

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

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Running title: Fertilization benefits facultative parasitic plants more than their hosts

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ABSTRACT

- **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.). Fertilisers are often proposed as control measure against hemiparasitic weeds but understanding of 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 subjected to different nutrient availability levels. Biomass measurements were used to assess if and how nutrient availability effects are expressed in the host and parasite.
- **Key Results.** Compared to parasite-free host plants, the biomass of parasite-infested plants was severely reduced and nutrient effects on host plant biomass were less pronounced. Inversely, increased nutrient availability did not have an effect on parasitic plants when grown alone, but when grown with a host the parasitic plant biomass proportionally increased. Grown together, the combined biomass of host plant and parasite was substantially lower than that of the host plant grown alone. The ratio in 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 parasite's production and reproduction benefits accrued from increased nutrient availability are restricted to conditions in presence of a host plant. Host presence and nutrient effects are thus observed to be synergistic; *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 more likely resulting from exploitation of the nutrient uptake capacity of the host plant it parasitizes on.

Key words: rice vampireweed, rice, *Rhamphicarpa fistulosa*, *Oryza sativa*, host-parasite interactions, hemiparasitism, parasitic weeds, fertiliser

1. 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 dependency on the host plant (Westwood et al., 2010). Achlorophyllous holoparasites have no leaf chlorophyll and are thus completely depending on a host for their carbon acquisition. These parasites are primarily phloem-feeding. Chlorophyllous obligate hemiparasites depend fully on a host during the underground stages, but only partly during the aboveground stages owing to the presence of leaf chlorophyll enabling them to assimilate part of their carbon needs themselves (Estabrook & Yoder, 1998). An even more independent group of parasitic plants are the facultative hemiparasites. They can fulfill their life cycle independently but whenever a suitable host plant grows in their vicinity they will parasitize it (Matthies, 1997). Compared to independently growing counterparts, facultative parasitic plants that parasitize on a host show more vigorous growth and seed production (Kabiri et al., 2016, Jiang et al., 2003, Klaren & Janssen, 1978). All hemiparasitic plants are predominantly xylem-feeding.

Across the range of parasitism types, root parasitic plants can have a large impact on the performance of their host. Worldwide, around 50 root parasitic plant species attack crops, and less than a 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 increasingly observed as 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 aboveground 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 Don, *Odontites Verna* (Bell) Dum. (syn. *Bartisia odontites*), *Melampyrum arvense* L., *Rhinanthus minor* L. and *R. angustifolius* C. Gmelin (syn. *R. serotinus*), but none of them is known to threaten food security at a comparable scale and increment as *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 fertiliser application (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 & Cox, 2014). Fertiliser applications (nitrogen in particular) have shown to control *Striga hermonthica* (Del.) Benth. and *S. 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 fertiliser 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 under 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 to (1) investigate if negative effects of parasitism on host biomass accumulation can be compensated by increased nutrient levels and (2) ascertain if biomass and reproduction of the facultative parasite *R. fistulosa* is suppressed or stimulated by increased nutrient availability.

2. MATERIALS AND METHODS

2.1. 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, the United Kingdom, both between April and late August. In both experiments rice (*Oryza sativa* L., subspecies indica, variety IR64) was used as host plant species and rice vampireweed (*R. fistulosa* [Hochst.] Benth., ecotype from Kyela, Tanzania, collected in 2010), as 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 x 0.55 mm (Rodenburg et al., 2015). The time from germination to the production of the first mature seeds is around 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 further involved a nutrient application treatment, with the equivalent of 0, 25, 50 or 100% of the recommended fertiliser application rate for lowland rice as provided by Wopereis et al. (1999). Experiment 1 had two additional fertiliser treatment levels, at 150% and 200%. Quantity and composition of the 100% nutrient treatment, expressed in weight (g) of essential plant nutrients per pot, is provided in Table 1.

2.2. Experiment 1 (WUR)

A first experiment, using a randomized complete block design in 5 blocks (replicates), with three plant levels and six fertiliser application levels as treatments (total: 90 pots), was conducted in a greenhouse of Wageningen University (51°59'4.40"N and 5°39'56.77"E). Screens were used to create 12 hours day length (08:00-20:00). 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 $\mu\text{mol m}^{-2} \text{s}^{-1}$, highpressure sodium lamps (SON-T Agro, 400W, 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/pot (approx. 0.018%).

Rice seeds were pre-germinated on a moistened filter paper in petri dishes for 48 hours. Seeds were then planted in the middle of the pots. At rice planting, one hundred (100) seeds of *Rhamphicarpa fistulosa* (germination rate: 41%) were mixed with sand and 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 avoid *R. fistulosa* seedlings already infecting the host were 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 prioritised smaller over larger plants. From experience, parasites growing closest to the host plant are the first to infect the host, and parasitising plants can be distinguished from autotrophic 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.

