RESEARCH ARTICLE

Host influence on germination and reproduction of the facultative hemi-parasitic weed *Rhamphicarpa fistulosa*

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Abstract

Rice Vampireweed, *Rhamphicarpa fistulosa*, was a minor parasitic weed until recently when rice cultivation in sub-Saharan Africa was expanded into marginal wetlands, that are the parasite's natural habitat. Unlike most of the parasitic weeds, *R. fistulosa* is facultative, meaning that the parasite is able to complete its life cycle without a host. However, when not connected to a host plant, its biomass and seed production is lower. Because very little is known regarding the germination ecology of the parasite, the main objective of our study was to identify the cues that favour germination. We hypothesised that, first, being a wetland species, germination of *R. fistulosa* is stimulated by light and high soil moisture. Second, we hypothesised that if host plant presence increases its reproductive output then a germination stimulatory effect from host presence is likely to have developed. A Petri-dish and pot experiment showed that light and completely saturated soils were a requirement for germination, demonstrating that germination requirements of *R. fistulosa* are typical of species that grow in environments with fluctuating water levels. A pot experiment in which five infestation levels of *R. fistulosa* were installed in the absence and presence of a rice plant, showed that host plant presence resulted in a 3.7 times higher seed production rate and a 15% larger average seed size. Despite this reproductive advantage, a pot experiment with three rice cultivars, selected because of their difference in strigolactone production, showed that host plant presence, regardless of the development stage, did not influence the emergence rate of *R. fistulosa*. In a follow-up study, the germination stimulation effect of root exudates collected from the same three rice cultivars and a treatment consisting of an artificial germination stimulant (GR24) was compared with a treatment consisting of plain water. In these treatments, seeds of *R. fistulosa* were compared with seeds of the obligate parasite *Striga hermonthica*. Germination of *S. hermonthica* was strongly advanced by the presence of root exudates and GR24 but was completely absent in water, whereas germination of *R. fistulosa* in all treatments was similar to that in plain water. The absence of a host recognition mechanism at the germination stage suggests that the regulation of germination through light and soil moisture is near optimal. Our finding might also indicate that for this facultative parasitic plant species, a more opportunistic germination strategy is superior. Implications of the findings for management of *R. fistulosa* in rice cultivation are discussed.

Introduction

The most common root hemi-parasitic plant species in Africa are from the family *Orobanchaceae* (Parker, 2013). All cultivated cereals and legumes can be parasitised by one or more of these species resulting in high yield reduction and even crop failure (Berner *et al.*, 1995; Ejeta & Gressel, 2007). The most important of these parasitic weeds are the well-known Witchweeds, *Striga hermonthica* (Del.) Benth. and *Striga asiatica* (L.) Kuntze. Recently, Rice Vampireweed, *Rhamphicarpa fistulosa* (Hochst.) Benth. (syn. *Macrosiphon fistulosus* Hochst.), has gained importance and is developing into a serious pest of rain-fed rice (Rodenburg *et al.*, 2015).

Rhamphicarpa fistulosa is an annual parasitic plant with reduced pinnatisect leaves and long white flowers that open only at night (Hansen, 1975; Ouédraogo *et al.*, 1999). Its seeds are produced in asymmetrical capsules that readily shatter after ripening (Ouédraogo *et al.*, 1999). *R. fistulosa* is native to sub-Saharan Africa (SSA) and is widely distributed in Sahelian countries, eastern Africa and southern Africa including Madagascar (Hansen, 1975; Rodenburg *et al.*, 2015). The complete host range has not been established, but *R. fistulosa* is able to parasitise cowpea, sorghum, maize and rice (Parker & Riches, 1993; Ouédraogo *et al.*, 1999; Rodenburg *et al.*, 2011). The species was a minor weed until recently when rice developed into an important food crop in SSA. Currently, the area of expansion of rice cultivation is in marginal wetlands, the natural habitat of the parasite (Rodenburg *et al.*, 2014). It is thus, the adaptation to ephemeral wet areas (Hansen, 1975; Ouédraogo *et al.*, 1999; Müller & Deil, 2005; Kabiri *et al.*, 2015) that makes *R. fistulosa* specifically problematic to rice, the sole staple food crop that can be grown in such environments. Till today, not much is known about the germination ecology of this parasitic plant, and for that reason the main objective of our research was to explore the cues that favour germination of *R. fistulosa*.

It has been demonstrated that seeds of species that grow in unpredictable habitats with fluctuating water levels remain non-dormant (Baskin & Baskin, 1988). At any time during the growing season when water recedes, the seeds can germinate at a high rate when exposed to light and non-limiting soil moisture (Baskin *et al.*, 1989). We therefore hypothesised that germination of *R. fistulosa* seeds is also favoured by light and non-limiting soil moisture. This hypothesis was evaluated through two germination experiments with either light or soil moisture as the main experimental factor.

Contrary to most of the parasitic plants of agricultural importance, *R. fistulosa* is facultative, meaning that the species can complete its life cycle in the absence of

