Ecology and Biology of *Rhamphicarpa fistulosa*, a New Parasitic Weed of Rain-fed Rice (*Oryza sativa*) in sub-Saharan Africa

Stella Kabiri

Thesis committee

Promotor
Prof. Dr N.P.R. Anten
Professor of Crop and Weed Ecology
Wageningen University & Research

Co-promotors
Dr L. Bastiaans
Associate Professor, Centre for Crop System Analysis
Wageningen University & Research

Dr J. Rodenburg
Agronomist, Africa Rice Center (CGIAR)
Côte d'Ivoire, Ivory Coast

Other members
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Dr M. Fernández Aparicio Ruiz, Spanish National Research Council, Madrid
Dr J. van Ruijven, Wageningen University & Research

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“An antelope is brown because it hangs around anthills”

African proverb

Were papa uwemwikhulu, nasimire naabi

Abstract

Stella Kabiri (2017). Ecology and Biology of *Rhamphicarpa fistulosa*, a New Parasitic Weed of Rain-fed Rice (*Oryza sativa*) in sub-Saharan Africa. PhD thesis, Wageningen University, Wageningen,The Netherlands**,** with summaries in English and Dutch, 130 pp.

Rice is an important staple food crop in Africa. The increasing scarcity of agricultural land has driven rice growers to expand into marginal areas that have natural infestations of *Rhamphicarpa fitulosa*. In return*, R. fistulosa* has increasingly become a serious problem to rice production in sub-Saharan Africa. To date, the understanding of the ecology and biology of the species and its dependence and effects on a host, is rather limited. The discrepancy between the emergence of this weed problem and the virtual absence of knowledge on the weed species motivated the study presented in this thesis.

In a field survey in Tanzania, *Striga asiatica* was observed in higher lying and drier fields, while *R. fistulosa* was observed in the lower lying wetter fields. Experiments confirmed that *S. asiatica* is favoured by free-draining soils and *R. fistulosa* by water-logged soils. These results imply that changes in climate, specifically moisture regimes, will be crucial for the future prevalence of both parasitic weed species. In a second investigation, I found that daylight and completely saturated soil conditions were prerequisites for germination, demonstrating that *R. fistulosa* is a typical species of environments with fluctuating water levels. Neither root exudates collected from rice host plants, nor the synthetic germination stimulant GR24, triggered germination of *R. fistulosa* seeds. Host plant presence resulted in a 3.7 times higher seed production rate and a 15% larger average seed size. The absence of a host recognition mechanism at the germination stage suggests that either the regulation of germination through light and soil moisture is near optimal, or that for this parasitic plant species an opportunistic germination strategy is superior. In a third study, I observed that infection by *R. fistulosa* led to significant reductions in leaf photosynthetic rate, stomatal conductance, the quantum efficiency of PSII (Φ_{PSII}) and chlorophyll content of rice. In addition, there was a 19-32% negative deviation of the linear relationship between quantum yield of CO_2 assimilation (Φ_{CO2}) and quantum efficiency of PSII (Φ_{PSII}) of infected plants in comparison to un-infected plants. This indicated a parasite induced influence on the photochemical process of the host. Furthermore, there was a considerable time lag between the parasite's gains in growth and the reduction of host photosynthesis. The reduction in host growth, coincided with suppression of host photosynthesis. This indicates that *R. fistulosa* affects host growth by first extracting assimilates and making considerable gains in growth, before it affects the host photosynthesis. In the final investigation, I examined how the interaction between host plant and parasite influenced growth and (re)production of *R. fistulosa* and rice. Infection by *R. fistulosa* increased root:shoot ratio and decreased plant height, leaf area and tiller number of rice. Reductions in light interception of the host were

followed by reductions in light use efficiency, causing 22-71% losses in host plant biomass and 78-100% losses in host kernel production. Parasitism eventually caused a complete standstill of host plant growth, while the parasite managed to gradually increase its share of the total host plant-parasite biomass up to 50-82%. This implies that ultimately the host plant was producing solely for the sake of the parasite.

In a final chapter, I discuss the implications of my findings for the future expansion of this parasitic weed, specifically in light of climate change. I also discuss how the divergent ecology and biology of *R. fistulosa* is likely to influence the effectiveness of measures that are currently applied to manage *Striga* spp. I argue that more than the current attention needs to be paid to *R. fistulosa,* specifically for the problems it causes to the rice sector in sub-Saharan Africa.

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Chapter 1

General Introduction

1.1 Background

Rice is an important crop to fight food insecurity in Africa where consumption of rice has grown faster than any other major staple crop (Seck *et al.*, 2013). In recent years the annual per capita consumption has increased by over 60 % (Diagne *et al.*, 2011). Countries with the highest consumption of rice (per capita per annum consumption ranging between 70-120 kg) include Madagascar, Guinea Bissau, Côte d'Ivoire, Senegal, Sierra Leone, The Gambia, Guinea and Gabon (Balasubramanian *et al.*, 2007; Seck *et al.*, 2012). Some of these countries with the highest consumption of rice also have a relative large share of their total area of arable land under rice. These include; Madagascar (1.2 million ha), Côte d'Ivoire (0.97 million ha) and Sierra Leone (0.60 million ha) (Diagne *et al.*, 2013). Nigeria with 1.9 million ha rice area is the largest rice producer in Africa, followed by Madagascar and Guinea (1 million ha). Other countries include Tanzania (0.94 million ha), Mali (0.64 million ha), and Egypt (0.56 million ha) (Diagne *et al.*, 2013). Currently, 9.9 million ha in sub-Saharan Africa (SSA) is under rice cultivation producing 25 million tons of rice per annum (Seck *et al.*, 2012). However, despite the large area under rice production, nearly 40% of the rice consumed in Africa is imported (Seck *et al.*, 2010). This is to meet the growing demand for food to feed the population that has tripled, from 279 million to 826 million, in the past forty years (AGRA, 2014; Haub and Kaneda, 2013).

The increasing scarcity of good arable land necessary to produce food for the growing population has driven rice growers to expand into marginal areas. Newly opened fields in marginal areas are often characterized by low or heterogeneous soil fertility, escalating the problem of weeds and parasitic weeds that thrive in areas of low fertility (Rodenburg and Johnson, 2009). Moreover, the agricultural exploitation of new land also implies a change in vegetation. Such a situation may cause an increase in specific weed problems. For instance, with respect to parasitic weeds, the change from 'natural' vegetation characterized by a high species diversity — with suitable hosts alternated by non-host species —, to a 'cultivated' vegetation characterized by mono-culture/one dominant species — a suitable host species for parasitic weeds —, creates ideal conditions to build up weed populations. These weeds become particularly problematic under conditions of subsistence farming characterized by low-input production methods. Consequently, cultivating suitable host crops in habitats of parasites well adapted to the natural ecosystem has raised the economic status of previously unimportant parasitic weeds.

Obligate parasites cannot germinate or survive without a haustorial connection to a host (Estabrook and Yoder, 1998) while facultative parasites can complete all life cycles even in the absence of a host (Gibson and Watkinson, 1989) but usually benefit considerably from attachment to a host (Ouédraogo *et al.*, 1999). These root hemiparasitic species are serious agricultural weeds of cereals and legume crops where they cause substantial yield losses (Parker, 2013; Ejeta and Gressel, 2007). Parasitic weed inflicted yield losses in cereals surpass

40% although the extent of losses varies among parasite species, crop species and cultivars (Ejeta and Gressel, 2007).

1.2 Parasitic weeds of rice and what we know about them

One of the most investigated genera of parasitic weed species, is *Striga* of the family Orobanchaceae. Of the 40 *Striga* species more than 55% are widespread in Africa (Olmstead *et al.*, 2001). Of the species in Africa, 11 are important parasitic weeds of agricultural crops (Berner *et al.*, 1995). *Striga hermonthica* (Del.) Benth. and *Striga asiatica* (L.) Kuntze. are the most widespread and damaging to cereal crops estimated to affect millions of hectares across SSA (Parker, 2009). Heavy infestation of *Striga* spp can be found throughout sub-Saharan Africa (Ejeta and Gressel, 2007).

