the host plant and parasite in

TABLE 2. ANCOVA output (F-values) for the treatment effects (nutrient supply, parasite presence and their interaction) on host biomass dry weight (dw) of stem, leaves, total shoot (Stem dw, Leaf dw, Shoot dw), roots, and roots as a fraction of total biomass (Root dw, Root frac.), and tiller numbers (Tiller #); d.f. = degrees of freedom.

Source of variation	Experiment 1							Experiment 2				
	d.f.	Stem dw	Leaf dw	Shoot dw	Tiller #	Root dw	Root frac.	d.f.	Stem dw	Leaf dw	Shoot dw	Tiller #
Nutrient (N)	1	255.01	290.78	331.74	69.58	56.92	1.76	1	93.57	62.17	73.81	87.71
Parasite presence (P)	1	1061.50	228.55	822.63	18.79	98.13	113.37	1	237.30	271.47	272.53	60.72
N × P	1	81.44	15.54	61.44	0.0008	0.59	1.23	1	6.44	5.92	5.82	0.54
Residuals	56							44				

White cells: not significant; light grey cells: significant at $P < 0.05$; dark grey cells: significant at $P < 0.001$.

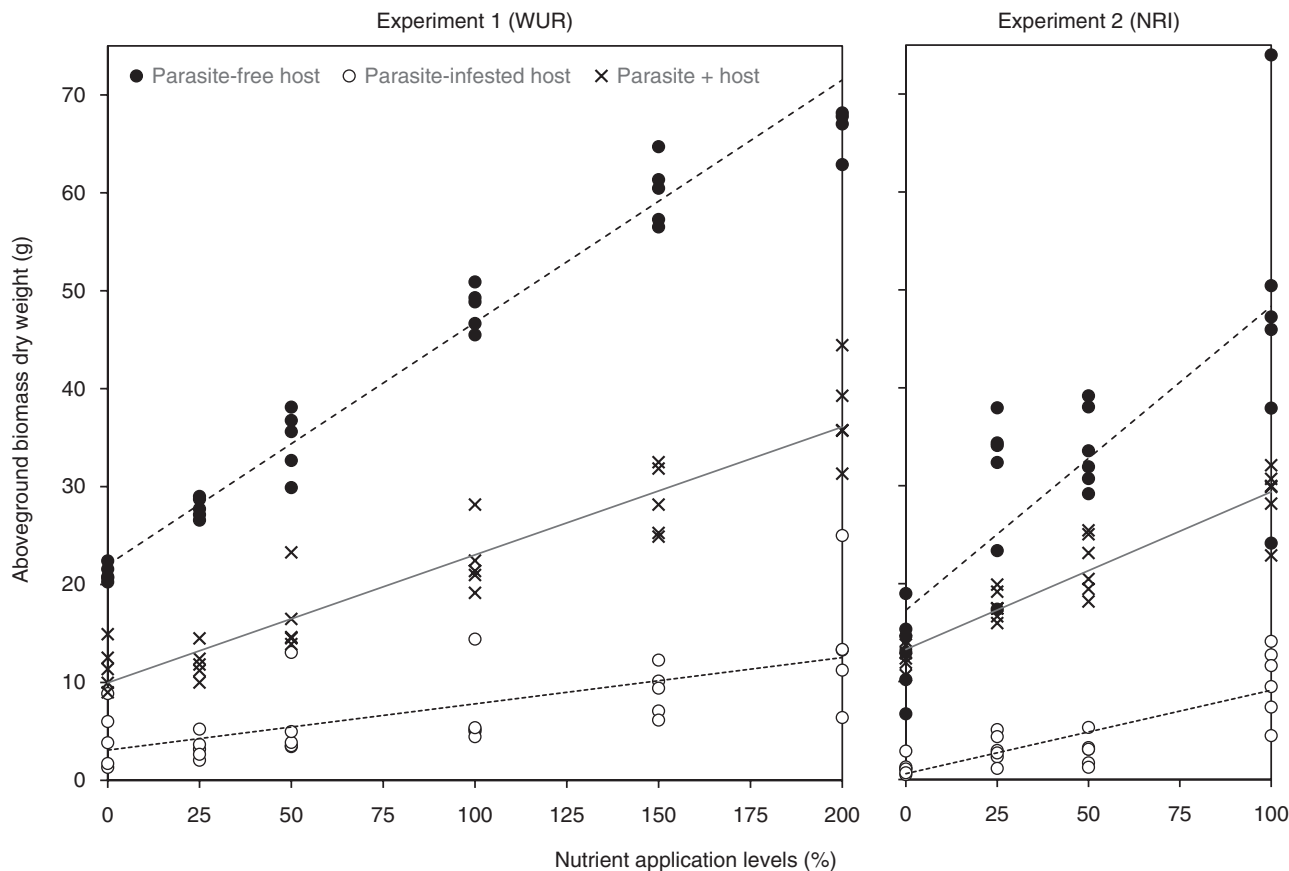


FIG. 1. Total above-ground biomass dry weights of parasite-free and parasite-infested host plants and of the parasite-infested host plant combined with its parasites (parasite + host) as a function of nutrient application levels as observed in experiment 1 (left) and experiment 2 (right). In both experiments, the intercept ($P < 0.001$) and the slopes (experiment 1: $P < 0.001$; experiment 2: $P < 0.05$) of the regression lines of the host-only biomass dry weights are significantly different.