a host. For obligate parasitic plants, like *Striga* spp., the presence of a host plant is fundamentally important for survival. It is therefore not surprising that obligate parasitic plants possess a host plant detection mechanism. This mechanism recognises strigolactones, the underground signalling molecules present in root exudates released by host plants. It is often suggested that the ecological significance of strigolactones is to stimulate mycorrhizal fungi to initiate a symbiotic interaction (Bouwmeester *et al.*, 2007). However, seeds of obligate parasitic plants make use of the same compounds to detect potential hosts, and it is well established that for obligate parasitic weeds, the presence of strigolactones is a prerequisite for germination (Xie *et al.*, 2010). Seeds of *R. fistulosa* are known to germinate without a host (Ouédraogo *et al.*, 1999). However, this does not exclude the possibility that host root exudates can enhance germination. Such a host recognition mechanism through host root exudates appears likely if the growth and seed production rate of a parasitic plant greatly benefits from its connection to a host plant. Although it has been observed that *R. fistulosa* plants that associate with a host plant grow taller and produce more seeds (Ouédraogo *et al.*, 1999), the extent of this advantage is not well documented. Linking reproductive output and stimulation of seed germination, we hypothesised that if the presence of a host plant results in a sizeable increase in reproduction of *R. fistulosa*, seed germination of the parasite is likely to be stimulated by the presence of a host plant. The reproductive advantage of the parasite following host plant presence was established in a pot experiment consisting of pots with and without a host plant. In this experiment, parasite infestation level was used as a second factor to be able to determine the reproductive advantage at the parasitic plant population level. The germination response to host plant presence was investigated both in a pot experiment with three rice cultivars at three development stages and in a Petri-dish experiment where seeds were exposed to root exudates collected from the same three rice cultivars.

Materials and methods

In the time period 2010–13, a series of laboratory and greenhouse experiments were conducted at Wageningen University, the Netherlands. Seeds of *R. fistulosa* used in these experiments were from a seed lot collected in 2009 from an infested rice field in Kyela, Tanzania. In all the experiments, rice (*Oryza sativa* L.) was used as the host plant species. All greenhouse experiments were conducted in the same greenhouse, in which screens were used to create a day length of 12 h (from 07:00 to 19:00 h). Growing conditions were set to 26°C/23°C for day/night temperatures, though during warm summer

days, daytime temperature was regularly higher, with a maximum of 32°C. Relative humidity varied between 50% and 70%. Supplementary lighting was provided by lamps (SON-T Agro, 400 W, Philips) that automatically switched on when photosynthetically active radiation outside the greenhouse dropped below 910 µE m^{-2} s^{-1}.

Influence of light and dark conditions on germination

In November 2010, a laboratory experiment (Experiment 1) was conducted to investigate whether germination of *R. fistulosa* seeds is stimulated by light. The experiment was a randomised two-factor Petri-dish trial with five replications. The first factor was the presence of light and dark (control) conditions, while the second factor was the presence or absence of an artificial germination stimulant (GR24). The GR24 is commonly used to trigger germination of obligate parasites (Mangnus *et al.*, 1992). As the influence of GR24 on seeds of facultative parasitic plants was unknown, this factor was included to find out if it would modify the response of the seeds to light/dark conditions. Seeds were surface sterilised in 1% NaOCl solution for 2 min, rinsed thoroughly and dried. After drying, 100 Petri-dishes with a diameter of 90 mm and a Whatman No. 1 filter paper at the bottom were each filled with 100 evenly spread seeds. Fifty Petri-dishes were moistened with 4 mL of demineralised water, while the other 50 Petri-dishes were moistened with 4 mL of 2 mg L^{-1} GR24 solution. All dishes were sealed with parafilm. Five Petri-dishes with GR24 and five with water were left unwrapped for the *Light* treatment. The remaining 90 represented the *Dark* treatment and were wrapped in two layers of aluminium foil to prevent light penetration. The higher number of dishes for the *Dark* treatment was needed because at each observation date, examined dishes were discarded because they had been exposed to light.

Petri-dishes were placed in a continuously lit incubator set at 30°C in a randomised design (no blocks). Observation of germinated seeds was carried out nine times during a period of 5 weeks. Seeds were considered germinated when a primary shoot was visible. At each observation date, germinated seeds from Petri-dishes of the *Light* treatment were removed, after which dishes with the remaining non-germinated seeds were put back into the incubator.

Influence of soil moisture on emergence

In 2011, a greenhouse experiment was conducted to establish suitable soil moisture conditions for the emergence of *R. fistulosa* (Experiment 2). The experiment was a two-factor, randomised complete block design in three replications. The first factor was soil moisture, while the second factor was soil texture (coarse and fine sand). Plastic coffee cups with a volume of 180 cm^3 were filled with 220 g of sand. Field capacity was determined by adding water to perforated cups till the soil was saturated, then left to drain freely for 2 days. After weighing, it was determined that field capacity of coarse sand contained 44 mL of water while field capacity of fine sand contained 68 mL of water per 220 g of soil. Accordingly, soil moisture treatments were adjusted to 10%, 25%, 50%, 100% and 150% of the amount of water required to reach field capacity. Fifty *R. fistulosa* seeds were evenly distributed on the surface of each cup and covered with the lid of Petri-dishes to minimise moisture loss through evaporation. Cups were placed on benches in the greenhouse. Twice a week, for a total period of 7 weeks, emerged seedlings were counted and removed.

Influence of a host on germination and emergence

In November 2012, the influence of the presence of a host on the emergence of *R. fistulosa* was investigated in a greenhouse experiment (Experiment 3). The soil used was a 1:1 mixture of arable loam and sand. This mixture has proven to work well for rice-*Rhamphicarpa* experiments (based on our previous work). Sixty pots, sealed at the bottom, were each filled with 8 kg of mixed soil. The pots had a 5 L capacity and were watered to soil saturation. The experiment was set up as a two-factor randomised complete block design with five replicates. The first factor was rice cultivar. Cultivars IAC165, IR64 and Shiokari, known to be high, medium and low root exudate producers, respectively (Jamil *et al.*, 2012) and control pots without a rice plant were used. The second factor involved the age of the rice plants at addition of the parasite seeds (0, 2 and 4 weeks after emergence). We varied the ages of host plants on the assumption that root exudates and strigolactone production might vary with the age of the rice plant. Rice plants were planted in succession in intervals of 2 weeks, to allow the introduction of the parasite seeds at one moment in time. Except for the control pots, all pots had one rice plant in the centre. On 26 November 2012, 100 seeds of *R. fistulosa* were mixed through 100 cm^3 of fine sand and evenly spread on the soil surface of each pot. Pots were watered daily with a fine spray of water and set on benches in the greenhouse. Emergence of *R. fistulosa* seeds was recorded from 3 Days after sowing (DAS) till 30 DAS, when no newly emerged seedlings had been observed for a period of 5 days.