Seeds of *Striga* spp. have a dormancy period of six months, an adaptation that prevents germination when host crops in the fields are not available after harvesting (Berner *et al.*, 1997). To germinate, the seeds require a preconditioning period with moist and relative warm conditions, and germination stimulants from root exudates secreted from a host (Ejeta and Gressel, 2007). Some non-host plants are known to release root exudates that can stimulate germination of *Striga* spp. In the absence of a host, endosperm nutrients can only sustain the seedling for up to 3 to 7 days after which the seedling dies (Worsham, 1987). When the seedlings are in close proximity with a host root they develop specialized structures known as haustoria, that can establish xylem-to-xylem connection with the host which facilitates the transfer of nutrients, carbon and water from host to parasite (Parker and Riches, 1993; Press *et al.*, 1987). After attachment to the host root, *Striga* plants grow underground for 3-7 weeks before they emerge above ground. *Striga* emergence can occur throughout the entire season. *Striga* infection symptoms on the host, range from stunting and resemblance to vascular chlorosis, to leaf necrosis and drought stress. Some of these symptoms are observed before the parasite emerges above ground, a characteristic to which its popular name ‘Witchweed’ refers (Ejeta and Gressel, 2007).

In contrast with *Striga* spp., we know much less of the other parasitic weed that constrain cereal production, *R. fistulosa.* This parasitic weed has become a more serious problem to rice production in SSA in recent years. What is known is that *R. fistulosa*, is a facultative root hemi-parasite of the family Orobanchaceae that is not only native to Africa but is widely distributed in the continent (Hansen, 1975; Kuijt, 1969; Parker and Riches, 1993). It favours open sunny grasslands but is well adapted to temporary wetlands or waterlogged soils near streams and can even be found in brackish water near the sea (Friis and Vollesen, 2005; Hansen, 1975; Ouédraogo *et al.*, 1999). The species most likely has a wide host range but is most often found parasitizing rice a crop that is ecologically well adapted to waterlogged environments (Andriesse and Fresco, 1991), a similar environment to where *R. fistulosa* is found. Since rice cultivation is expanding, *R. fistulosa* has the potential to become more problematic in the near future through regional spread. To date, the understanding of the ecology and biology of the species *R. fistulosa*, and its dependence and effects on a host, is rather limited, in particular when compared to the widely studied *Striga* species. A more profound understanding would be instrumental to assess the extent of the problem and the directions it can evolve in to, and it will lay the necessary foundation for the development of effective management strategies. Therefore the study of this thesis is dedicated to unravel the ecology, biology and host-interactions of the facultative *Rhamphicarpa fistulosa*.

1.3 Problem Statement

Rhamphicarpa fistulosa has been observed to parasitize cowpea, sorghum, maize and rice (Ouédraogo *et al.*, 1999; Parker and Riches, 1993). Being facultative, *R. fistulosa* can complete its life cycle as a free living plant but benefits considerably from attachment to a host (Ouédraogo *et al.*, 1999). The facultative nature of the parasite may limit management options. For instance the obligate parasitic weed *Striga* that cannot survive without attachment to a host, can be managed by crop rotation or intercropping with trap and catch crops (Parker and Riches, 1993; Khan *et al.*, 2002; Showemimo *et al.*, 2002; Cechin and Press, 1993). These agronomic practices of controlling *Striga* based on host plant absence or suicidal germination caused by false hosts are ineffective for controlling *R. fistulosa* because the species can survive without a suitable host. In fact, the design of control methods that are effective require a more thorough understanding of the seed ecology and biology as well as the interaction with its host. For instance, there is an incomplete understanding of the environmental factors that favour the germination and prevalence of this parasitic weed. Moreover, the timing and mechanisms of the host-parasite interactions between *R. fistulosa* and its host are yet unknown.

1.4. Thesis Objectives

The overall objective of this study was to unravel the ecology of the facultative *Rhamphicarpa fistulosa*. I investigated the effect of light and germination stimulants on germination and the effects of soil moisture on overall parasite growth by examining how each phase of the parasite’s life cycle directly or indirectly interacts with the host to cause an eventual effect on its productivity. These stages of the life cycle include germination, parasite growth and reproduction. The specific objectives of thesis are described.

1.4.1 Objective 1: To understand the ecological niche of Rhamphicarpa fistulosa

The distribution of *R. fistulosa* in Africa is widely dispersed such that the distribution of *Striga asiatica* overlaps in *R. fistulosa* areas (Hansen, 1975; Mohamed *et al.*, 2001; Ouédraogo *et al.*, 1999). Despite their distribution in similar regions on the continent, and the assumed negative effects they have on the rice sector, more precise information concerning their ecological plasticity in terms of soil-water status, soil fertility, association with other weed species or whether their habitats are mutually exclusive is not known. Knowing this would be of importance for the design and development of management strategies and guidelines and policy plans on a higher hierarchical level (e.g. national crop protection services). Moreover, as the area used for rice production expands, these weeds may spread and establish into new areas. Whether or not such spread is limited by the ecological requirements of these species is not known. Hence, to get a better understanding of the risks of future expansion, it is important to determine the habitat ranges of these weeds. Knowing this range is particularly relevant in the face of climate change, where rainfall patterns have been predicted to change and to become more erratic (IPCC, 2007).

1.4.2 Objective 2: To investigate the influence of a rice host on the germination and reproduction of Rhamphicarpa fistulosa

The complete host range of *R. fistulosa* has not been established, but the parasitic weed has been observed to parasitize cowpea, sorghum, maize and rice (Ouédraogo *et al.*, 1999; Parker and Riches, 1993; Rodenburg *et al.*, 2011). Currently, the area of expansion of rice cultivation is in marginal wetlands, the natural habitat of the parasite (Rodenburg *et al.*, 2014a). It is thus the adaptation to ephemeral wet areas (Hansen, 1975; Muller and Deil, 2005; Ouédraogo *et al.*, 1999) that makes *R. fistulosa* specifically problematic to rice, the sole staple food crop that can be grown in such environments.

According to Ouédraogo (1999) the germination biology of *R. fistulosa* seeds bears a resemblance to seeds of *Striga angustifolia*, characterized by Krause (1990), *Castilleja coccinea* and *Buchnera hispida*, characterized by Logan and Stewart (1992). They postulate the need for light to stimulate germination as a compensation for the absence of host-derived stimulants. However it is not clear why *R. fistulosa* does not respond to host-derived stimulants. As *R. fistulosa* plants that associate with a host plant grow taller and produce more seeds (Ouédraogo *et al.*, 1999) one could assume that it would be more beneficial for the parasite to have a germination mechanism depending on host presence. This would ensure that its precious seeds only germinate when the likelihood of parasitizing a suitable host is high. The second objective of this thesis is therefore to investigate the host-dependency of *R. fistulosa* at different stages, to gain insights in the more opportunistic strategies involving germination and reproduction employed by a facultative parasitic plant.

1.4.3 Objective 3: To investigate the mechanism involved in the interaction between R. fistulosa and its host and the eventual effect of this interaction to its host.

Parasitism of *R. fistulosa* has a negative effect on the host plant, resulting in severe yield reductions. However, little is known about the mechanisms involved in the interaction between *R. fistulosa* and its host. In some host-parasite associations, the negative impact parasitic plants have on their hosts is practically restricted to withdrawal of assimilates (Jiang *et al.*, 2003b). Some of the Orobanchaceae also reduce the photosynthetic rate of their host, thereby hampering host plant biomass accumulation (Press and Stewart, 1987; Cechin and Press, 1993; Watling and Press, 2001). In other cases, the assimilate production of host plants has been reported to be inhibited through a direct negative impact of the parasite on the photosynthetic capacity of its host (Watling and Press, 2001; Cameron *et al.*, 2005) whereby the extent of this impact varies between host species (Graves *et al.*, 1989; Watling and Press, 2001; Hibberd *et al.*, 1998a). Other studies demonstrate that most members of the Orobanchaceae family manipulate their hosts to allocate more carbon to the roots which, in turn, is partly withdrawn by the parasite (Hibberd *et al.*, 1998b; Watling and Press, 2001). This withdrawal of assimilates and nutrients by the parasite is at the cost of host plant growth. The interaction between host plant and parasite ultimately determines the reduction in biomass accumulation and kernel production of the host plant. Whether the interaction between *R. fistulosa* and rice can be described by either of these mechanisms is yet unknown.

Therefore, understanding the impacts of *R. fistulosa* on photosynthesis of its rice host and the magnitude of these impacts and the mechanisms behind it will provide valuable insights in the virulence potential of the parasite. Such information in turn may eventually be used to formulate, design and develop mitigation strategies, such as the identification or generation of rice genotypes that are able to better withstand infection.