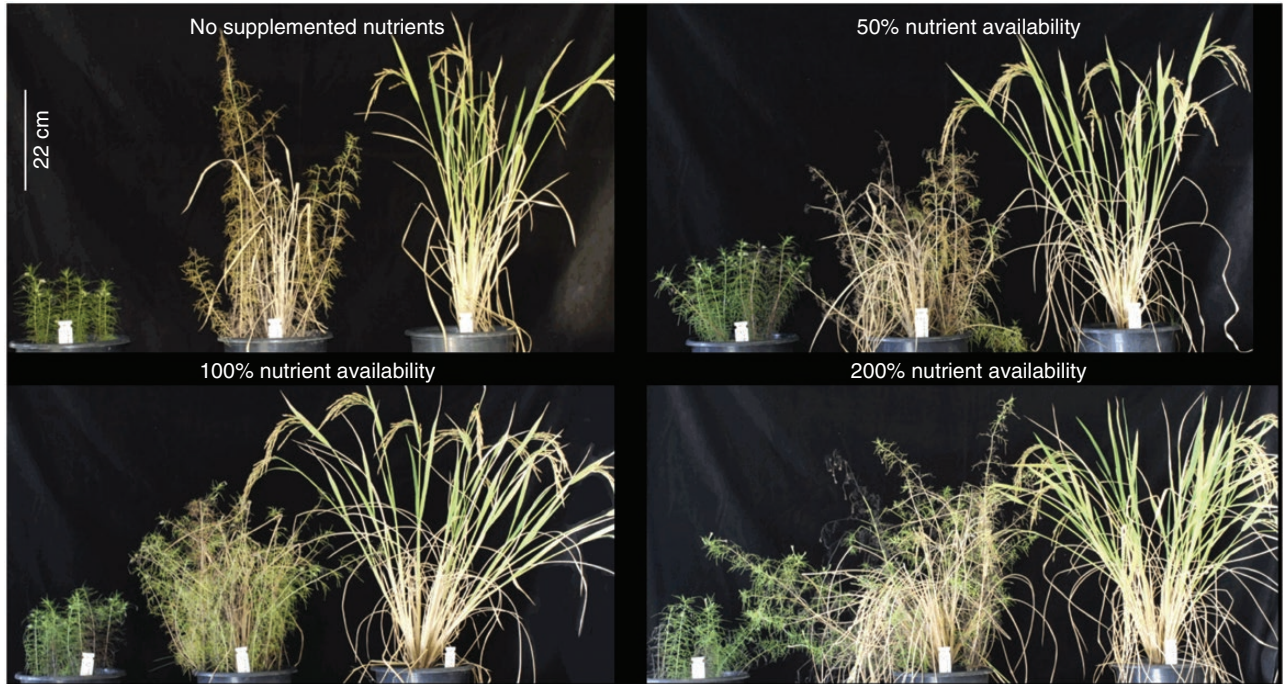


FIG. 2. Photographs of parasite (left) and host (right) plants growing alone and together (middle) following 0 (no supplemented nutrients), 50, 100 and 200 % nutrient application levels, in experiment 1 at 120 days after sowing.