In 2013, a follow-up experiment (Experiment 4) was conducted to specifically further investigate the influence of rice root exudates on the germination of *R. fistulosa*. In

this experiment, plants of the same three rice cultivars as in the previous experiment (IAC165, IR64 and Shiokari) were planted in pots in a greenhouse in six replications. The pots used were unperforated 5 L capacity pots filled with 1:1 mixture of dry arable soil and sand. To stimulate strigolactone production the rice plants were watered with a P deficient (0% P) nutrient solution (Jamil *et al.*, 2013; Koltai, 2013). At 50 DAS, 12 mL of a solution of root exudates was collected from each cultivar, according to the methodology described by Jamil *et al.* (2011).

Part of the original root exudate solutions was diluted with demineralised water to produce 20 mL of each of the following three concentrations: 10%, 1% and 0.1%. Additionally, a 100 mL stock solution of GR24 (10 mg L^{-1}) was prepared by dissolving 1 mg of GR24 powder in 1 mL of acetone and adding demineralised water till a total volume of 100 mL. Part of this stock solution was further diluted to obtain two additional concentrations of 10% and 1%. Fibre glass filter papers (Sartorius, Göttingen, Germany) were cut into 10 mm discs. The discs were moistened with 75 µL of the prepared solutions (GR24 and root exudates of the three rice cultivars at three concentrations) including water as an additional control. Moistened discs were placed in Petri-dishes in groups of five. Twenty to 30 seeds of *R. fistulosa* were spread on each disc, resulting in 100–150 seeds per Petri-dish. Discs with seeds were then covered by a second disc. In a similar set of Petri-dishes with exactly the same treatments, *S. hermonthica* seeds were used. The *S. hermonthica* seeds had been conditioned for 20 days by placing them between two moistened filter papers in Petri-dishes at 30°C. Each treatment was replicated three times, resulting in a total of 90 Petri-dishes laid out in a randomised complete block design. All Petri-dishes were sealed with parafilm. Dishes containing *S. hermonthica* were wrapped with aluminium foil while those containing *R. fistulosa* seeds were left unwrapped. All dishes were placed in a continuously lit incubator, set at 30°C for 7 days, after which germinated seeds were counted.

Two millilitres of the original root exudate solution from each cultivar was passed through solid phase extraction (SPE) columns (C18-Fast, 500 mg 3 mL^{-1}; Grace pure) following the method described by Jamil *et al.* (2011), to establish the amount of strigolactones contained in the solutions. This was merely carried out to obtain a confirmation on the earlier established differences in strigolactone production between rice cultivars.

Influence of a host on growth, development and seed production

A final greenhouse experiment (Experiment 5), conducted in 2013, was used to establish the influence of a host on growth, development and seed production of *R. fistulosa*. In this experiment, parasite seed infestation level was included as an additional factor, as an increased growth of the parasite in the presence of a rice host might easily result in an increased level of intraspecific competition. Consequently, the size of the reproductive advantage might be density dependent and inclusion of parasite seed infestation level as a factor allows studying this phenomenon. The experiment was laid out as a two-factor, randomised complete block design with five replicates. *R. fistulosa* seed infestation level was the first factor and consisted of 16, 32, 64, 128, 256 seeds pot^{-1}. Presence or absence of a host was the second factor. A 1:1 soil mixture of arable loam and fine sand was put into 5 L capacity, non-perforated pots. Densities of parasite seeds were manually counted, thoroughly mixed in 100 cm^3 of soil in a container and evenly spread on the soil surface (660 cm^2). Pregerminated rice seeds of rice cultivar IR64 were sown in half of the pots on 28 May 2013. The pots were placed on benches and watered daily with a fine spray of water. A first destructive sampling took place at 60 DAS, followed by a second sampling at rice maturity (110 DAS). Data collection focused on the parasite and consisted of seedling emergence, seedling survival, plant height, days to flowering, number of capsules produced, flower and capsule mass and total above-ground dry weight. Capsules were harvested as soon as a capsule ripened to avoid seed shattering. Total above-ground biomass weight was obtained after the aerial plant parts were dried in an oven for 48 h at 70°C. Capsules and flowers were stored in labelled papers envelopes, which were air dried on benches in the greenhouse. After weighing, seeds were separated from capsules and flowers by using a sieve with a 0.5 mm mesh size. The total seed weight was determined and 1000 seeds were counted manually to obtain 1000-seed weight.

Statistical analysis

Data were subjected to analysis of variance (ANOVA) using statistical software package GenStat for Windows 16th Edition (VSN International, 2013). In Experiment 1, ANOVA was conducted separately for each observation day, as in the *Light* treatment repeated measures on the same Petri-dish were conducted. In Experiment 4, data on germination of *S. hermonthica* and *R. fistulosa* were transformed prior to analysis to meet assumptions of ANOVA, using $\log(x+1)$, where x is the original observation (Sokal & Rohlf, 1995). In Experiment 5, the same procedure was followed for data on maximum emergence of *R. fistulosa*, number of *R. fistulosa* plants that reached reproductive maturity and above-ground biomass dry weight. The ANOVA was followed by comparison of means by least

significant difference. All data are presented in original scale with respective SE. Means and SE of transformed data were back-transformed from $\log(x+1)$, using Delta method for SE (Onofri *et al.*, 2010).