2.3. Experiment 2 (NRI)

A second experiment, using a split-plot design in six replicates with a fertiliser treatment at four steps on the plot level and a plant treatment at three steps on the sub-plot level (total: 72 pots), was conducted in a greenhouse at the Medway campus of the University of Greenwich, Chatham Maritime, UK (51°23'50.7"N and 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), 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/pot (approx. 0.023%). Pots were sealed at the bottom to avoid drainage.

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

Table 1. Nutrient application of the 100% treatment in each experiment (Exp. 1 at WUR, Exp. 2 at NRI) in g/pot (macronutrients) or mg/pot (micronutrients), using an equivalent of 120 kg N/ha as benchmark (grey). Other nutrients varied between experiments, depending on fertiliser composition.

Macronutrients	Exp. 1	Exp. 2	Micronutrients	Exp. 1	Exp. 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 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			

2.4. Observations and measurements

Rice plant tiller numbers were counted at 95 (Exp. 1) and 94 (Exp. 2) DAS. *Rhamphicarpa fistulosa* plant height was measured at 109 (Exp. 1) and 108 (Exp. 2) DAS on all 10 plants and then averaged. *Rhamphicarpa fistulosa* seed capsules were removed upon ripening, on a daily basis, from all 10 plants growing in a pot. Removed capsules were air-dried for three weeks and weighted. Upon maturity, opening and drying, seed capsules harvested in Exp. 2 were also sieved using analytical sieves (mesh sizes: 500 μm - 1 mm) to extract and weight seeds. All plants were destructively harvested at 122 days after sowing (DAS), when the majority of host and parasitic plants reached physiological maturity. Aboveground biomass dry weights were obtained for both the host and the parasite (again, all 10 plants growing in a pot) whether growing alone or in combination. Plants were cut off at the soil surface level.

For rice, stem and leaves were separated before drying. In Exp. 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 in Kabiri et al. (2017).

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

2.5. Statistical analyses

Data analyses were done in R, Version 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 fertiliser treatments. Host plant and parasite height and biomass (both aboveground and roots) were analysed using a linear analysis of covariance model, with treatment (host alone, parasite alone, host and parasite) as the threelevel categorical factor, and nutrient dose as a covariant. Analysis of covariance (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. Mangiafico, 2015, McDonald, 2014). Host-plant tiller numbers were analysed with a generalised linear model (GLM), which had a log link and quasi-binomial errors, to allow for overdistribution. 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.

3. RESULTS

3.1. 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 absence and presence of the parasite, aboveground host biomass increased with nutrient level and at any nutrient level host biomass was significantly higher in absence than in presence of the parasite (Fig. 1 & 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 parasitefree 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 absence of plant nutrition (Fig. 1 & 2). The combined dry weight of host plant and parasite under parasite-infested conditions was markedly lower than the host dry weight under parasite-free conditions (Fig. 1 & 2). The ratio between host and parasite dry weight under parasite-infested conditions was hardly affected by nutrient application rate. The parasite contributed 66 to 76% to the combined host and parasite biomass (Fig. 3).

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

Source of variation	Exp. 1							Exp. 2				
	Df	Stem dw	Leaf dw	Shoot dw	Tiller #	Root dw	Root frac.	Df	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 x 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 $P < 0.001$