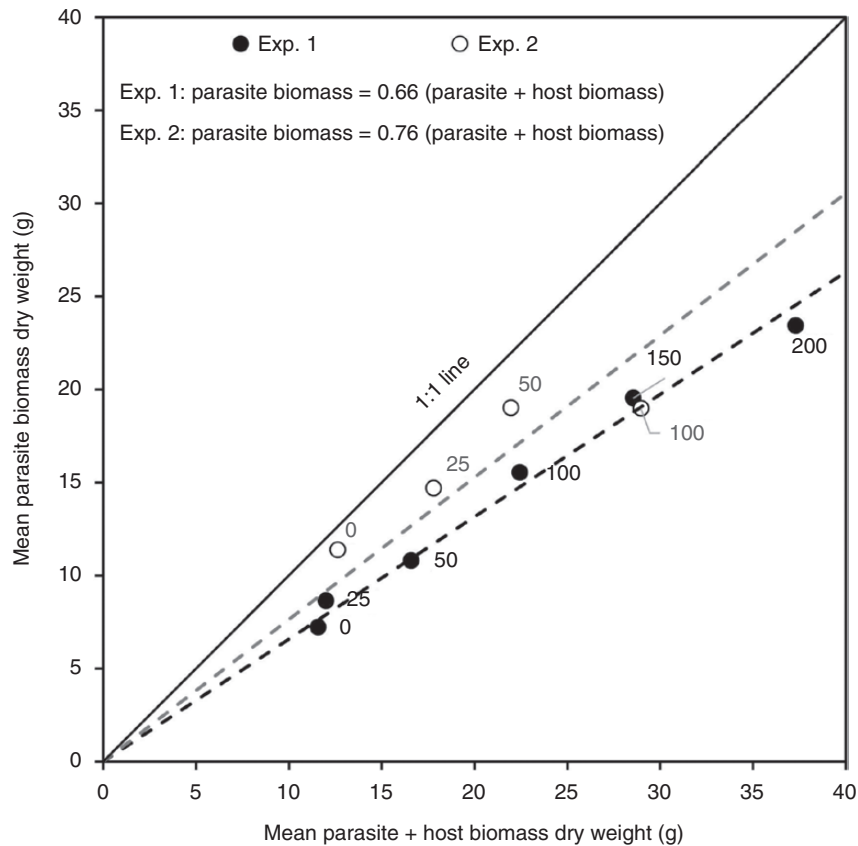


FIG. 3. The relationship between total (parasite + host) and parasite biomass dry weight, when host and parasite were grown together at different nutrient application levels (labels 0–200) for experiment 1 (filled symbols; $R^2 = 0.999$) and experiment 2 (open symbols; $R^2 = 0.991$).