Results

Germination and emergence

In Experiment 1, throughout the entire experiment, none of the *R. fistulosa* seeds in the *Dark* treatment germinated, regardless of whether they had been treated with GR24 or water. Germination of *R. fistulosa* seeds in the *Light* treatment was first observed at the second observation date, at 12 DAS (Fig. 1). From then on, the percentage of germinated seeds steadily increased until just over 30% at the final observation date at 36 DAS. The difference between germination percentage of seeds treated with artificial germination stimulant and those treated with water was not significant on any of the observation dates (Table 1).

Experiment 2 showed that emergence of *R. fistulosa* was strongly influenced by soil moisture status ($F_{5,22} = 233.42$; $P < 0.001$). The highest emergence (42%) was attained under saturated soil moisture conditions, with cups containing 150% of the amount of water required to reach field capacity. At field capacity, emergence was on average 12%, thus, 3.5 times lower. The parasite did not emerge under conditions drier than field capacity. Emergence of *R. fistulosa* did not differ significantly between coarse and fine sand ($F_{1,22} = 2.88$; $P = 0.104$).

In Experiment 3, average emergence of *R. fistulosa* at 30 DAS was 52% (Fig. 2). The presence of a rice plant, regardless of the root exudate producing ability of the variety, did not significantly influence the emergence ($F_{3,44} = 0.46$; $P = 0.713$). Also, age of host plant at introduction of the parasite seeds did not influence emergence ($F_{2,44} = 0.42$; $P = 0.660$).

In Experiment 4, the results of the SPE confirmed the significant dependence of strigolactone production on rice cultivar ($F_{2,17} = 9.04$; $P = 0.006$). Strigolactone production by cultivar IAC165 (6.65 pmol mL^{-1}) was significantly higher than that of cultivar IR64 (2.98 pmol mL^{-1}) and cultivar Shiokari (1.84 pmol mL^{-1}). These differences in strigolactone production between cultivars were reflected in differences in germination of *S. hermonthica* evoked by root exudate dilutions of 10% and 1% (Fig. 3A). At a dilution of 0.1%, hardly any germination of *Striga* seeds was observed. A similar response to concentration was observed with GR24. Evidently, germination of seeds of *S. hermonthica* was significantly stimulated by root exudates and GR24 ($F_{8,28} = 6.18$; $P < 0.001$). In line with this, no germination occurred in plain water. For *R. fistulosa*,
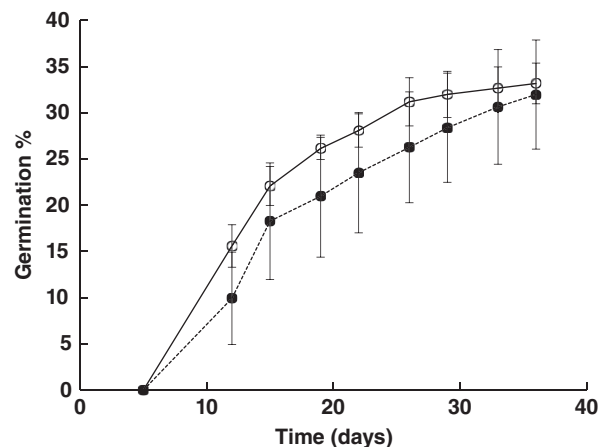

Figure 1 Progression of germination of *Rhamphicarpa fistulosa* seeds over time treated with GR24 and water under light conditions in Petri-dishes ($n = 5$). Vertical bars are SEs of mean. Seeds treated with GR24 and water exposed to dark conditions are not included, as no germination was observed (Experiment 1).

Table 1 Germination percentage (G%) of *Rhamphicarpa fistulosa* seeds over time averaged over seeds treated with GR24 and water under light conditions (*R. fistulosa* did not germinate under dark conditions). *F*-value and corresponding *P*-probability refer to the contrast between GR24 and water, which was not significant at any of the observation dates (Experiment 1).

DAS	Mean	$F_{1,4}$	P
7	(no germination)		
12	12.8	2.27	0.206
15	20.2	0.69	0.453
19	23.6	0.82	0.415
22	25.8	0.84	0.412
26	28.7	1.61	0.274
29	30.2	0.77	0.429
33	31.7	0.19	0.687
36	32.6	0.08	0.796

the results were completely different. Seed germination of this facultative plant species was not at all influenced by rice root exudates or GR24, regardless of the concentration ($F_{8,28} = 0.39$; $P < 0.917$) (Fig. 3B). The average germination rate with rice root exudates or GR24 was 74% and similar to that obtained in plain water.

Growth, development and seed production

Similar to what was observed in the third experiment, presence of a host did not influence maximal emergence of *R. fistulosa* in Experiment 5 ($F_{1,36} = 1.89$; $P = 0.178$) (Fig. 4A). Although the number of emerged parasites increased significantly with seed infestation level ($F_{4,36} = 19.94$; $P < 0.001$), the fraction of maximal emergence gradually decreased from 35% at 16 seeds per
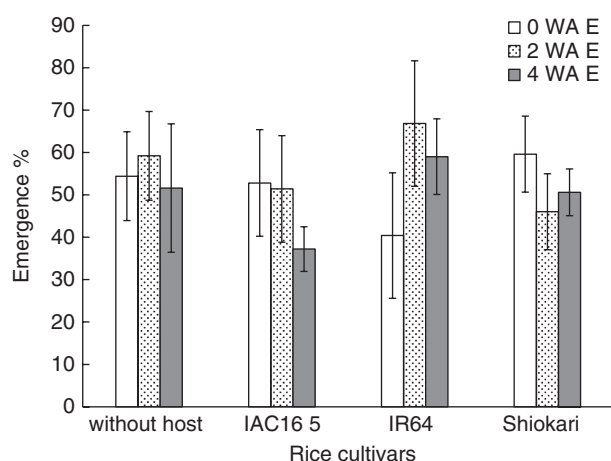
Figure 2 Emergence of *Rhamphicarpa fistulosa* at 30 DAS in pots without a host plant and in pots with rice plants of cultivar IAC165, IR64 and Shiokari that were either 0, 2 or 4 weeks old at the introduction time of parasite seeds ($n = 5$). Vertical bars are SEs of mean (Experiment 3).

pot to 18% at 256 seeds per pot. A significant effect of seed infestation level was still present at maturity of the host plant ($F_{4,35} = 16.45$; $P < 0.001$) (Fig. 4B). Seed infestation level did not have any effect on survival rate (i.e. fraction of plants that reached reproductive maturity) ($F_{4,35} = 0.35$; $P = 0.841$). The presence of the rice host had a significant negative effect on the number of mature parasites ($F_{1,35} = 10.57$; $P = 0.003$). This was mainly because of a significant difference in survival rate ($F_{1,35} = 9.40$; $P = 0.004$), with an average survival rate of 53% in presence of a host, compared with 72% in absence of a host.