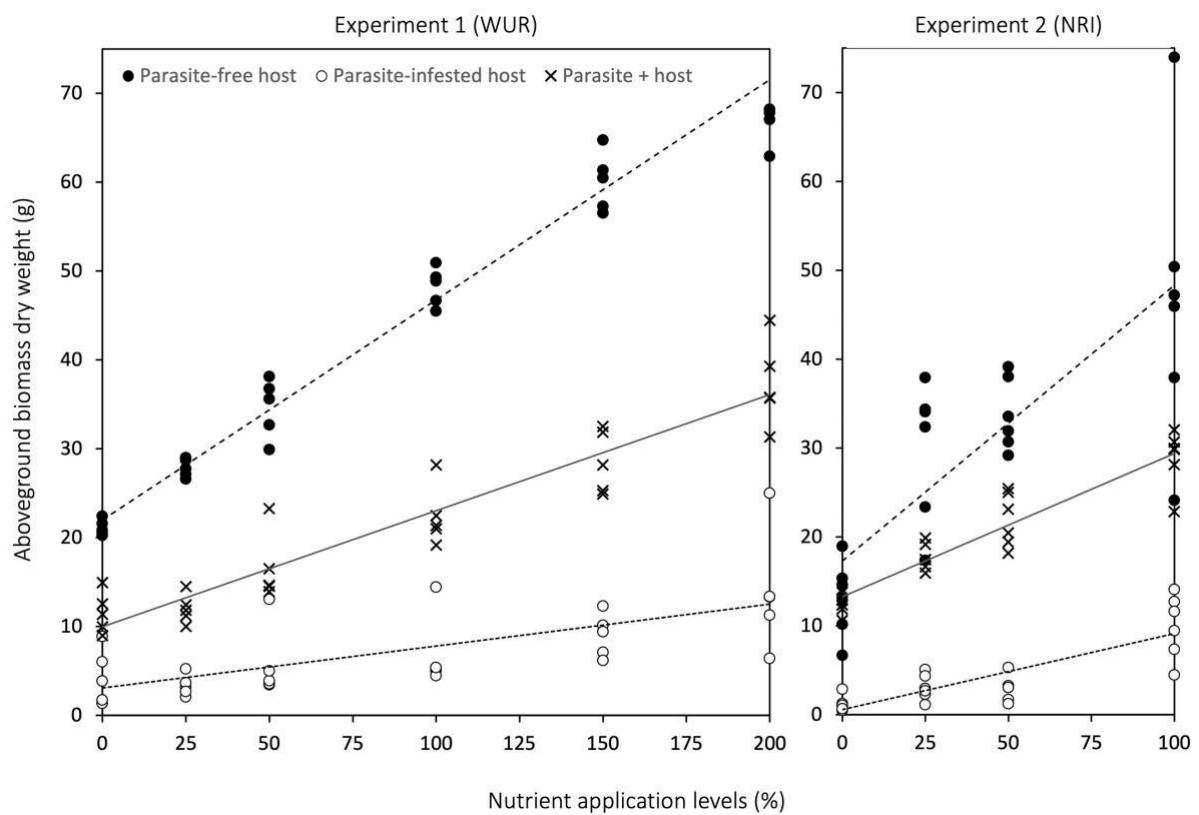


Fig. 1. Total aboveground biomass dry weights of parasite-free and parasite-infested host plants and of the parasite-infested host plant combined with its parasites (parasite + host) as a function of nutrient application levels as observed in Experiment 1 (left) and Experiment 2 (right). In both experiments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

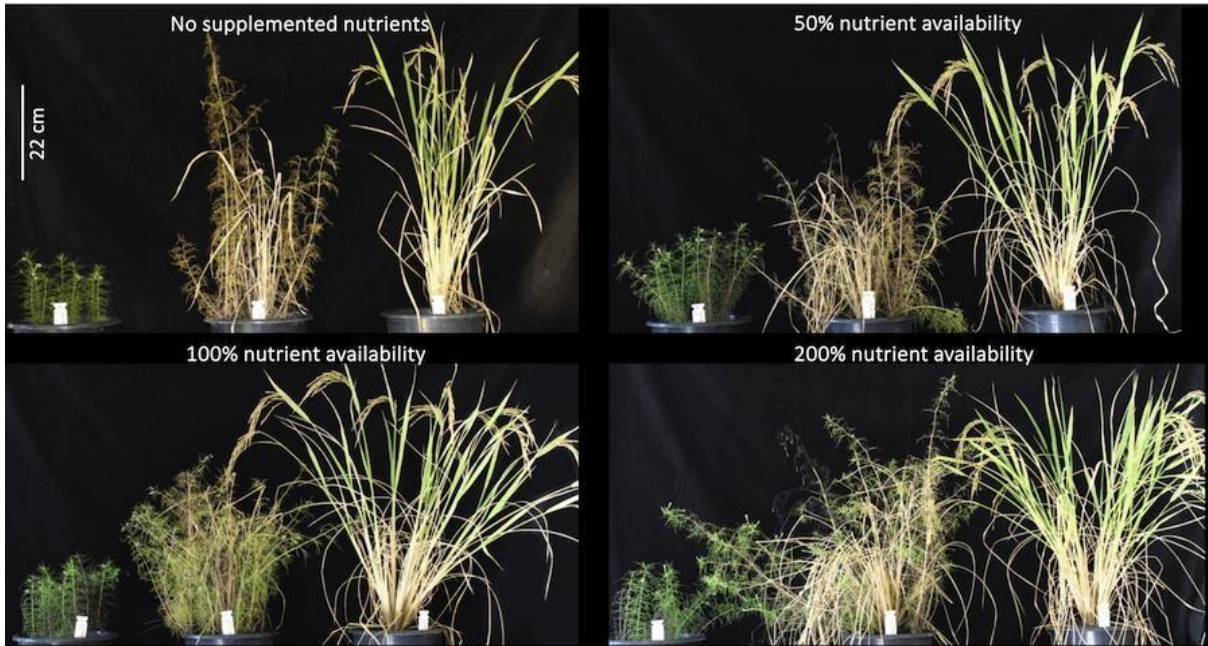


Fig. 2. Photos of parasite (left) and host (right) plants growing alone and together (middle) following 0% (no supplemented nutrients), 50%, 100% and 200% nutrient application levels, in Exp. 1 at 120 days after sowing.