Not much is known about how facultative parasites influence their hosts, particularly those facultative parasites that can become weedy. To characterize the timing and extent of consequences of parasite infection, the host plant biomass accumulation and partitioning need to be studied. How does host-damage relate to parasite infestation level and how does the interaction between host plant and parasite develop over time? Such characterisation of parasite – host interaction is one of the necessary first steps to formulate, design and develop possible solutions to this problem and to advise affected farmers on the timing of control.

1.5 Research Questions of this Thesis

Based on the research objectives mentioned above, this thesis seeks to answer the following research questions.

1. What is the habitat range and ecological niche of *Rhamphicarpa fistulosa*?
2. Is seed germination of *R. fistulosa* stimulated by the presence of a host plant?
3. What is the magnitude of benefit for *R. fistulosa* from association with a host plant?
4. How and to what extent does *R. fistulosa* impact the photosynthetic capacity of its host?
5. What are the consequences of *R. fistulosa* attachment to biomass accumulation of its host and the timing of these consequences to the host?

Chapter 2

Can the parasitic weeds *Striga asiatica* and *Rhamphicarpa fistulosa* co-occur in rain-fed rice?

Stella Kabiri[1], Jonne Rodenburg[2], Juma Kayeke[3], Aad van Ast[1], Derick W. Makokha[2], Saidi H Msangi[4], Runyambo Irakiza[2] & Lammert Bastiaans[1]

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[1]Centre for Crop Systems Analysis, Wageningen University & Research, the Netherlands
[2]Africa Rice Center (AfricaRice), Dar es Salaam, Tanzania
[3]Mikocheni Agricultural Research Institute, Dar es Salam, Tanzania
[4]Sokoine University of Agriculture, Morogoro, Tanzania

ABSTRACT

Striga asiatica and *Rhamphicarpa fistulosa* are important parasitic weeds of rain-fed rice, partly distributed in similar regions in sub-Saharan Africa (SSA). It is not evident whether their ecologies are mutually exclusive or partially overlapping. In Kyela, a rice growing area in south Tanzania where both parasites are present, three transects of about 3 km each across the upland-lowland continuum were surveyed in June 2012 and 2013. A total of 36 fields were categorised according to their position on the upland-lowland continuum as *High, Middle* or *Low* and soil samples were taken. In each field, parasitic and non-parasitic weed species were identified in three quadrats. Additionally, in two pot experiments with four different moisture levels ranging from wilting point to saturation, influence of soil moisture on emergence and growth of parasites was investigated. *Striga asiatica* was observed in higher lying drier fields, while *R. fistulosa* was observed in the lower lying wetter fields. Furthermore, non-parasitic weed species that were exclusive to *S. asiatica*-infested fields are adapted to open well-drained soils, while species that were exclusive to *R. fistulosa* fields are typical for wet soils. The experiments confirmed that *S. asiatica* is favoured by free-draining soils and *R. fistulosa* by waterlogged soils. These results imply that changes in climate, specifically moisture regimes, will be crucial for future prevalence of these parasitic weeds. The non-overlapping ecological range between their habitats suggests that their distribution and associated problems might remain separate. Thus, management strategies can be focused independently on either species.

Keywords: Parasitic weed species, climate change, niche, ecology, agro-ecosystems, soil moisture.

2.1 Introduction

Striga asiatica (L.) Kuntze and *Rhamphicarpa fistulosa* (Hochst.) Benth. are parasitic weeds causing considerable yield losses to rain-fed rice in sub-Saharan Africa (SSA) (Johnson, 1997; Mohamed *et al.*, 2001; Ouédraogo *et al.*, 1999; Rodenburg *et al.*, 2011). It is partly due to such production constraints that a large gap remains between regional production and consumption in SSA (Seck *et al.*, 2010). Both *S. asiatica* and *R. fistulosa* are root hemi-parasites of the family Orobanchaceae that extract host assimilates through a developed attachment organ known as haustorium, but also produce assimilates independently through photosynthesis (Cochrane and Press, 1997; Ouédraogo *et al.*, 1999). *Striga asiatica* is an obligate parasite and cannot survive without attachment to a host (Cochrane and Press, 1997), while *R. fistulosa* is facultative, as it can complete its life cycle as a free living plant but benefits considerably from attachment to a host (Ouédraogo *et al.*, 1999).

The rain-fed rice (*Oryza sativa* L and *O. glaberrima* Steud.) area in SSA covers 7 million hectares (Diagne *et al.*, 2013). Rice consumption in the last decade has increased by 4.6%, which is twice as much as the continent's 2.6% population growth (USDA, 2012). Despite increased rice production, this demand has not been met and as a result, SSA has become increasingly dependent on rice imports (Seck *et al.*, 2010). Furthermore, cultivation of rice has been expanded into marginal rain-fed uplands and lowlands (Rodenburg *et al.*, 2014a) which are often natural habitats of parasitic weeds (Raynal Roques, 1994). *Striga asiatica* is mainly distributed in eastern and southern Africa (Mohamed *et al.*, 2001), whereas *R. fistulosa* is distributed in Sahelian regions (Senegal to Ethiopia), East Africa, South Africa and Madagascar (Hansen, 1975; Ouédraogo *et al.*, 1999).

Despite their distribution in similar regions on the continent and the assumed negative effects they have on the rice sector, quantitative information on habitats of these parasitic weed species is scarce. As the area used for rice production expands, these weeds may spread and establish into new areas. If we are to find out whether areas of these species are likely to expand in future, it is important to determine their habitat range. Knowing this range is particularly relevant in the face of climate change, where rainfall patterns have been predicted to become more erratic (IPCC, 2007). In order to understand habitat characteristics of both *S. asiatica* and *R. fistulosa* in rain-fed rice, a field study was carried out in Kyela, a major rice growing area in southern Tanzania. Supplementary pot experiments were conducted to substantiate the role of soil moisture. We tested the hypothesis that the ecological niches of the two parasites are mutually exclusive, resulting in rain-fed rice ecosystems that will form a habitat for either *S. asiatica* or *R. fistulosa.*

2.2 Materials and Methods

2.2.1 Field survey

In June 2012 and June 2013, field surveys were conducted in Kyela, in southern Tanzania, between 35º41’30’’ E and 9º25’40’’ S. Kyela lies around 500 m above sea level where the main rice cultivation months range from January to June. Rainfall is of the unimodal type with an annual average of around 3000 mm, while average temperatures range from 19˚C to 23˚C in cool months (May to October) and 29˚C to 31˚C in hotter months (November to April). Rice is grown along the upland–lowland continuum without clear boundaries between upland, hydromorphic and lowland zones.

Rainfall data were collected during rice growing seasons of 2012 and 2013 from rain gauges installed in *Striga*-infested areas and in *Rhamphicarpa*-infested areas and averaged over the two measuring points. An additional seven-year period of rainfall data from 2005 to 2011 was provided by the office of the District Agricultural Livestock Development Officer (DALDO) of Kyela.

During the surveys, three transects of about 3 km each in length were aligned across the upland-lowland continuum, from the road to the river (Fig. 1). Each transect covered 12 rice fields ranging from 0.5 to 1 ha in size. A total of 36 rice fields were thus surveyed. The rice fields in each transect were subdivided into high, middle and low categories according to their location on the continuum, i.e. Fields 1 to 4 positioned close to the road were denoted as *High*, low lying fields located closer to the river, 9 to 12, were denoted as *Low*, and fields in between *High* and *Low*, 5 to 8, were denoted as *Middle*. This resulted in a total of 12 rice fields for each category.

In 2012, three quadrats (1 × 1 m) were randomly placed within each field. Location and elevation of each quadrat was recorded using a global positioning system (GPS) device (eTrex Legend; GARMIN International Inc., USA). A 440 cm^3 soil sample was taken from the top 10 cm using closable 10 cm length PVC pipes. Wet and dry weights of each soil sample were recorded to determine soil moisture content. One soil sample from each of the 36 rice fields was analysed for texture, organic matter (OM%), nitrogen (N%), pH, electrical conductivity (EC), cationic exchange capacity (CEC), phosphorus (P), potassium (K), magnesium (Mg), calcium (Ca) and sodium (Na). Variation of soil characteristics of rice fields of *High*, *Middle* and *Low* categories were tested via one-way ANOVA using statistical software GenStat for Windows 15th Edition (GenStat, 2000-2013).