Parasites in pots with a host (23.7 ± 2.09 cm) were significantly taller than in pots without a host (10.7 ± 2.09 cm) ($F_{1,33} = 39.25$; $P < 0.001$) (Fig. 5). Regardless of host presence, average height of parasites decreased with increasing *R. fistulosa* seed infestation level ($F_{4,33} = 3.17$; $P = 0.026$). In the absence of a host, the tallest plants were found in the category 18–24 cm (16 seeds pot^{-1}) or 12–18 cm (256 seeds pot^{-1}) (Fig. 6). In the presence of a host, some *R. fistulosa* grew as tall as 54–60 cm and consequently, variation in individual plant height was much larger. At low infestation levels, parasites were evenly distributed over height classes that ranged from 0 to 6 cm till 54 to 60 cm. At high infestation levels, the distribution was skewed such that there were more plants in the lower plant height categories.

Both at 60 DAS and at 110 DAS, dry total above-ground biomass of plants that developed on a host was around 3.1 times higher than that of host-less plants ($P < 0.001$) (Fig. 7). In addition, a significant effect of seed infestation level on *R. fistulosa* biomass was observed, both at 60 DAS ($F_{4,33} = 9.57$; $P < 0.001$) (Fig. 7A) and at 110
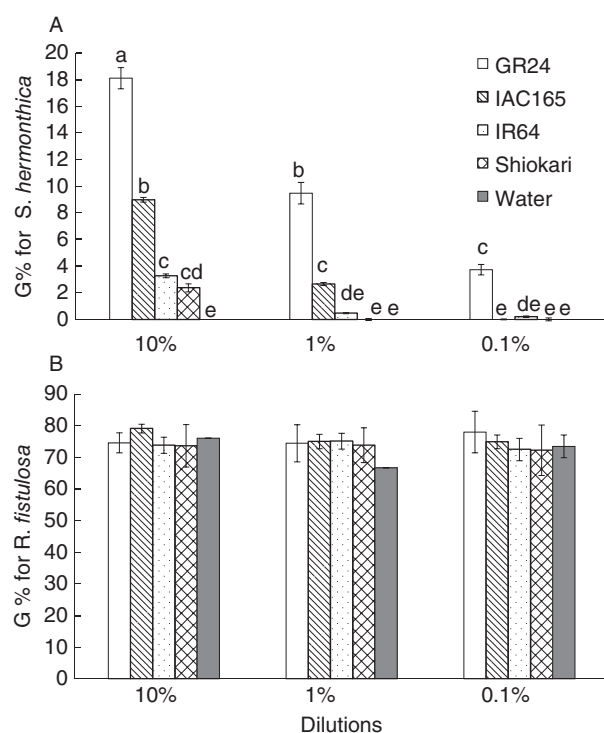

Figure 3 Germination percentage (*G*%) of *S. hermonthica* (A) and *Rhamphicarpa fistulosa* (B) treated with dilutions of artificial germination stimulant GR24 and root exudates extracted from rice cultivars IAC165, IR64 and Shiokari and water ($n = 3$). Vertical bars are SEs of mean. Least significant difference test was used to test whether differences between treatments were significant at $P < 0.05$. Non-similar letters indicate significant differences. Means and SE are back-transformed from $\log(x + 1)$ data (Experiment 4).

DAS ($F_{4,33} = 4.95$; $P < 0.003$) (Fig. 7B). This effect of seed infestation level was particularly evident at 60 DAS, with an average 0.5 g of shoot biomass at the lowest infestation level, compared with 2.6 g of shoot biomass at the highest infestation level. At 110 DAS, the range was smaller with an average 5.5 and 6.5 g of shoot biomass at the lowest and highest infestation levels, respectively.