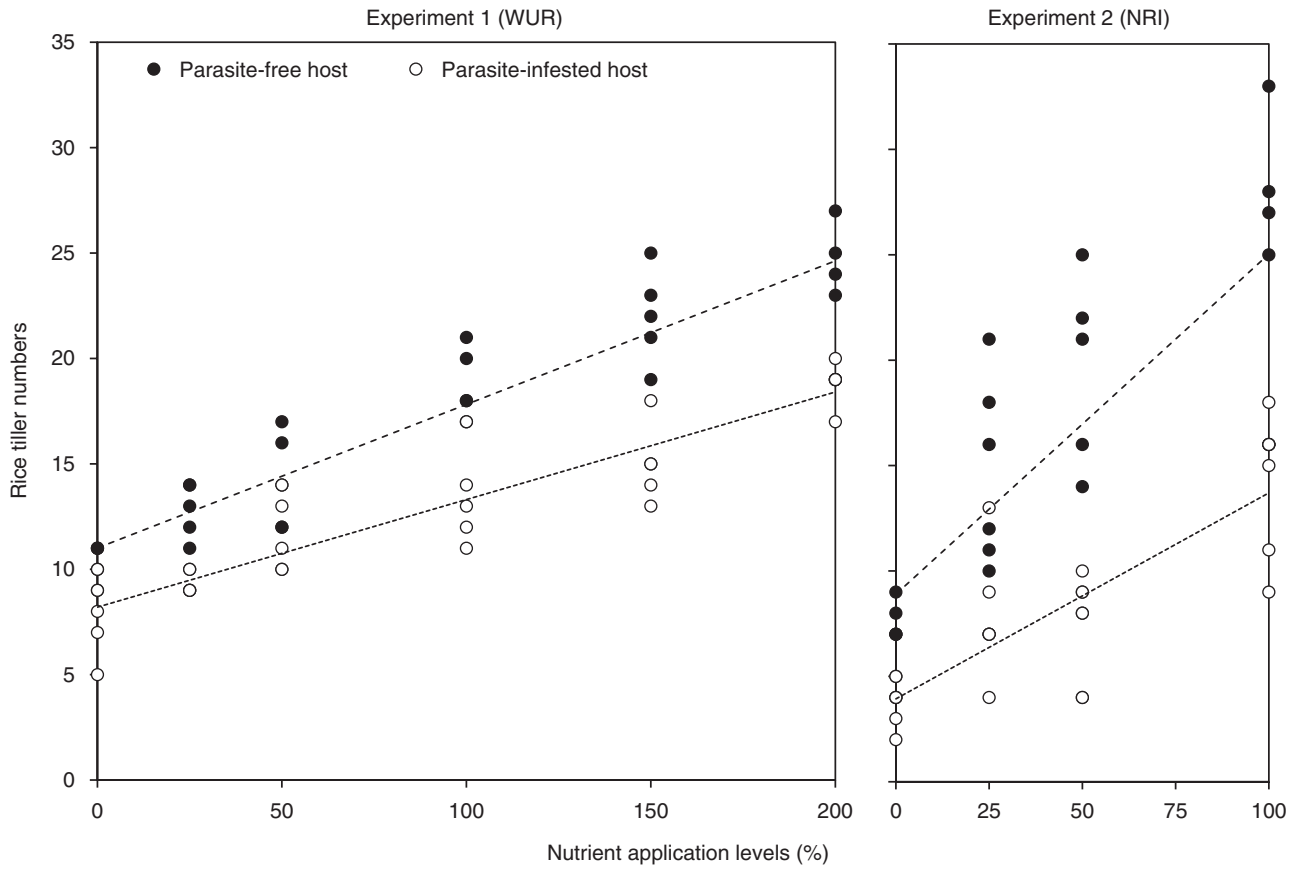


FIG. 4. Host (rice) plant tiller numbers (at 95 DAS for experiment 1 and 94 DAS for experiment 2) of parasite-free and parasite-infested plants as a function of nutrient application levels as observed in experiment 1 (left) and experiment 2 (right). In both experiments, the intercepts are significantly ($P < 0.001$) different.

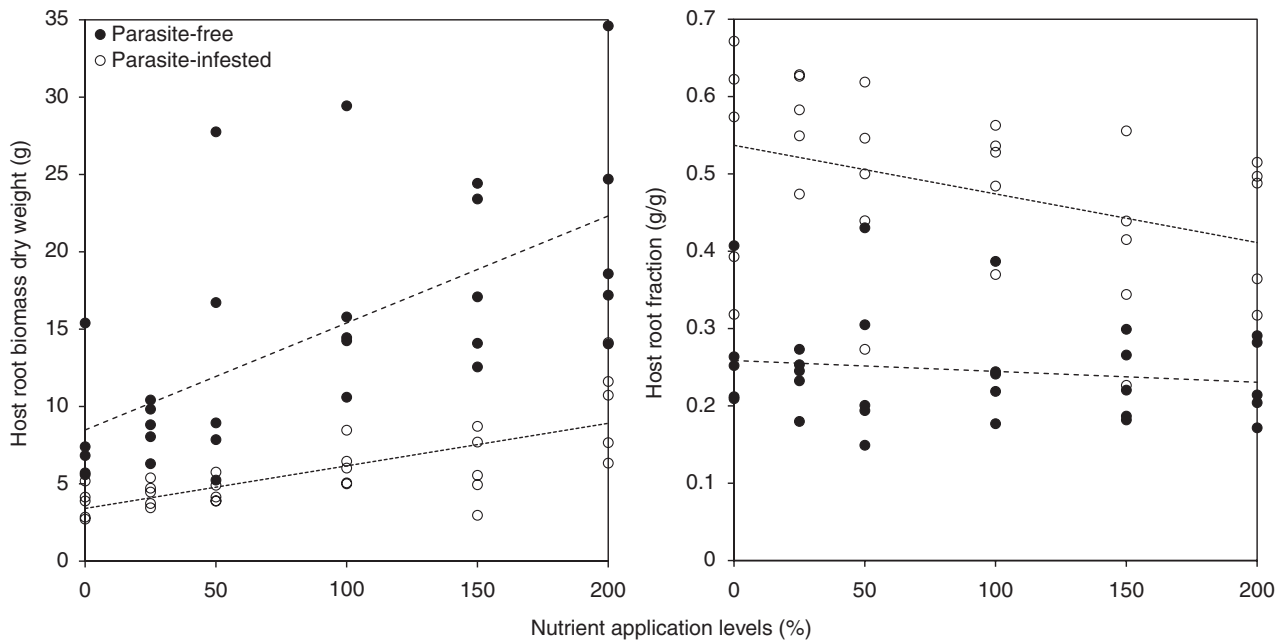


FIG. 5. Host (rice) root biomass dry weights and fraction of root to total biomass of parasite-free plants and parasite-infested plants as a function of nutrient application levels as observed in experiment 1. For both the root biomass dry weight and the root fraction, only the intercept of parasite-free and parasite-infested plants is significantly ($P < 0.001$) different.

TABLE 3. ANCOVA output (F-values) for the treatment effects (nutrient supply, host presence and their interaction) on parasite biomass dry weight (Shoot dw), capsule dry weight (Capsule dw), mean parasite plant height (Height), parasite root biomass (Root dw) and roots as a fraction of total biomass (Root frac.). The last data were arcsin-transformed before analyses; d.f. = degrees of freedom.

Source of variation	Experiment 1					Experiment 2				
	d.f.	Shoot dw	Capsule dw	Height	Root dw	Root frac.	d.f.	Shoot dw	Capsule dw	Height
Nutrient (N)	1	227.15	60.07	4.917	25.89	4.96	1	13.27	0.21	0.54
Host presence (H)	1	968.49	275.08	395.657	134.78	264.45	1	786.86	67.82	358.21
N × H	1	215.56	54.70	10.263	53.54	1.82	1	0.01	0.26	0.49
Residuals	56						44			

White cells: not significant; light grey cells: significant at $P < 0.05$; intermediate grey cells: significant at $P < 0.01$; dark grey cells: significant at $P < 0.001$.