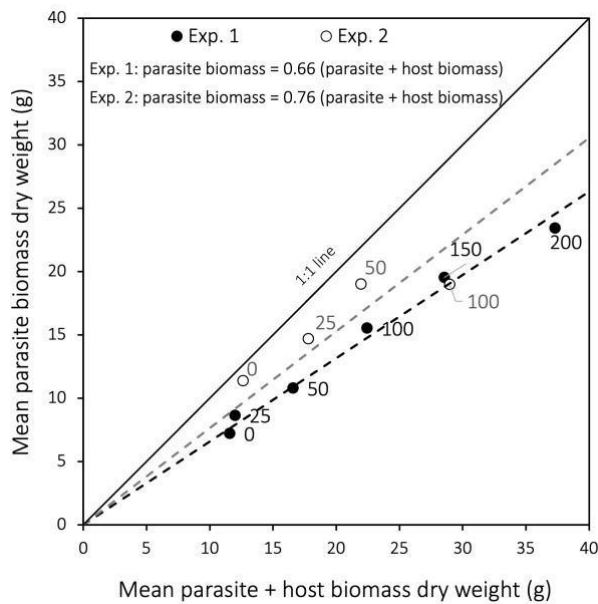


Fig. 3. The relation between total (parasite + host) and parasite biomass dry weight, when host and parasite were grown together at different nutrient application levels (labels 0 to 200) for Experiment 1 (closed symbols; $R^2=0.999$) and Experiment 2 (open symbols; $R^2=0.991$).

In both experiments, total host shoot biomass correlated highly significantly to host tiller numbers (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.

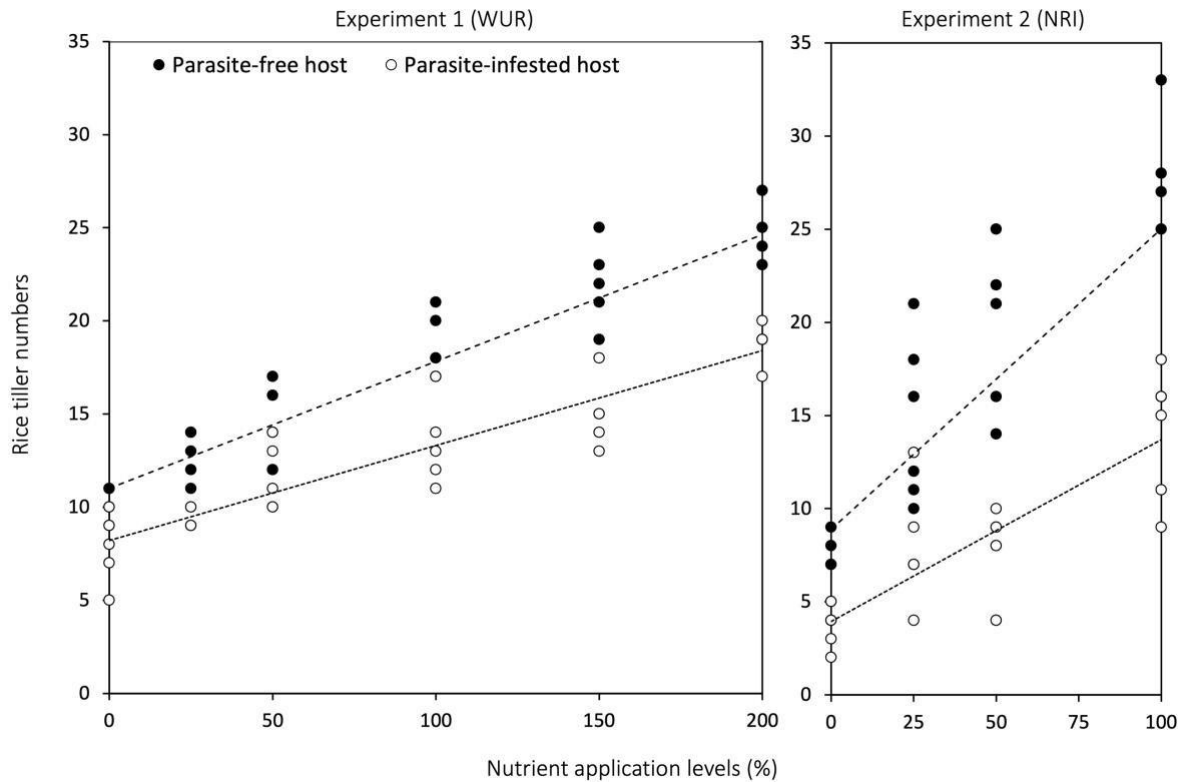


Fig. 4. Host (rice) plant tiller numbers (at 95 DAS for Exp. 1 and 94 DAS for Exp. 2) of parasite-free and parasite-infested plants as a function of nutrient application levels as observed in Experiment 1 (left) and Experiment 2 (right). In both experiments the intercepts are significantly ($P < 0.001$)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