First flowering was observed at around 90 DAS and was the same for parasite plants that developed with and without a host. Capsule number ($F_{1,33} = 72.49$; $P < 0.001$), capsule and flower weight ($F_{1,33} = 160.51$; $P < 0.001$), total seed weight ($F_{1,31} = 144.36$; $P < 0.001$) and 1000-seed weight ($F_{1,31} = 27.89$; $P < 0.001$) (residual values of the F statistic may vary because of the missing values) were all significantly influenced by the presence of a host (Table 2). All of these characteristics greatly benefited from the presence of a rice host plant. Plants of *R. fistulosa* that grew in the presence of rice produced on average 48 000 seeds per pot, compared with 13 000 seeds per pot in the absence of rice. Apart from this 3.7 times
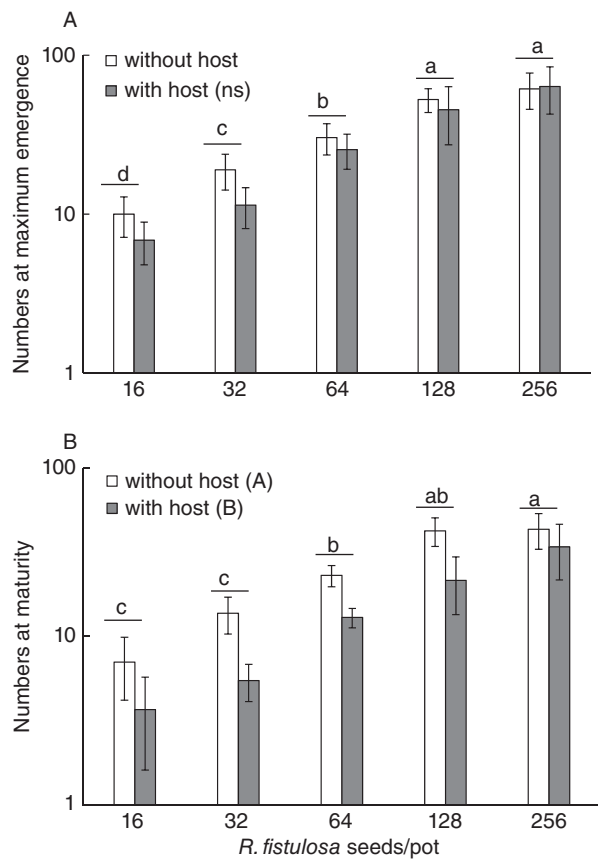
Figure 4 Number of *Rhamphicarpa fistulosa* plants in the absence and presence of a rice host plant at a range of *R. fistulosa* seed infestation levels, that emerged (A) ($n = 5$) and at maturity (B) ($n = 5$). Vertical bars are SEs of mean. Different letters on the legend indicate significant differences ($P < 0.05$) between plants with and without a host, while *ns* indicates non-significance. Different letters on horizontal bars indicate significant differences between parasite densities. Means and SE are back-transformed from $\log(x + 1)$ data (Experiment 5).

increase in seed production, these seeds were on average 15% heavier than those produced in pots without a host. A significant effect of seed infestation level was observed for capsule production ($F_{4,33} = 2.75$; $P = 0.044$) and 1000-seed weight ($F_{4,31} = 3.33$; $P = 0.022$).

Discussion

We found out that germination of *R. fistulosa* is stimulated by abundant soil moisture and light. These findings demonstrate what is commonly found for species in wetland areas (Baskin *et al.*, 1989). The requirement for saturated soil moisture conditions serves as an indicator for the start of the wet season, which coincides with the availability of potential host plants. These results are in line with a previous study, where emergence and growth of *R. fistulosa* were found to be favoured by waterlogged
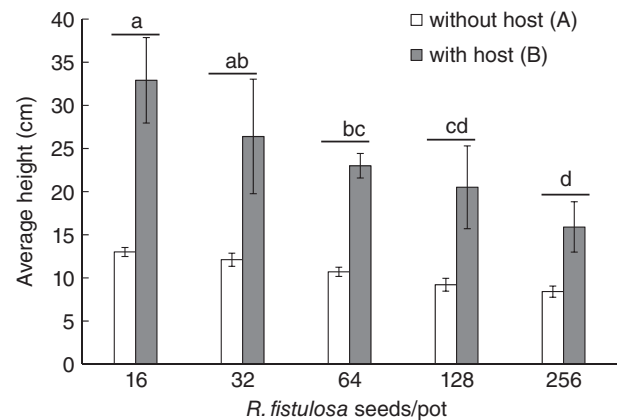

Figure 5 Average height of *Rhamphicarpa fistulosa* plants with and without a host at a range of *R. fistulosa* seed infestation levels, at maturity (110 DAS) ($n = 5$). Vertical bars are SEs of mean. Different letters on the legend indicate significant differences between plants with and without a host, whereas different letters on horizontal bars indicate significant differences between parasite densities ($P < 0.05$) (Experiment 5).

conditions (Kabiri *et al.*, 2015). Germination was completely inhibited by dark conditions, implying that *R. fistulosa* seeds are only able to germinate on or near the soil surface. Such an adaptation is important for species with a small seed mass (*R. fistulosa* 1000-seed weight ≈ 0.0115 g). The influence of light on seed germination has been interpreted as a soil depth indicator that prevents germination deeper in the soil (Schütz *et al.*, 2002). Germination close to the soil surface ensures that the germinated seed is able to quickly emerge and start photosynthesis before its storage reserves are exhausted (Bewley & Black, 1994).

In the presence of a host plant, the average height of *R. fistulosa* plants increased significantly. This increased average was associated with a much wider range of plant heights, as part of the parasite population grew much taller. Clearly, presence of a host plant resulted in some taller parasitic plants dominating the population. Probably, these parasites are attached to the host at a relatively early stage, thereby out-competing the non-attached and later attached individuals because of intraspecific competition. The reduction in average plant height of *R. fistulosa* with increasing parasite infestation level indicates that intraspecific competition was stronger with an increased number of parasites. The same trend was observed in the absence of a host. Furthermore, data on parasite biomass showed that intraspecific competition among parasite plants at 60 DAS was far less pronounced than at 110 DAS, irrespective of the host plant presence. At 60 DAS, parasite biomass still steadily increased with the increasing infestation level, whereas at 110 DAS, parasite biomass at higher infestation levels reached a plateau. This plateau represents the carrying capacity and

Table 2 Analysis of variance of reproductive characteristics of *Rhamphicarpa fistulosa* plants in the presence and absence of a rice host plant and at a range of seed infestation levels ($n = 5$). Non-similar letters indicate significant differences (Experiment 5).