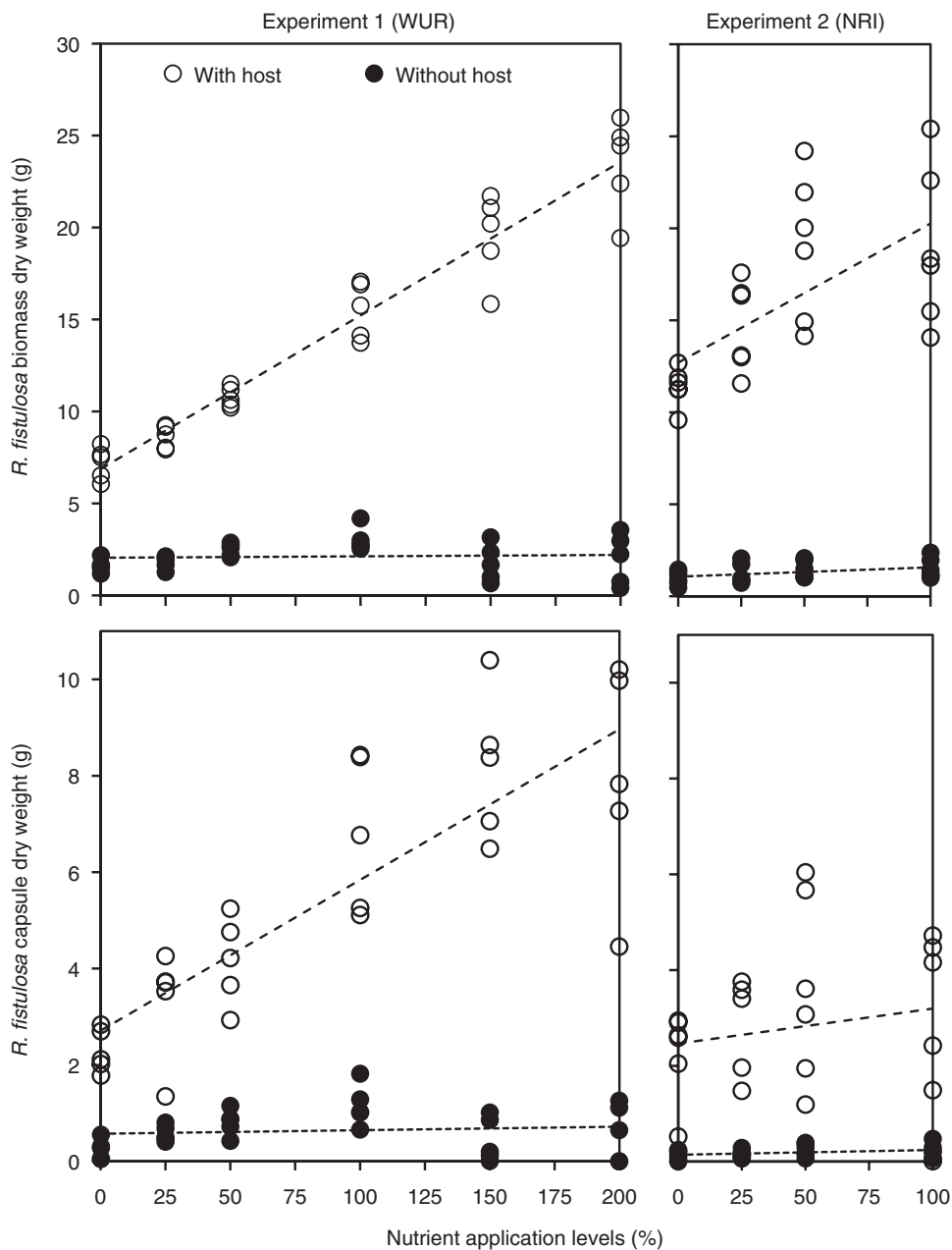


FIG. 6. Parasite total above-ground and seed capsule biomass dry weight production, when grown with or without a host, as a function of nutrient application levels as observed in experiment 1 (left) and experiment 2 (right). In both experiments, the intercept ($P < 0.001$) of the regression lines are significantly different. The slopes were significantly different only in experiment 1 ($P < 0.001$).

parasite-infested conditions was markedly lower than the host dry weight in parasite-free conditions (Figs 1 and 2). The ratio between host and parasite dry weight in parasite-infested conditions was hardly affected by the nutrient application rate. The parasite contributed 66–76 % to the combined host and parasite biomass (Fig. 3).

In both experiments, total host shoot biomass exhibited a highly significant correlation with host tiller numbers (Supplementary Data Table S1). Both nutrient application and parasite presence had highly significant effects on host plant tiller numbers in both experiments (Table 2; Fig. 4). Tiller number increased with nutrient application rate, whereas parasite infestation caused a reduction.