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

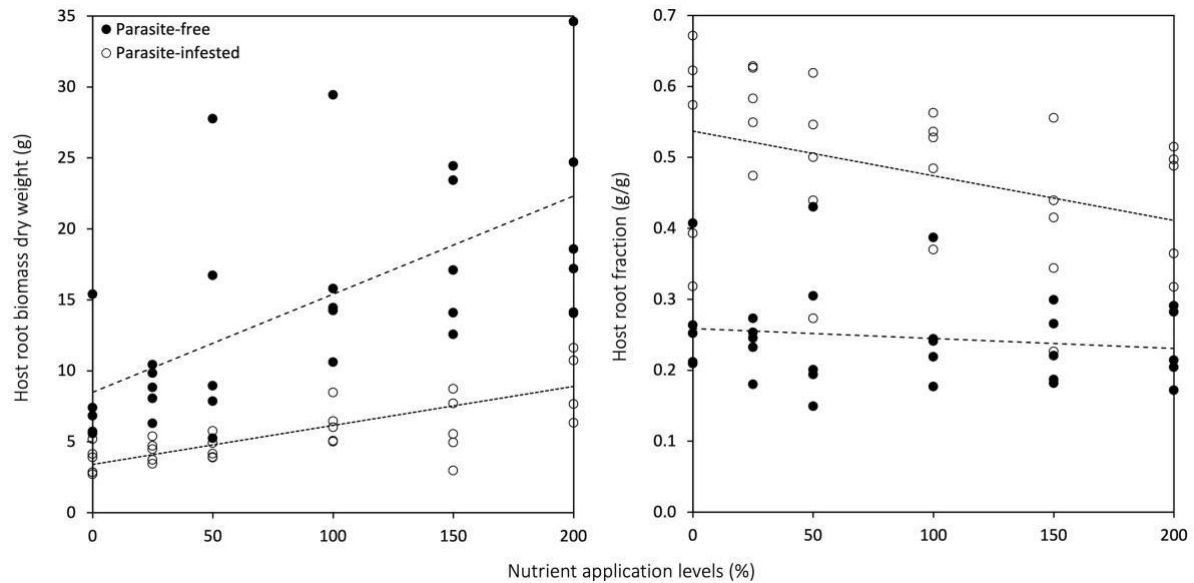


Fig. 5. Host (rice) root biomass dry weights and fraction root to total biomass of plants parasite-free and parasite-infested plants as a function of nutrient application levels as observed in Experiment 1. For both the root biomass dry weight and the root fraction only the intercept of parasite-free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

3.2. Nutrient availability effects on the parasite with and without a host

In Exp. 1, significant nutrient by host-presence interaction effects were observed on aboveground *R. fistulosa* biomass, seed capsule dry weight and plant height, assessed on all 10 plants growing a pot (Table 3). All these parameters significantly increased by host plant presence. Following increasing nutrient quantities, increases in parasite biomass, reproductive output and height were only observed when the parasites were grown together with the host plant (Fig. 6; Suppl. Fig. 1). In 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 9fold. In presence of a host, nutrient application caused a more than 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). Nutrient and host-presence main effects were observed on aboveground parasite biomass production, while both parasite seed capsule weight and parasite plant height were only significantly (positively) affected by host plant presence (Fig. 6, 2, Suppl. Fig. 1). 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 correlated highly significantly with parasite seed capsule dry weight (both experiments) and with parasite seed dry weight (Exp. 2; Table S1). Parasite seed capsule dry weight correlated highly significantly with seed weight (Table S1), hence the above presented shoot and seed capsule weights provide a reliable indication of the parasite's seed production.