Seeds/pot		16	32	64	128	256	Average			Infestation level	Host influence	Infestation level × host influence
No. of capsules	(−) host	16.8	19.4	29.0	31.0	31.4	25.5[b]	F		2.75	72.49	0.32
	(+) host	45.8	47.6	57.6	51.4	54.6	51.4[a]	P		*0.044*	*<0.001*	*0.862*
	Average	31.3[b]	33.5[b]	43.3[a]	41.2[ab]	43.0[a]						
Flower and capsule weight (g)	(−) host	0.459	0.489	0.676	0.657	0.820	0.620[b]	F		1.63	160.51	0.73
	(+) host	1.701	1.888	2.128	1.669	1.926	1.862[a]	P		*0.191*	*<0.001*	*0.578*
	Average	1.080	1.188	1.402	1.163	1.373						
Total seed weight (g)	(−) host	0.139	0.078	0.168	0.164	0.161	0.142[b]	F		1.74	139.74	1.35
	(+) host	0.613	0.587	0.759	0.469	0.656	0.617[a]	P		*0.167*	*<0.001*	*0.274*
	Average	0.376	0.333	0.463	0.317	0.408						
1000-seed weight (g)	(−) host	0.0123	0.0115	0.0111	0.0103	0.0103	0.0111[b]	F		3.33	27.89	0.53
	(+) host	0.0136	0.0125	0.0130	0.0126	0.0122	0.0128[a]	P		*0.022*	*<0.001*	*0.712*
	Average	0.0129[a]	0.0120[ab]	0.0120[ab]	0.0114[b]	0.0113[b]						

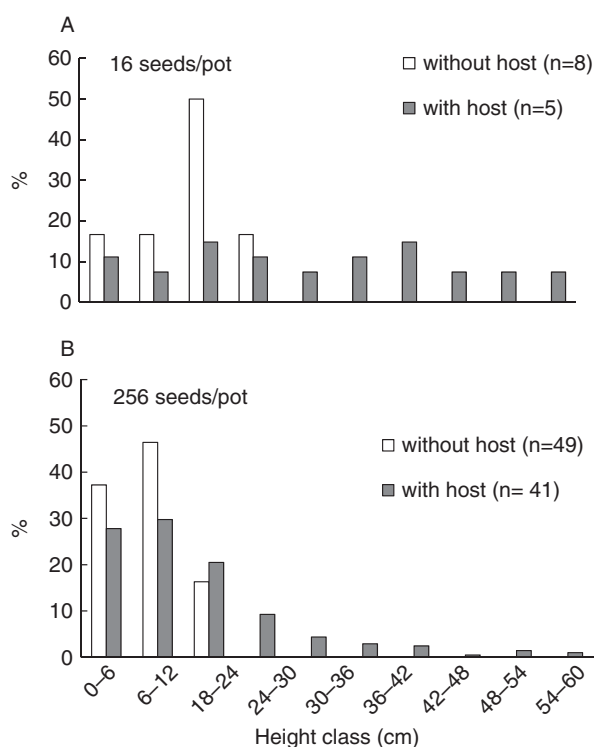Bold italic and italic values indicate significance differences at $P < 0.05$.

Figure 6 Plant height distribution of *Rhamphicarpa fistulosa* plants with and without a host at seed infestation levels of 16 and 256 seeds per pot, using 10 height classes with a width of 6 cm. Number of plants is expressed as a percentage to facilitate comparison between treatments. The actual number of established parasite plants (*n*) is mentioned in the legend (Experiment 5).

indicates that at this stage resource availability rather than number of individuals determines the dry matter production of the population (i.e. the law of constant final yield; Kira *et al.*, 1953). Most important for our study is the observation that the carrying capacity of the parasite population in the presence of a host was about three times higher than the carrying capacity in the absence of a host. The presence of a host plant thus greatly facilitates resource availability to the parasite, and this resulted in an even bigger difference in seed production. In the presence of a host, plants of *R. fistulosa* produced around 48 000 seeds per pot, compared with 13 000 seeds per pot in the absence of a host. On top of that, individual seed weight in the presence of a host was increased by 15%. Evidently, reproductive output of *R. fistulosa* greatly benefited from parasitism. Similar advantages of interaction with a host have also been reported for other facultative hemiparasites, like *Rhinanthus minor*, *Euphrasia frigida* and *Melampyrum sylvaticum* (Seel & Press, 1993).

Because seed production rate was significantly increased in the presence of a host, one might expect adaptations to have evolved that increase the likelihood of a successful attachment of the parasite to a host plant. Non-facultative parasitic plants, for which the presence of a host plant is a prerequisite for their survival, typically possess a host plant recognition mechanism at the germination stage. Accordingly, host plant root exudates have evolved into an absolute requirement for seed germination, in this way preventing seeds of the parasite from suicidal germination (Brown *et al.*, 1949; Xie *et al.*, 2010). In a similar manner, host root exudates could have evolved into a stimulatory agent for germination of facultative parasites, in this case not being an absolute germination requirement, but a means for lifting the reproductive output of the parasite. In our experiments, we did, however, not find any indication for such a stimulatory effect. In pot experiments, we did not find an increased emergence of *R. fistulosa* seeds in the presence of a rice host. This finding was supported by
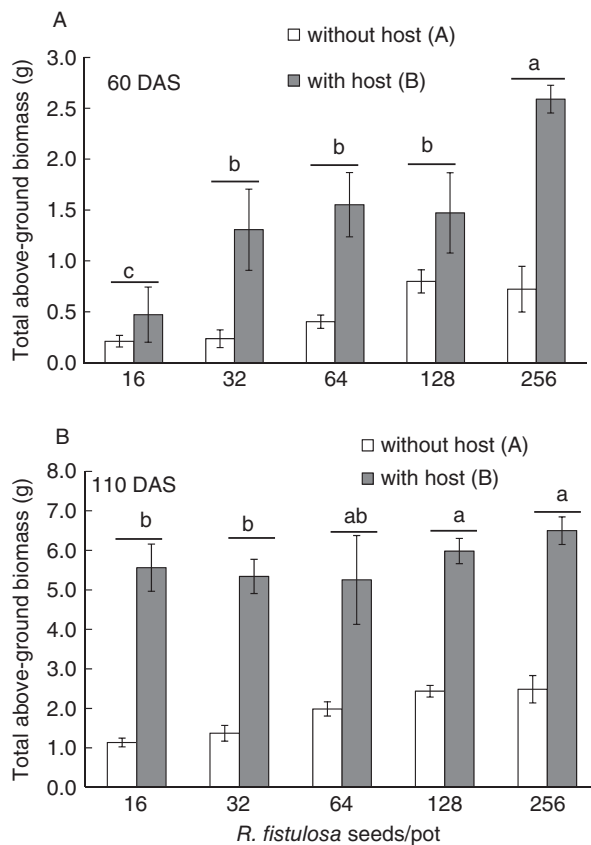
Figure 7 Total above-ground biomass of *Rhamphicarpa fistulosa* plants with and without a host at a range of *R. fistulosa* seed infestation levels, at 60 DAS and at maturity (110 DAS) (*n* = 5). Vertical bars are SEs of mean. Different letters on the legend indicate significant differences (*P* < 0.05) between plants with and without a host, whereas different letters on horizontal bars indicate significant differences between parasite densities (*P* < 0.05). Means and SE are back-transformed from log(*x* + 1) data (Experiment 5).