Host shoot biomass exhibited a highly significant correlation with host root biomass (Supplementary Data Table S1). Significant effects of nutrients and parasite presence were observed on host root biomass, whereas the fraction of root to total biomass was influenced only by parasite presence (Table 2). In absolute terms, the root biomass of parasite-free hosts was greater than that of parasite-infested hosts, and in both conditions the host plant root biomass increased with nutrient application (Fig. 5). In relative terms (fraction of root to total biomass), the host root biomass increased in the presence of the parasite (intercept) but was unaffected by increased nutrient availability.

Effects of nutrient availability on the parasite with and without a host

In experiment 1, significant nutrient by host-presence interaction effects were observed on above-ground *R. fistulosa* biomass, seed capsule dry weight and plant height, assessed on all ten plants growing in a pot (Table 3). All these parameters increased significantly with host plant presence. Following

increasing nutrient quantities, increases in parasite biomass, reproductive output and height were observed only when the parasites were grown together with the host plant (Fig. 6; Supplementary Data Fig. S1). In the absence of a host, the parasites remained relatively small, with a low reproductive output, and no nutrient-induced changes in growth, production and reproduction were observed (Fig. 6). Without supplemented nutrients, host presence increased mean parasite biomass 5-fold and reproductive output of the parasite 9-fold. In the presence of a host, nutrient application caused a >3-fold increase in vegetative and reproductive biomass of the parasite.

In experiment 2, no significant nutrient by host-presence interaction effects were observed on parasitic plants (Table 3). Main effects of nutrients and host presence were observed on above-ground parasite biomass production, while both parasite seed capsule weight and parasite plant height were significantly (positively) affected only by host plant presence (Fig. 6; Supplementary Data Fig. S1). Averaged over nutrient levels, host presence caused a 13-fold increase in mean parasite biomass and seed capsule production. Parasite shoot dry weight and parasite plant height exhibited a highly significant correlation with parasite seed capsule dry weight (both experiments) and with parasite seed dry weight (experiment 2; Supplementary Data Table S1). Parasite seed capsule dry weight exhibited a highly significant correlation with seed weight (Supplementary Data Table S1), hence the shoot and seed capsule weights presented above provide a reliable indication of seed production by the parasite.

Parasite shoot dry weight and parasite seed capsule dry weight both exhibited a highly significant correlation with parasite root dry weight (Supplementary Data Table S1). Similar to above-ground *R. fistulosa* biomass, significant nutrient by host-presence interaction effects were observed on root biomass of the parasite (Table 3; assessed only in

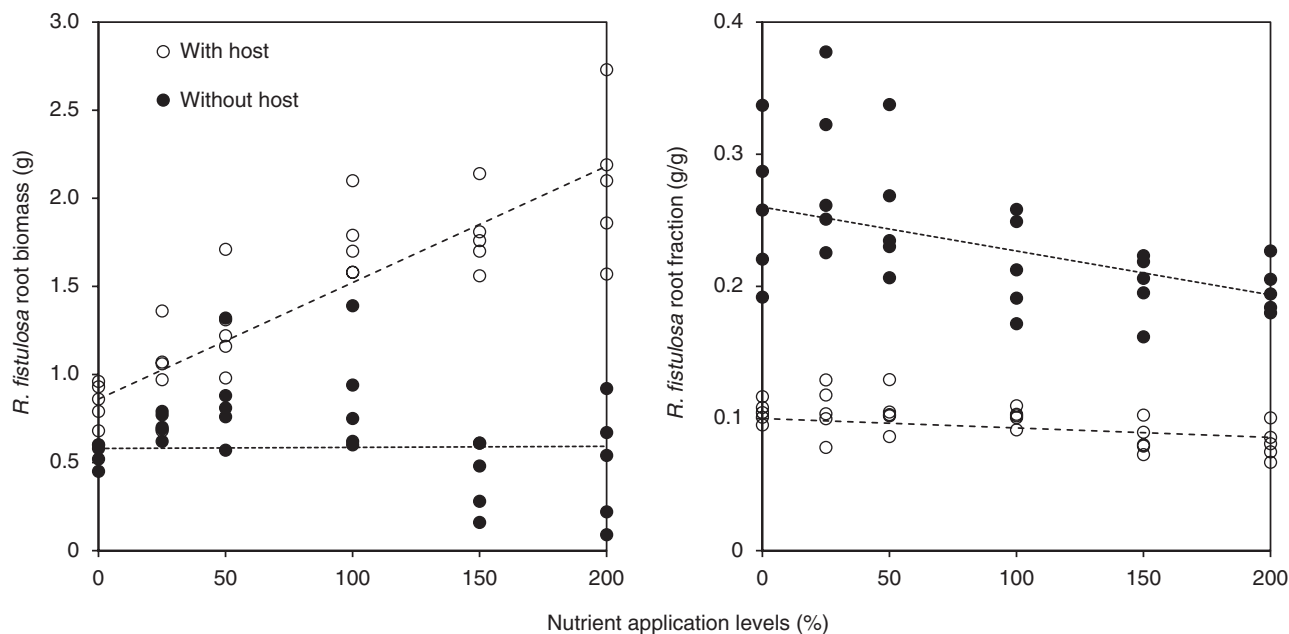


FIG. 7. Parasite root biomass and fraction of root to total biomass, when grown with or without a host, as a function of nutrient application levels as observed in experiment 1. In both cases, the intercepts of the regression lines are significantly different. The slopes were significantly different for root biomass ($P < 0.001$).