2.2.2 Species diversity

Each rice field was visually surveyed for infestation of *S. asiatica* and *R. fistulosa* and, if present, their average cover scored on a 1- 5 scale of Braun-Blanquet (1932) (Table 1). Based on infestation, rice fields were classified as *Striga*-infested, *Rhamphicarpa*-infested, *Striga* and-*Rhamphicarpa* infested or parasite-free.

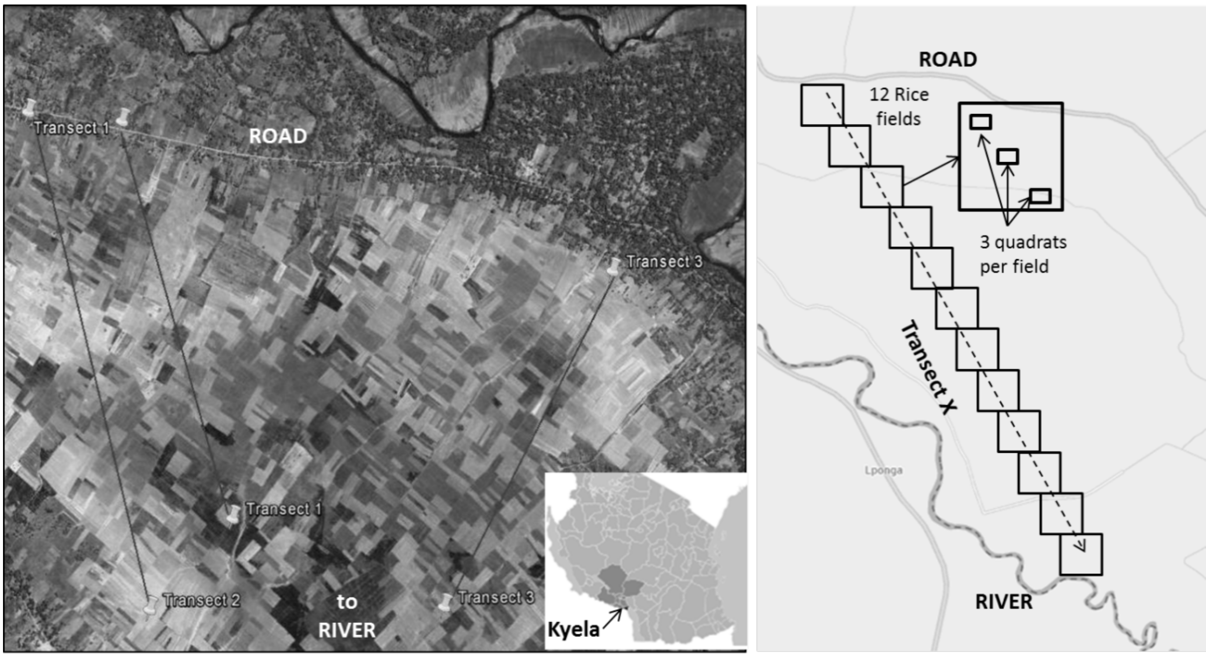

Figure 1. A Google Earth image showing three transects taken in Kyela district, south of Tanzania in June 2012 and 2013. The transects walks started from the road connecting the three rice cultivating villages, Kilasilo, Mbako and Kaziba, and trailed from upland to lowland (road to river). On the side of the figure, a schematic transect is shown covering 12 rice fields in which 36 quadrats were installed.

Presence of both parasitic and non-parasitic weed species were identified and recorded per quadrat (n=108) using the AFROweeds identification tool (CIRAD-AfricaRice, 2012) and the field guide of Johnson (1997). Data on occurrence of plant species obtained from the quadrats were used to calculate the Shannon-Wiener plant species diversity Index (H') for *Striga*-infested, *Rhamphicarpa*-infested and parasite-free rice fields following equation 1:

$$\mathrm{H}' = -\sum_{\mathrm{i}=1}^{\mathrm{s}} (P_i)(\ln P_i) \qquad (1)$$

where $P_i = n_i / \sum_{i=1}^{s} n_i$, with n_i = number of quadrats in which species *i* was observed and *s* refers to total number of observed plant species. The H' value indicates diversity of species present in the set of quadrats, and may range from 0, when just one species is present in all quadrats, to about 4.5 for a wide range of species (Molles, 1948). The relationship between species was determined by a distance and similarity measure generated from Hierarchical Cluster Analysis (HCA) with Pearson's correlation as the proximity procedure using statistical software SPSS version 20 (SPSS, 2011).

Table 1. Species cover classes of Braun Blanquet's five point scale (Braun-Blanquet, 1932)

Cover Class	% of species coverage
0	No parasite species observed
1	< 5 %
2	5 – 25 %
3	25 – 50 %
4	50 – 75 %
5	75 – 100 %

2.2.3 Pot experiments

Two pot experiments were conducted, the first (Experiment 1) in a screen house at Mikocheni Agricultural Research Station (MARI), located in Dar es Salaam in Tanzania, from January to June 2012. Experiment 2 was established in a screen house at Sokoine University of Agriculture (SUA), located in Morogoro in Tanzania from September 2012 to January 2013. The design was a split-plot with five replicates containing four moisture levels at main plot level and parasitic weed species (*S. asiatica* and *R. fistulosa*) at subplot level. A total number of 40 pots, sealed at the bottom, were used in each experiment. The pots had a capacity of 15 L with a diameter of 26 cm and were filled with a dry mixture of sand and loam soil (5:1 v/v) (≈ 17 kg soil mixture). To determine field capacity, ten pots with the same dry mixture of sand and loam soil, but with holes at the bottom, were weighed and then gradually filled with water, until addition of any extra water created a tiny flood layer. The pots were then left to drain freely for 48 hours and weighed again. This way it was established, that pots at field capacity [FC] contained around 2 L of water. Accordingly, moisture levels were installed in the pots without holes by adding 1 litre of water per pot [FC-1 L] (resulting in drier soil conditions), 2 litres of water per pot [FC] (resulting in field capacity conditions), 3 litres of water per pot [FC+1 L] (resulting in a moderately saturated soil) and 4 litres of water per pot [FC+2 L] (resulting in a totally saturated soil). The weight of each pot was then recorded and pots were regularly weighed and watered to retain the intended moisture level.

The parasitic weed seeds used were collected in Kyela in 2009 from farmers' fields. Germination percentages were 40% (for *S. asiatica*) and 60% (for *R. fistulosa*). In the first experiment, about 2400 viable seeds of *S. asiatica* (0.022 g) were mixed through the upper 10 cm of the pot. For *R. fistulosa*, with seeds requiring daylight to germinate, about 150 viable seeds (0.00315 g) were thoroughly mixed with 100 cm^3 of soil and spread evenly on the soil surface in each pot. In Experiment 2, the added amount of seed was doubled for both parasites because in Experiment 1 the achieved emergence was sub-optimal. Two rice seeds were then sown in the centre of each pot. Around 2 weeks after emergence, rice seedlings were thinned to one rice plant per pot. Above-ground *S. asiatica* and *R. fistulosa* numbers were counted every three days, starting from 7 days after sowing (DAS). At 60 DAS, all above-ground plant parts of *S. asiatica* and *R. fistulosa* were cut and dried at 70°C for 48 hours. Root systems of rice with *S. asiatica* and rice with *R. fistulosa* were washed and placed in plastic bags and cooled at 4°C for 2 days. This was to enable both *S. asiatica* and *R. fistulosa* roots to turn into a dark blue shade, which permitted separation from rice roots that retained a lighter hue. *Striga asiatica* and *R. fistulosa* roots were then separated from rice roots and dried at 70°C for 48 hours to determine dry weights.

Data on emergence and dry weight of both parasites was subjected to Analysis of Variance (ANOVA) using statistical software package GenStat for Windows 15th Edition (GenStat, 2000-2013). Count data (i.e. number of emerged parasite seedlings) were transformed prior to analyses to meet assumptions of ANOVA, using log(x+1), where x is the original observation (Sokal and Rohlf, 1995). The analysis was then followed by comparison of means by Least Significant Difference (LSD).