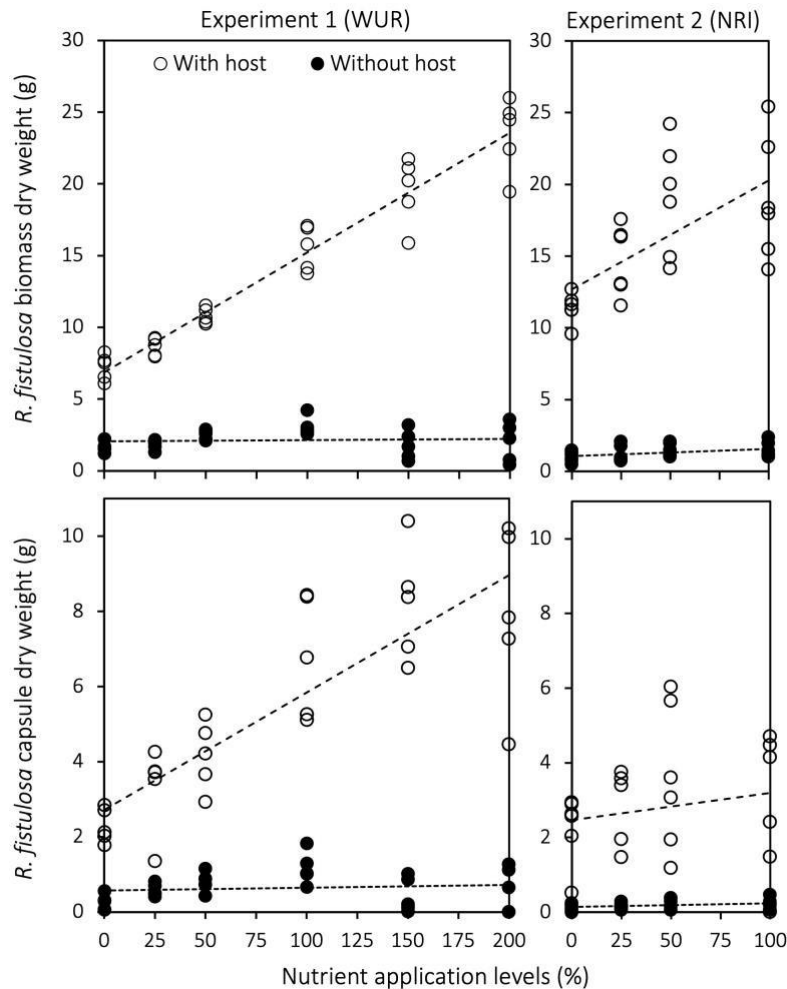


Fig. 6. Parasite total aboveground and seed capsule biomass dry weight production, when grown with or without a host, as a function of nutrient application levels as observed in Experiment 1 (left) and Experiment 2 (right). In both experiments the intercept ($P < 0.001$) of the regression lines are significantly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

Parasite shoot dry weight and parasite seed capsule dry weight both correlated highly significantly to parasite root dry weight (Table S1). Similar to aboveground *R. fistulosa* biomass, significant nutrient by host-presence interaction effects were observed on root biomass of the parasite (Table 3; only assessed in Exp. 1). Root biomass of parasites in presence of a host was always greater than in absence of a host. In addition, in presence of a host the parasite root biomass responded positively to nutrient application, whereas in 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 to parasites growing alone, the root fraction was significantly smaller in presence of a host (Fig. 7). This implies that while both below and aboveground parasite biomass significantly increased due 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.

Table 3. ANCOVA output (F values) for the treatment effects (nutrient supply, host presence, interaction) on parasite biomass dry weight (Shoot dw), capsule dry weight (Capsule dw),

mean parasite plant height (Height), parasite root biomass (Root dw) and fraction root to total biomass (Root frac.). The latter data were arcsin transformed before analyses; Df = degrees of freedom.

Source of variation	Exp. 1					Exp. 2				
	Df	Shoot dw	Capsule dw	Height	Root dw	Root frac.	Df	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 x 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 $P<0.001$

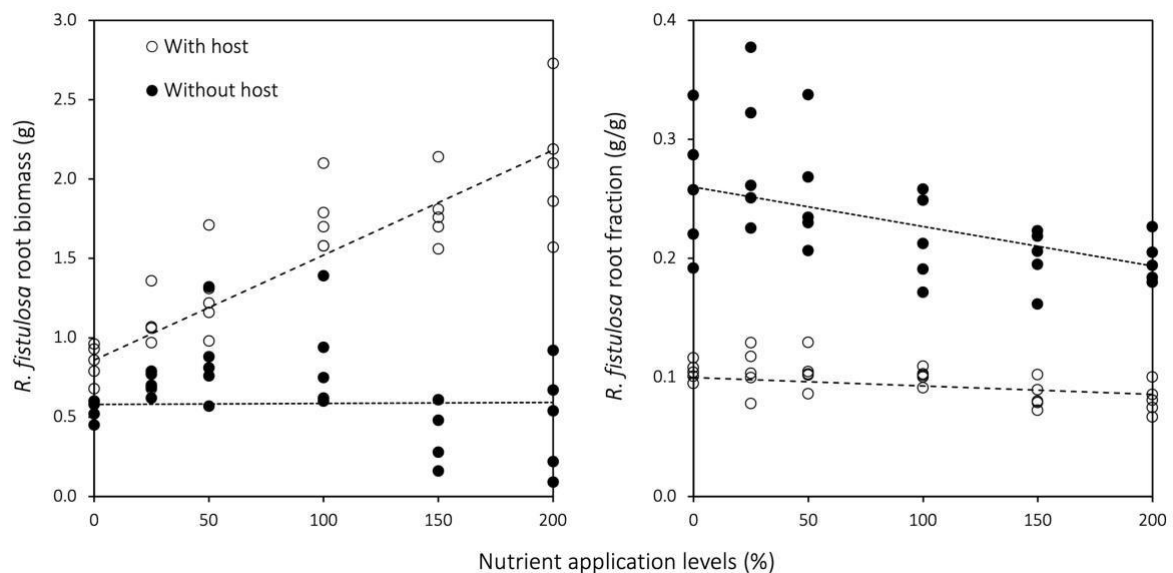


Fig. 7. Parasite root biomass and fraction root to total biomass, when grown with or without a host, as a function of nutrient application levels as observed in Experiment 1. In both cases the intercepts of the regression lines are significantly different. The slopes were significantly different for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

4. DISCUSSION

The aim of this study was to enhance our understanding of the effects of increased nutrient availability on the facultative parasitic weed *Rhamphicarpa 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).