experiment 1). Root biomass of parasites in the presence of a host was always greater than in the absence of a host. In addition, in the presence of a host the parasite root biomass responded positively to nutrient application, whereas in the absence of a host there was no such response (Fig. 7). The parasite's root fraction (i.e. ratio root biomass to total biomass) was also affected by host plant presence (Table 3); compared with parasites growing alone, the root fraction was significantly smaller in presence of a host (Fig. 7). This implies that although both below- and above-ground parasite biomass increased significantly owing to host presence, the shoot biomass increased disproportionately more than the root biomass. To a lesser extent, the root fraction also decreased with increasing nutrient application levels, but this was not significantly affected by host presence.

DISCUSSION

The aim of this study was to enhance our understanding of the effects of increased nutrient availability on the facultative parasitic weed *R. fistulosa* and the interaction with its host, rice. This is both timely and relevant because the status of this parasitic plant species as a weed of tropical cereals has gained importance in sub-Saharan Africa in the last three decades (Houngbedji et al., 2014; Rodenburg et al., 2015; Schut et al., 2015).

Although application of fertilizer has, in the past, been proposed as a control measure against *R. fistulosa* (N'Cho et al., 2014; Rodenburg et al., 2011), recent insights obtained from field experiments cast doubts on the efficacy of fertilizers because parasitic plant biomass increased after fertilizer application (Tippe et al., 2020). Given that parasitic plant biomass is highly correlated with seed (capsule) production (Rodenburg et al., 2006), uninformed fertilizer application could contribute to the build-up of a parasitic weed seed bank and thereby increase weed problems and jeopardize crop productivity in the future.

Compensating parasitic effects on the host by increased nutrient availability

The parasite had severe negative effects on host plant performance, as expressed in changes in host biomass accumulation of both above- and below-ground parts. Based on root fraction, the above-ground parts of the host plant were relatively more negatively affected than the below-ground parts. This was observed before, with rice plants infected by the obligate hemiparasite *S. hermonthica*, and was thought to be caused by a greater relative inhibition of shoot growth (Cechin and Press, 1994). The severe effects of *R. fistulosa* on its host observed here are in agreement with findings of previous studies (Rodenburg et al., 2016a; Kabiri et al., 2017), including studies on other facultative parasites and hosts, e.g. sorghum infested by *Buchnera hispida* (Nwoke and Okonkwo, 1974) and barley infested by *Rhinanthus minor* (Jiang et al., 2004). The loss in host plant biomass attributable to parasitism was not compensated by the gain in parasite biomass, because the overall above-ground plant biomass of the two plant species growing together was always lower than that of the host when grown alone. This is a common phenomenon with facultative parasitic plants

(Matthies and Egli, 1999; Rodenburg et al., 2011) because they cause more damage to their hosts than merely the extraction of assimilates and nutrients. Indeed, *R. fistulosa* infection leads to severe reductions in host-plant photosynthesis, associated with reduced stomatal conductance, electron transport and overall leaf chlorophyll contents (Kabiri et al., 2021). Overall system productivity can approach parasite-free host productivity through improved nutrient availability, as shown in the present study, confirming observations on *Rhinanthus alectorolophus* by Matthies and Egli (1999).

Improved nutrient availability through fertilization improves the biomass of parasite-infested host plants but never attains the biomass levels of parasite-free hosts. Even at the highest nutrient application level (equivalent to 200 % of a recommended fertilizer application rate), the biomass of the parasite-infested host is lower than that of the parasite-free host without additional fertilizer. A recent report from the field confirms that with application of fertilizers, *R. fistulosa*-induced rice yield losses could be reduced but not completely compensated (Tippe et al., 2020).