2.3 Results

2.3.1 Rainfall

Average total rainfall in the growing season of the last 7 years (2005-2011) was 2326 mm. The rainfall distribution showed that the rainy season in Kyela begins in January, peaks in April, declines in May and finally drops in June (Fig. 2). The rainfall pattern in 2012 showed a similar trend with the exception of May that was above the 7-year average. In 2013, rainfall was clearly below the 7-year average for the month of April. Total rainfall received during the rice growing season was 2260 mm in 2012 and 1255 mm in 2013 creating a 1005 mm difference in precipitation between the years.

2.3.2 Transects

During the transect walks, it was observed that soils in the lowlands (*Low*) were completely flooded, while those in the uplands (*High*) were dry. In addition, soils in the transition between lowlands and uplands (*Middle*) were moderately wet. For all three transects, there were clear differences in elevation, i.e. 514 m to 529 m (Transect 1), 516 m to 533 m (Transect 2) and 513 m to 525 m (Transect 3). The elevation differed significantly, with *Low* fields on average 1.7 and 3.0 m lower than *Middle* fields and *High* fields, respectively.

All fields had saline acidic soils, with low levels of nitrogen, phosphorus and organic matter, while they had high levels of sodium, potassium, calcium and magnesium. Soil moisture was significantly higher in *Low* fields and lowest in *High* fields (Table 2). In addition to this obvious difference in soil moisture content, some other differences were observed. Soil calcium (Ca) and sodium (Na) contents and cationic exchange capacity (CEC) were

Table 2. Mean values of soil characteristics from rice fields located at *High* (n=12), *Middle* (n=12) and *Low* (n=12) elevations

					mgkg^{-1}							
Position on the slope	Rep	Moisture (g/dm^3)	OM %	N %	P	K	Ca	Mg	Na	pH	C.E.C (meq/100)	E.C. (S)us/ cm
High	12	598^{b*}	2.18	0.14	6.05	267^a	433^b	101^b	59^b	5.33	6.7^b	51.2
Middle	12	617^b	2.11	0.11	6.45	199^b	544^b	111^{ab}	75^b	5.45	7.2^b	50.8
Low	12	774^a	2.19	0.13	4.71	199^b	706^a	143^a	99^a	5.45	9.1^a	65.2
	p	0.023	*n.s.***	*n.s.*	*n.s.*	*0.043*	*0.006*	*0.042*	*0.004*	*n.s.*	*0.007*	*n.s.*

* means followed by a different letter are significantly different between elevations
** *n.s.* indicates not significant

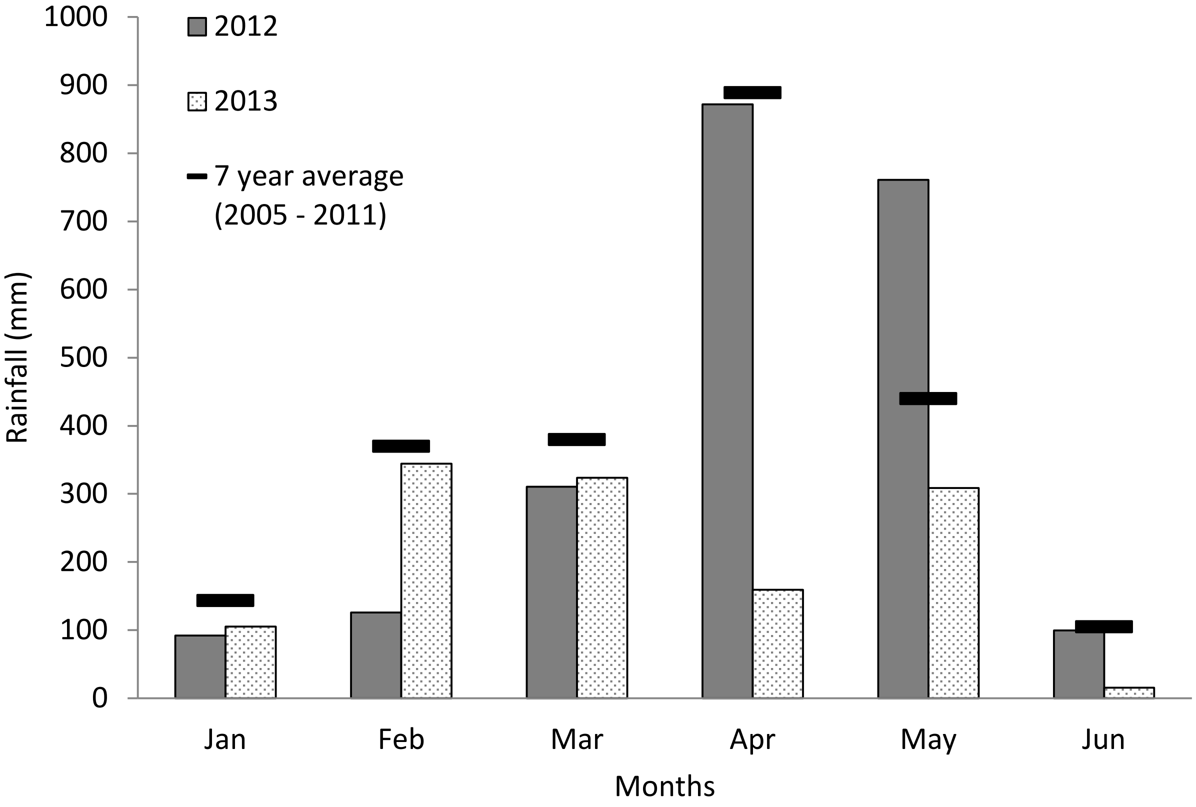

Figure 2. Rainfall distribution of Kyela during the main rice crop calendar of the years 2012 and 2013. The black bar (▬) represents 7-year monthly average rainfall received in the first six months of the years 2005 to 2011.

significantly higher in *Low* fields than in *High* fields. Soil potassium (K) content was highest in *High* fields, while it did not differ between *Low* and *Middle* fields. Soil pH, organic matter (OM%), nitrogen (% total N) and phosphorus (P) contents did not differ between the three positions on the slope.

In 2012, in all three transects, *S. asiatica* was observed in 92% of rice fields located in the *High* parts of the slope and 42% of fields in the *Middle* part of the slope. *Striga asiatica* was not observed in the *Low* parts of the slope. *Rhamphicarpa fistulosa* on the other hand was observed in 100% of the low-lying fields (*Low*), 8% in the *Middle* part of the slope, but was not observed in the *High* part of the slope (Fig. 3). There were 16 *Striga*-infested fields, 13 *R. fistulosa* infested fields and seven parasite-free fields. Coverage of parasitic weeds was generally higher in *Rhamphicarpa*-infested fields than in fields infested with *S. asiatica*. Just over 85% of *Rhamphicarpa*-infested fields had a parasite cover between 50 - 100%, whereas 69% of *Striga*-infested fields had a parasite cover of less than 5%. In between parasite-infested fields there were parasite-free fields, showing a clear demarcation between the two species. In 2013, *S. asiatica* was observed in 100% of the fields located in the *High* parts of