While fertiliser application 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 fertilisers as parasitic plant biomass increased after fertiliser application (Tippe et al., 2020). As parasitic plant biomass is highly correlated with seed (capsule) production (Rodenburg et al., 2006), uninformed fertiliser application could contribute to the build-up of a parasitic weed seed bank and thereby increase weed problems and jeopardise crop productivity in the future.

4.1. *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 belowground parts. Based on root fraction, the aboveground parts of the host plant were relatively more negatively affected than the belowground parts. This was observed before with rice plants infected by the obligate hemiparasite *Striga hermonthica*, and was thought to be caused by a greater relative inhibition of shoot growth (Cechin & Press, 1994). The severe effects of *R. fistulosa* on its host observed here agree 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 & Okonkwo, 1974) and barley infested by *Rhinanthus minor* (Jiang et al., 2004). The loss in host plant biomass due to parasitism was not compensated by the gain in parasite biomass as the overall aboveground 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 & Egli, 1999, Rodenburg et al., 2011) as they exert 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 the current study showed, confirming observations on *Rhinanthus alectorolophus* by Matthies and Egli (1999). Improved nutrient availability through fertilisation 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 fertiliser application rate), the biomass of the parasite-infected host is lower than that of the parasite-free host without additional fertiliser. A recent report from the field confirms that with application of fertilisers *R. fistulosa*-induced rice yield losses could be reduced but not completely compensated (Tippe et al., 2020).

4.2. *Effects of increased nutrient availability on *Rhamphicarpa fistulosa* with or without a host*

Rhamphicarpa fistulosa growth, production and reproduction was boosted by host presence. The positive host effect was previously observed with *R. fistulosa* (Kabiri et al., 2016) as well as with other facultative parasites, such as *Rhinanthus minor* (Jiang et al., 2003, Jiang et al., 2004) and *R. serotinus* (Klaren & Janssen, 1978). Consistent positive nutrient effects on parasite biomass were only observed in the presence of a host plant. Such fertiliser effects on attached parasites were previously observed in the field by Tippe et al. (2020). The current study showed that increased nutrient availability also results in increased parasite seed production. The observed positive fertiliser effects on *R. fistulosa* contrasts with the frequently observed fertiliser effects on related parasitic weeds. Fertiliser applications are generally found to decrease infection levels of cereal crops by the obligate parasitic weed *Striga 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 striga seed germination stimulants. *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 fertiliser 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 biomass of independently growing plants only showed a slight increase following nutrient application, while in Experiment 1 these plants showed no noticeable change. This minor difference in nutrient response observed between the two experiments could be due to differences in environmental conditions (e.g., light, temperature, humidity) but more importantly to differences between supplied fertiliser types in terms of other nutrients than nitrogen. The fertilisers of Experiment 2 had a relative higher concentration of phosphorus than the fertiliser used in Experiment 1. Biomass accumulation of independently growing plants of another facultative parasite, *Rhinanthus minor*, previously showed to be only benefiting from phosphorus fertiliser, while 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 hypothesised that unattached parasites have a low nitrogen uptake and use efficiency (Seel & Jeschke, 1999) and that the phosphorus response is a result 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 & Cameron, 2009). With our experiments, although both conducted on relatively poor soils (1:1 soil-sand mixture), we could only confirm the latter to be the case for *R. fistulosa* as well; parasitic plants produced relatively low root biomass, independent of nutrient levels.

The positive host presence effect on general parasite performance and the additional nutrient effect can be explained by an increased resource uptake capacity by attached parasites, compared to independently growing parasites. The uptake capacity is increased because parasite root biomass of attached parasites is increased, as shown in the current study. This confirms previous findings on another facultative parasitic plant, *Rhinanthus minor*, that root growth of parasitising plants doubles compared to autotrophic plants (Jiang et al., 2004). In addition, the parasite can exploit the root system of the host plant it parasitizes on. Root hemiparasites are considered to take up water primarily through their hosts rather than directly by their own root system (Stewart & 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 & Van de Dijk, 1976). The current study showed that root biomass of *R. fistulosa* is very small, and therefore it seems likely that water uptake for this species is mainly through the host as well. Together with the water, hemiparasitic plants will take up host-derived mineral nutrients and some C (Shen et al., 2006, Press & Whittaker, 1993, Seel & Jeschke, 1999). Parasitic individuals of *Rhinanthus serotinus* showed increased levels of nitrogen, phosphorus and potassium compared to unattached plants of this species (Klaren & 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 benefiting more from supplemented nutrients when they are attached to a host plant as shown in the current study.

4.3. Conclusions

This study showed that whereas 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. While the parasite benefits from the increased carrying capacity of the host following fertilisation, the gains from increased nutrient availability to the host are far from sufficient to compensate the parasite-induced losses. Increased fertilisation may not be an effective solution for rice crops infested by the parasitic weed *Rhamphicarpa fistulosa* as it increases the reproductive output which could worsen infestation rates in the following crop season.