Effects of increased nutrient availability on R. fistulosa with or without a host

Rhamphicarpa fistulosa growth, production and reproduction was boosted by host presence. The positive host effect was observed previously with *R. fistulosa* (Kabiri et al., 2016) and with other facultative parasites, such as *Rhinanthus minor* (Jiang et al., 2003, 2004) and *Rhinanthus serotinus* (Klaren and Janssen, 1978). Consistent positive nutrient effects on parasite biomass were observed only in the presence of a host plant. Such fertilizer effects on attached parasites were observed previously in the field by Tippe et al. (2020). The present study showed that increased nutrient availability also results in increased parasite seed production. The observed positive effects of fertilizer on *R. fistulosa* contrast with the frequently observed effects of fertilizer on related parasitic weeds. Fertilizer applications are generally found to decrease infection levels of cereal crops by the obligate parasitic weed *S. hermonthica* (Jamil et al., 2012). The most studied and cited mechanism behind this is the reduced exudation of strigolactones by host roots following increased N and P nutrition (Jamil et al., 2011). Some strigolactones are known potent stimulants of striga seed germination. *Rhamphicarpa fistulosa* is, however, a facultative parasite, and seed germination of this species is independent of the presence of such stimulants (Kabiri et al., 2016). Another explanation for reduced striga infection levels following fertilizer application could be that macronutrient supply also increases post-attachment resistance in host plants (Mwangangi et al., 2023). It is not clear why improved host-plant nutrition does not enhance resistance against *R. fistulosa*.

In experiment 2, the biomass of independently growing plants showed only a slight increase following nutrient application, whereas in experiment 1 these plants showed no noticeable change. This minor difference in nutrient response observed between the two experiments could be attributable to differences in environmental conditions (e.g. light, temperature, humidity), but more importantly, to differences between supplied fertilizer types in terms of nutrients other than nitrogen. The fertilizers

of experiment 2 had a relatively higher concentration of phosphorus than the fertilizer used in experiment 1. Biomass accumulation of independently growing plants of another facultative parasite, *Rhinanthus minor*, was previously shown to benefit only from phosphorus fertilizer, whereas nitrogen and potassium had no effect (Seel et al., 1993). A similar response to phosphorus was observed before, with the facultative parasite *Pedicularis tricolor* growing without a host (Li et al., 2013). It is hypothesized that unattached parasites have a low nitrogen uptake and use efficiency (Seel and Jeschke, 1999) and that the phosphorus response results from the inherent low plant-available P contents of soils, the absence of mycorrhizal symbiosis, a high demand for P and a limited root system of the parasitic plant (Irving and Cameron, 2009). With our experiments, although both were conducted on relatively poor soils (1:1 soil-sand mixture), we could only confirm the latter to be the case for *R. fistulosa* i.e. parasitic plants produced relatively low root biomass, independent of nutrient levels.

The positive effect of host presence on general parasite performance and the additional nutrient effect can be explained by an increased resource uptake capacity by attached parasites, in comparison to independently growing parasites. The uptake capacity is increased because parasite root biomass of attached parasites is increased, as shown in the present study. This confirms previous findings on another facultative parasitic plant, *Rhinanthus minor*, that root growth of parasitizing plants doubles compared with autotrophic plants (Jiang et al., 2004). In addition, the parasite can exploit the root system of the host plant it parasitizes. Root hemiparasites are considered to take up water primarily through their hosts rather than directly by their own root system (Stewart and Press, 1990). It has been shown that roots of *Rhinanthus serotinus* have a high water uptake resistance, rendering direct water uptake less important than that through its host plant (Klaren and Van de Dijk, 1976). The present study showed that the root biomass of *R. fistulosa* is very small, and therefore it seems likely that water uptake for this species is mainly through the host. Together with the water, hemiparasitic plants will take up host-derived mineral nutrients and some carbon (Press and Whittaker, 1993; Seel and Jeschke, 1999; Shen et al., 2006). Parasitic individuals of *Rhinanthus serotinus* showed increased levels of nitrogen, phosphorus and potassium in comparison to unattached plants of this species (Klaren and Janssen, 1978), and the proportion of this nutrient gain can be relatively high, as shown with *Rhinanthus minor*, with 87 % of its nitrogen coming from the host (Jiang et al., 2004). Improved nutrient uptake through parasitism could therefore explain why *R. fistulosa* plants are benefitting more from supplemented nutrients when they are attached to a host plant, as shown in the present study.

Conclusions

This study showed that although increased nutrient availability increases parasite-free rice, this has no effect on host-free *R. fistulosa* plants. When growing with a host, *R. fistulosa* can accrue more vegetative and reproductive biomass following nutrient application, demonstrating the host dependence of the parasite for nutrient uptake from the soil. The parasite benefits from the increased carrying capacity of the host following

fertilization, whereas the gains from increased nutrient availability to the host are far from sufficient to compensate for the parasite-induced losses. Increased fertilization might not be an effective solution for rice crops infested by the parasitic weed *R. fistulosa* because it increases the reproductive output, which could worsen infestation rates in the following crop season.

SUPPLEMENTARY DATA

Supplementary data are available at *Annals of Botany* online and consist of the following.

Figure S1: parasite plant height, when grown with or without a host, as a function of nutrient application levels as observed in experiment 1 (left) and experiment 2 (right). Table S1: Pearson correlation coefficients between host (H), parasite (P) and host and parasite (H + P) parameters for experiments 1 and 2.

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