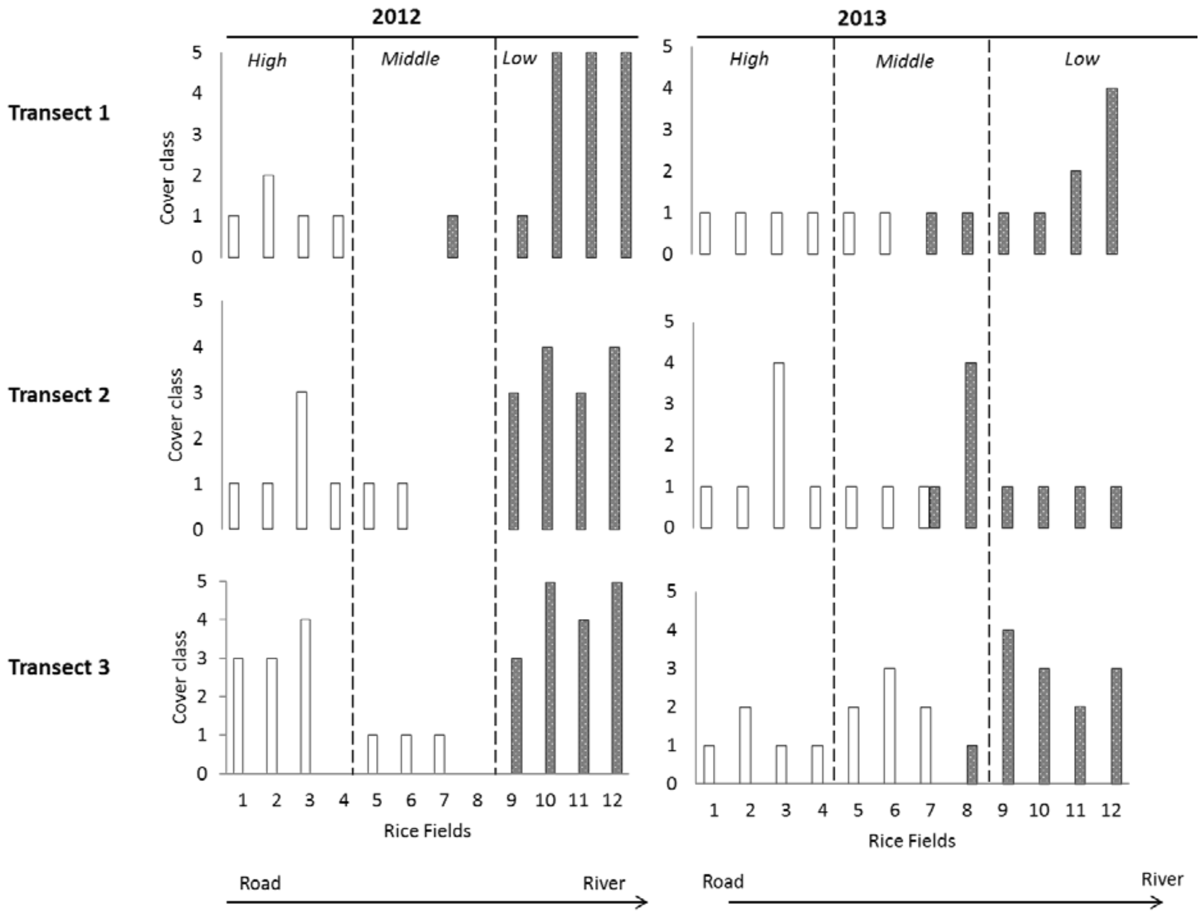

Figure 3. Parasitic weed cover class (0 = no parasite species observed, 1 = < 5%; 2 = 5 – 25%; 3= 25 - 50%; 4 = 50 - 75%; 5 = 75 - 100%) for *Striga asiatica* and *Rhamphicarpa fistulosa* in three transects in Kyela in 2012 and 2013. The light bars indicate the cover class of *S. asiatica*-infested fields, while the dark bars indicate the cover class of *R. fistulosa*-infested fields. Numbers refer to the position of the rice field along the transect. 1, 2, 3 and 4 are rice fields in the uplands (*High*), 5, 6, 7 and 8 are fields at middle elevation (*Middle*) while 9, 10, 11 and 12 are fields in the lowland (*Low*).

the slope and in 67% of the fields in the *Middle* parts while the species was not observed in the *Low* parts. *Rhamphicarpa fistulosa* on the other hand was observed in 100% of the fields classified as *Low*, in 17% of the fields classified as *Middle* and in none of the fields classified as *High* (Fig. 3). In 2013, a parasite-free zone was not observed. There were 19 *Striga*-infested rice fields and 16 *R. fistulosa* infested fields. In just one occasion, both parasites were observed in the same field, though at opposite ends. Apart from this field, a clear separation between *Striga*-infested and *Rhamphicarpa*-infested fields was maintained. Coverage of *S. asiatica* was comparable with that in the previous year, whereas *R. fistulosa* clearly had a lower coverage, with only 18% of *Rhamphicarpa*-infested fields with a cover score above 50%.

2.3.3 Species diversity and abundance

A total of 43 non-parasitic weed species were encountered in 108 quadrats installed in 36 rice fields. Non-parasitic species most frequently encountered were *Oldenlandia herbacea* (Linn.) Roxb, *Fimbristylis ferruginea* (L.) Vahl, *Pycreus lanceolatus* (Poir.) C.B.Clarke, *Eragrostis tremula* Hochst., *Melochia corchorifolia* (Linn.) and *Hyptis spicigera* (Lam.) Marubio. These species were observed in 30% to 45% of the quadrats. The Shannon-Wiener (H') index was relatively high and did not differ significantly between *S. asiatica* (3.08), *R. fistulosa* (3.06) and parasite-free (3.14) fields.

The cluster analysis showed a division of non-parasitic weed species that were more frequently associated with either one of the two parasitic weed species (Fig. 4). *Ammannia auriculata* Willd., *Oryza longistaminata* A. Chev. & Roehr., *Scleria vogelii* C.B. Clarke, *Fimbristylis littoralis* Gaud. and *Cyperus distans* L. were exclusively encountered in *Rhamphicarpa*-infested fields, while *Spermacoce octodon* (Hepper) Hakki., *Pennisetum polystachion* (L.) Schult., *Mitracarpus hirtus* (L.) DC. and *Rottboellia cochinchinensis* (Lour.) Clayton, were only encountered in *Striga*-infested fields. None of the species were exclusive to parasitic weed-free fields.

2.3.4 Pot experiments

In Experiment 1, emerged plants of both *S. asiatica* and *R. fistulosa* were observed at all moisture treatments. However, *S. asiatica* emergence gradually decreased ($P= 0.009$) at the higher moisture levels (Fig. 5a), while *R. fistulosa* emergence steadily increased $(P< 0.001)$ with increasing moisture levels (Fig. 5b). The highest emergence for *S. asiatica* was at the 2 L moisture level (FC). In Experiment 2, the response of emergence to moisture was largely similar to that observed in Experiment 1, except that *S. asiatica* did not emerge at the highest moisture level (4 L). The highest emergence was observed at both 1 L and 2 L ($P< 0.001$) (Fig. 5a). *Rhamphicarpa fistulosa* had the highest emergence at both 3 L and 4 L, while the lowest emergence was observed at 1 L and 2 L ($P< 0.001$) (Fig. 5b).

In both experiments, total biomass dry weight of *S. asiatica* decreased with increasing moisture levels (Fig. 5c), while biomass of *R. fistulosa* peaked at the higher moisture level (Fig. 5d). The highest biomass dry weight for *S. asiatica* was observed at both 1 L and 2 L moisture levels, while no biomass was produced at the 4 L moisture level (Experiment 1; $P=0.004$ and Experiment 2; $P<0.001$). For *R. fistulosa*, the highest biomass dry weight was produced at the 4 L moisture level, while there was no difference between the biomass produced at the other moisture levels (Experiment 1; $P=0.034$) and Experiment 2; $P<0.001$).