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Table S1. Pearson correlation coefficients between host (H), parasite (P), host and parasite (H+P) parameters for Experiment 1 and 2 (Shoot dw= aboveground plant biomass dry weight; Root dw = belowground plant biomass dry weight; Root frac. = belowground biomass dry weight relative to total biomass dry weight; Tiller # = host tiller number; Height = mean parasite plant height; Capsule dw = parasite seed capsule dry weight; Seed dw = parasite seed dry weight).

		Host				Parasite					
Exp. 1		Shoot dw	Tiller #	Root dw	Root frac.	Shoot dw	Heig ht	Capsule dw	Seed dw	Root dw	Root frac.
H	Tiller #	0.86									
	Root dw	0.87	0.81								
	Root frac.	-0.57	-0.49	-0.17							
P	Shoot dw	0.63	0.86	0.68	-0.32						
	Height	0.42	0.50	0.29	-0.49	0.57					
	Capsule dw	0.34	0.67	0.43	-0.17	0.88	0.52				
	Root dw	0.47	0.78	0.55	-0.22	0.89	0.42	0.94			
	Root frac.	-0.50	-0.54	-0.53	0.22	-0.75	0.55	-0.55		-0.42	
H+	Shoot dw	0.85	0.94	0.83	-0.46	0.94	0.56	0.76		0.83	-0.68

Exp.		Shoot dw	Tiller #	Root dw	Root frac.	Shoot dw	Heig ht	Capsule dw	Seed dw	Root dw	Root frac.
H	Tiller #	0.91									
P	Shoot dw		0.28			0.64					
	Height	0.18									
	Capsule dw	-0.02	0.11			0.66	0.55				
	Seed dw	0.01	0.08			0.40	0.44	0.81			
	dw	0.19	0.31								
H+	Shoot dw	0.68	0.72			0.84	0.46	0.49	0.40		
P	dw										

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

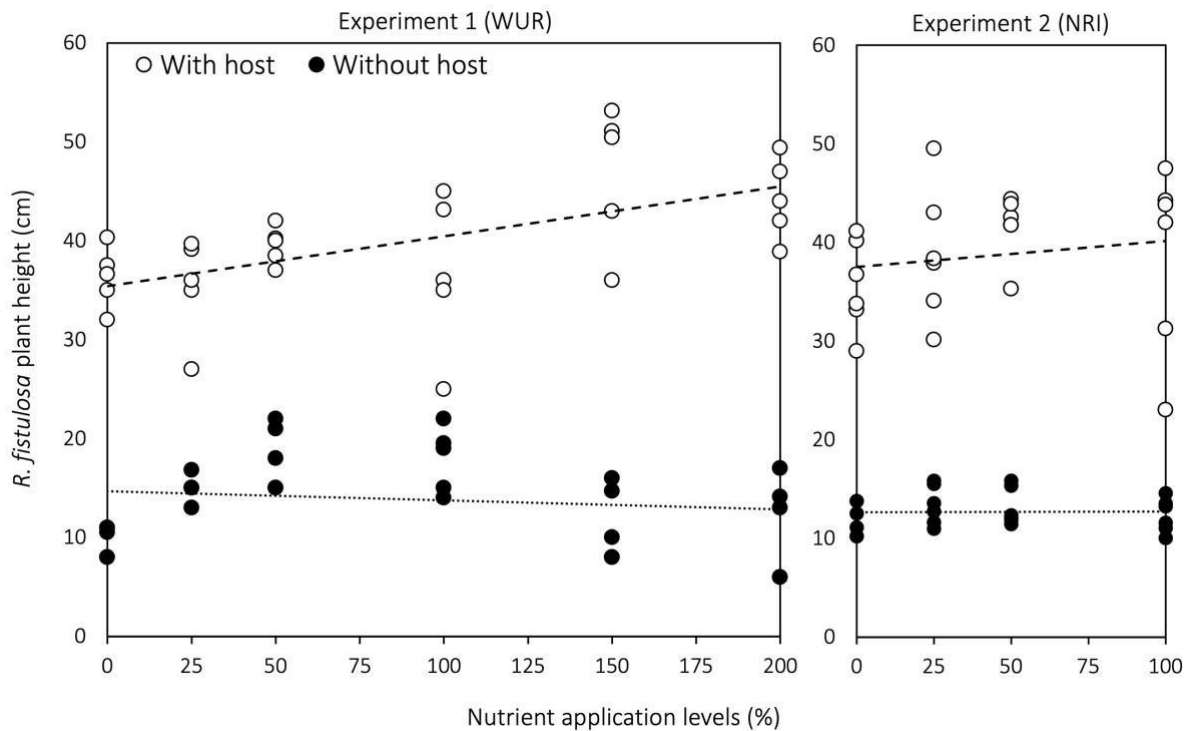


Fig. S1. Parasite plant height, when grown with or without a host, as a function of nutrient application levels as observed in Experiment 1 (left) and Experiment 2 (right). In both experiments the intercept ($P<0.001$) of the regression lines are significantly different. The slopes were only significantly different in Experiment 1 ($P<0.001$).