the germination experiment in Petri-dishes, in which the artificial germination stimulant GR24 and root exudates of three rice cultivars varying in exudate production rate were used. Germination percentages of *R. fistulosa* seeds in the presence of GR24 and the root exudates were not different to that of seeds exposed to plain water. The strong response of seeds of the obligate parasite *S. hermonthica* to GR24 and the root exudates, combined with the absence of germination in plain water, strengthens the validity of these results.

There might be different reasons for the absence of a stimulatory effect of host plant presence on *R. fistulosa* seed germination. One explanation is that the regulation of germination through the abiotic factors light and soil moisture is close to optimal. The presence of light prevents that germination is followed by failure of establishment, and sensitivity to water guarantees germination to occur when conditions for establishment and growth of

potential host species are also optimal. Particularly, if the parasite has a wide host range, the likelihood of the presence of a suitable host to attach to is quite large, minimising the added value of a specific host plant recognition mechanism. Till today, the actual host plant range of *R. fistulosa* has, however, not been established (Rodenburg *et al.*, 2015). Another explanation for the increased reproductive output not to have resulted in the evolvement of a specific host plant recognition mechanism might be the superiority of an opportunistic strategy. Particularly, if seed longevity is not extremely long, it might be more rewarding to germinate and produce seeds in the absence of a host, than to wait for a host plant that only appears after a large fraction of seeds has already deteriorated. Although Gbéhounou & Assigbé (2004) reported that seeds of *R. fistulosa*, remain viable for approximately 1 year, which would support this last explanation, a large body of research conducted on this topic is currently lacking.

The adaptation of *R. fistulosa* to saturated soils renders this parasitic weed problematic for rice production because rice is the only major food crop that can be grown under waterlogged soil conditions (Andriesse & Fresco, 1991). Such soil conditions are typically found in the rain-fed lowland rice production systems practised in inland valleys. In SSA, it is expected that there will be an intensification of such inland-valley land-use (Rodenburg *et al.*, 2014), implying a shift from natural wetland vegetation to a seasonal coverage by a rice crop. If this occurs, the problem of *R. fistulosa* may rapidly increase, as the density of suitable hosts for this parasite is much higher in a homogeneous rice crop situation compared with natural vegetation types that are commonly characterised by species mixtures. On the other hand, the fact that *R. fistulosa* seeds require light to germinate may imply that the plant is best adapted to natural vegetation without soil disturbance. In agricultural fields, part of the seeds shattered on the soil surface may be lost to unfavourable deeper soil layers because of seasonal tillage. Zero- or minimum-tillage crop production systems may form the exception. These systems, observed by Chauhan & Johnson (2009) to be unfavourable to a number of other common weeds, may benefit *R. fistulosa* as the soil is not disturbed and, consequently, weed seeds are left on the soil surface.

Our observations also enable the identification of potentially effective and ineffective management strategies of *R. fistulosa*. Deep ploughing as part of the land preparation may, for instance, be an effective method to bury newly produced seeds and prevent them from germination. The implication of the adaptation to wet soil conditions is that when farmers sow rice at the onset of rains, the parasite will emerge together with

its host, facilitating the parasite to attach to the roots of its host at the earliest stage possible and to benefit optimally from host assimilates. This principle can be used in the 'stale seedbed' technique, a common weed management strategy in lowland rice (Bond *et al.*, 2005; Rodenburg & Johnson, 2009). If, at the onset of rains, crop establishment is postponed for a few weeks, the first flush of *R. fistulosa* seedlings can be killed or removed before sowing the rice crop. The lack of host dependence for seed germination, observed with this facultative parasite, also disqualifies a certain category of management strategies that are effective against obligate parasites, that is those that make use of the stimulating effect of root exudates and other germination stimuli. For instance, the control of the obligate parasitic weed species of *Striga* can be achieved with the use of so-called false host species, grown in rotation or as intercrop with the cereal crop (Oswald *et al.*, 2002) that stimulate *Striga* seed germination without subsequently supporting successful parasitism. This mechanism, known as suicidal germination, cannot be applied to control *R. fistulosa*.

Our study showed novel insights in the germination ecology and reproduction biology of *R. fistulosa*. This in turn is useful for the identification and generation of potentially effective and ineffective *R. fistulosa* management strategies as shown above. Because the species is increasing in importance while generation of effective and affordable management strategies for affected rice farmers is lagging behind (Rodenburg *et al.*, 2011; Houngbedji *et al.*, 2014; Schut *et al.*, 2015), such insights and ideas are highly relevant for future rice production in SSA, particularly in the rain-fed lowland production systems.

Acknowledgements

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