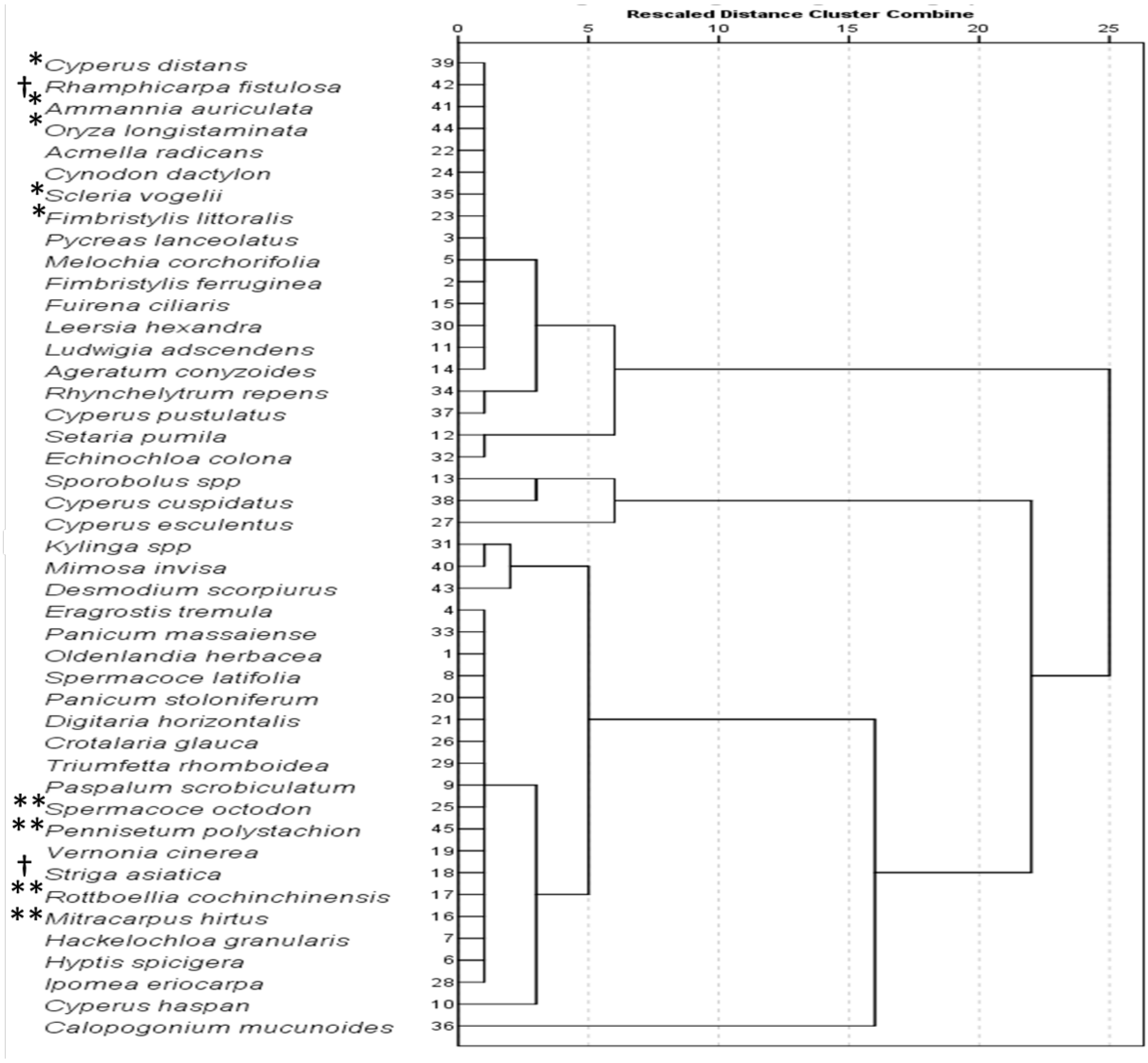

Figure 4. Dendogram showing the relation between frequency of occurrence of non-parasitic (*n* = 43) and parasitic weed species (*n* = 2) in the study area in 2012. Distance and similarity measures were generated from Hierarchical Cluster Analysis, using Pearson's correlation as the proximity procedure. The symbol † shows position of parasitic weed species, ** represents non-parasitic weed species that were exclusive to the *Striga asiatica* habitat while *represents non-parasitic weed species that were exclusive to the *Rhamphicarpa fistulosa* habitat.

2.4 Discussion

There were significant differences in soil moisture between *Striga*-infested and *Rhamphicarpa*-infested fields. *Striga asiatica* was exclusively observed in high lying fields with free draining upland soils, while *R. fistulosa* was observed in low-lying wetter fields, ranging from hydromorphic to temporarily flooded soils. This confirms what has previously been described as favourable growing environments of these species (Ouédraogo *et al.*, 1999).

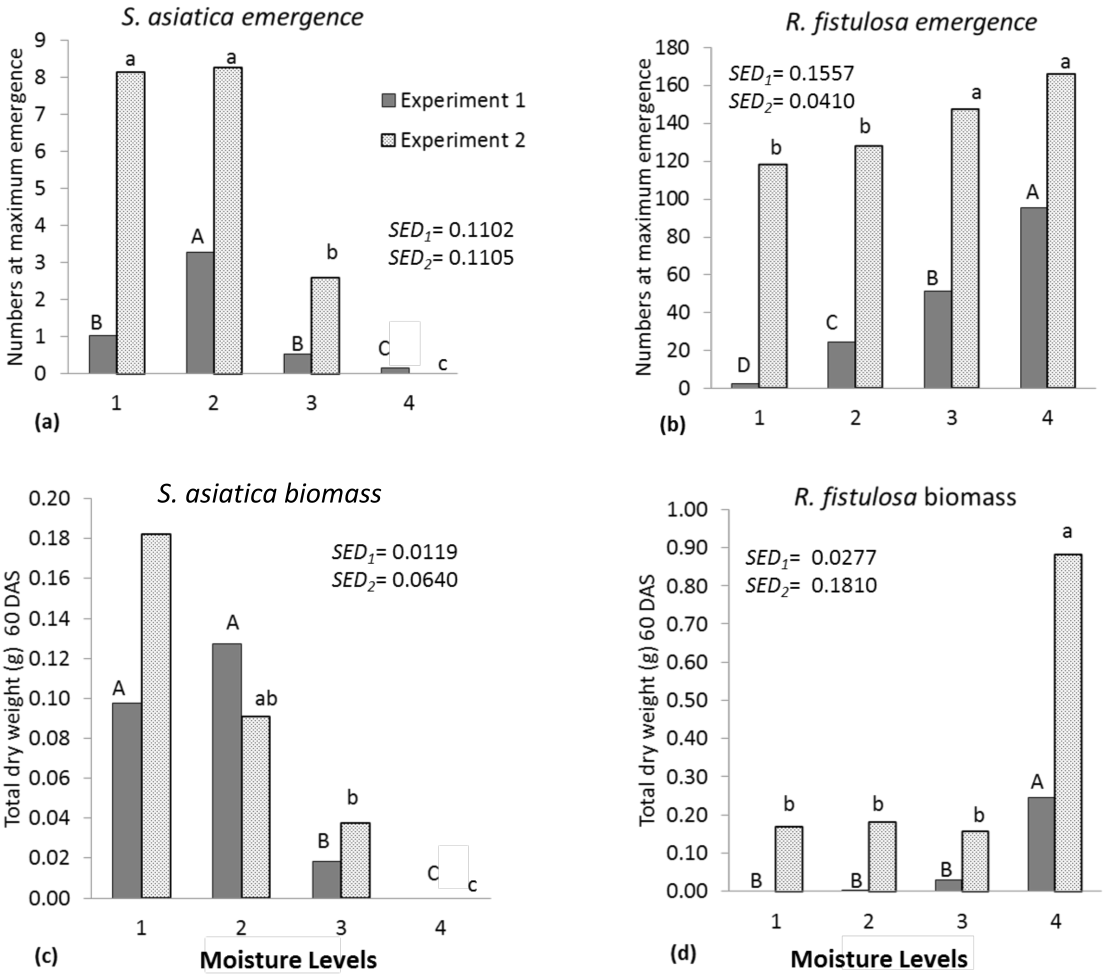

Figure 5. Maximum emergence of *Striga asiatica* (a) and *Rhamphicarpa fistulosa* (b) and total dry weight of *S. asiatica* biomass (c) and total dry weight of *R. fistulosa* biomass (d) at four moisture levels in Experiment 1 and 2. Soil moisture levels within each experiment with a different letter on top of the bar differed significantly ($P < 0.05$) (Experiment 1: capital; Experiment 2: small). SED-values for both experiments are presented in the graphs. Note that SED values are provided on log-scale.

In the drier second year of observation, even though both parasitic weeds had extended into fields that had been free of parasites the year before, there was still a clear demarcation between *S. asiatica* and *R. fistulosa* fields. Soil analyses further revealed differences between habitats of each parasitic weed species. CEC and extractable cations such as Ca and Na were markedly higher in *Rhamphicarpa*-infested fields than in *Striga*-infested habitats. This implies that *R. fistulosa* can thrive in more saline conditions, confirming findings by Hansen, (1975) who observed the parasitic species in briny waters near the sea. Soil OM, N, P, pH and EC were not significantly different between habitats, yet K was markedly higher in *Striga*-infested habitats. The N, P and K values could give an indication of the land use and how farmers respond to these parasitic weeds. Farmers in uplands tend to apply inorganic fertilizers, while those in lower parts of the catena found this practice unfeasible due to regular floods (M. Saidia, pers. com.). Prevalence of both parasitic weeds in the area implies that soil N, P, K levels in the study area are generally too low to affect their incidence. There is evidence that N, P, K fertilisation can dramatically reduce incidence of *S. asiatica* and *R. fistulosa* (Jamil *et al.*, 2013; Rodenburg *et al.*, 2011). The lack of exclusivity of the fields in the middle zone in terms of soil characteristics enables it to act as an ecological transition zone that can be prone to either of the two parasitic species, depending on prevailing environmental conditions. Seeds of both species are likely to be present here, but it is the annual specifics of the environmental conditions that determine whether one of the two species will occur, or whether the fields remain parasite-free, like in 2012. The Shannon-Wiener diversity Index (H') of the community of weed species observed was high. This indicates that there was a large diversity of species encountered in the study area (Molles, 1948). Crop type, crop sequence and tillage systems have been shown to strongly influence weed community composition (Bastiaans *et al.*, 2008; Bohan *et al.*, 2011). From field observations and informal surveys, it was established that farmers used hand weeding as a weed control measure and had only one cropping season per year.

Though species diversity were similar across habitats (based on H'), weed community composition was more related to particular parasite habitats. Following cluster analysis, non-parasitic weeds clustered into two major groups that were more closely, but not necessarily exclusively, related to either *S. asiatica* or *R. fistulosa* habitats. Non-parasitic weed species that were exclusive to *S. asiatica* habitats typically are indicator species of less fertile areas and frequently observed as weeds in rain-fed rice systems. For instance *Pennisetum polystachion* (e.g. Akobundu *et al.*, 1999; Kent *et al.*, 2001), *Rottboellia cochinchinensis* (e.g. Ampongnyarko and Dedatta, 1993) and *Mitracarpus hirtus*, syn. *M. villosus* (e.g.Chikoye and Ekeleme, 2001) are well adapted weeds of poor upland soils and marginal habitats. Similarly, non-parasitic weed species that were exclusive to *R. fistulosa* are indicator species of vegetation commonly found in moist areas. *Ammannia auriculata* (e.g.Caton *et al.*, 1997), *Cyperus distans* (e.g. Kent *et al.*, 2001; Rodenburg *et al.*, 2009), *Fimbristylis littoralis* and *Oryza longistaminata* (Rodenburg and Johnson, 2009), are all common weeds of lowland rice.

Differences in sensitivity of the two parasites to moisture were further revealed by results from pot experiments. *Striga asiatica* clearly performed better under drier conditions, while *R. fistulosa* thrived under wetter conditions. In the first experiment, both parasites emerged at all moisture levels but could not always reach maturity when moisture levels were suboptimal. In the second experiment, *S. asiatica* did not emerge at saturated moisture conditions at all. Of the two species, *R. fistulosa* seemed to have the broadest ecological range, as it was able to produce biomass under each of the four moisture conditions, even the less favourable ones, while *S. asiatica* was unable to produce any biomass under saturated conditions. Weed species with such a broad ecological range are potentially invasive and may spread and adapt to ecosystems beyond the original or most favourable ones (Booth *et al.*, 2010).

The clear demarcation in occurrence and success of each parasite in a specific ecological setting suggests that their niches are strictly separated. In between those two niches is a transition zone, in which seeds of the two species are likely to be present and the prevailing environmental conditions determine the occurrence of either of the two species. The occupation of this transition zone may differ from year to year and might even be largely parasite-free, as was the case in 2012. The occurrence of these parasite-free fields in 2012 could be attributed to the rainfall pattern, with an unusual wet month of May. The high rainfall months, might well have resulted in conditions that prolonged preconditioning period critical for *S. asiatica* germination in that year. Some *Striga* species are known to stay dormant when in wet and poorly aerated soil, only to germinate when conditions get dryer (Mohamed *et al.*, 2006). Germination of *Striga* spp. is regulated by specific chemical signals exuded by roots of host plants (Matusova *et al.*, 2004). To be responsive to these chemical signals (germination stimulants), the seeds require a moist environment for a certain preconditioning period at a suitable temperature. Matusova *et al.* (2004) observed that the length of this preconditioning time had a substantial effect on the sensitivity of *Striga* seeds to germination stimulants. The optimum preconditioning period was found to be between 21 and 28 days for *Striga* seeds. Being a drier year, 2013 might well have favoured germination of *S. asiatica* seeds that lay dormant in 2012 or the previous years. A farmer who owned one of the fields that were parasite free in 2012, claimed that he had never encountered either parasite before 2013, when his field was infested by *S. asiatica*. In 2013, *R. fistulosa* extended into previously parasite-free fields. Extension of this species into previously parasite-free fields could not be clearly explained, but the fact that its coverage was remarkably reduced in the drier year, implies that soil moisture variation, was a likely cause.

The influence of soil moisture on growth and success of the two parasitic species implies that changes in climate and specifically moisture regimes will be crucial for their future prevalence. As impacts of climate change on agriculture now become evident, it is anticipated that weed species' distribution and prevalence within weed and crop communities will be affected (Rodenburg *et al.*, 2011a). Changes in climate are likely to favour parasitic weed species of the Orobanchaceae family (Mohamed *et al.*, 2006), depending on prevailing rainfall patterns or incidences of drought and floods predicted to occur in Africa (IPCC, 2007). Besides moisture and nutrients, the distribution of *S. asiatica* and *R. fistulosa* is influenced by their hosts. The continuous rice cultivation in the study area sustains the continuity of these parasites, but it is also possible that some of the non-parasitic weed species can be potential hosts. The host range of *Striga* spp. include cereal crops and grasses of the *Poaceae* family (Johnson, 1997). Of the 17 non-parasitic weed species that segregate along with *S. asiatica* (Fig. 4), eight are of the family *Poaceae*. In the case of *R. fistulosa*, the entire host range has not been confirmed, but wild rice (*Oryza longistaminata*) that was exclusive to *R. fistulosa* habitat, is a known host species (Hansen, 1975).

Our results show a clear, non-overlapping, ecological range between *S. asiatica* and *R. fistulosa* niches. This clear separation implies that management strategies can typically be focused independently on one of the two species. For instance, in *Striga* areas, the use of phosphorus and nitrogen fertilizer (Jamil *et al.*, 2011), resistant and tolerant cultivars (Rodenburg and Bastiaans, 2011) or intercropping with legumes (e.g.Van Mourik *et al.*, 2008) can be promoted. In the case of *R. fistulosa*, a lesser-known parasite, more studies are required to find effective control strategies.

Chapter 3

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

Stella Kabiri[1], Aad van Ast[1], Jonne Rodenburg[2] & Lammert Bastiaans[1]

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[1] Centre for Crop Systems Analysis, Wageningen University & Research, the Netherlands

[2] Africa Rice Center (AfricaRice), Dar es Salaam, Tanzania

ABSTRACT

Rice Vampireweed, *Rhamphicarpa fistulosa* (Hochst.) Benth., was a minor parasitic weed until recently when rice cultivation in sub-Saharan Africa (SSA) was expanded into marginal wetlands, that are the parasite's natural habitat. Unlike most 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. Since 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 hypothesized that, being a wetland species, germination of *R. fistulosa* is stimulated by light and high soil moisture. Secondly, we hypothesized 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 revealed 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, revealed that host plant presence, regardless of 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, while 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.

Key words: Rice Vampireweed, *Oryza sativa*, *Striga*, seed production, weed management, rain-fed lowland rice, infestation level.

3.1 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 parasitized by one or more of these species resulting in high yield reduction and even crop failure (Berner *et al.*, 1995; Ejeta and 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). *Rhamphicarpa fistulosa* is native to sub-Saharan Africa (SSA) and is widely distributed in Sahelian countries, eastern Africa and southern Africa including Madagascar (Rodenburg *et al.*, 2015a). The complete host range has not been established, but *R. fistulosa* is able to parasitize cowpea, sorghum, maize and rice (Parker and 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.*, 2014a). It is thus the adaptation to ephemeral wet areas (Ouédraogo *et al.*, 1999; Muller and Deil, 2005; Hansen, 1975; 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*.

Contrary to most 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 recognizes 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 seems likely if the growth and seed production rate of a parasitic plant greatly benefits from its connection to a host plant. Though 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 hypothesized 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.

3.2 Materials and methods

In the time period 2010-2013, 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 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 0700 h to 1900 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 $\mu Em^{-2}s^{-1}$.

3.2.1 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 randomized two factor Petri-dish trial with five replications. The first factor was presence of light and dark (control) conditions, while the second factor was presence or absence of an artificial germination stimulant (GR24). 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 sterilized 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. 50 Petri-dishes were moistened with 4 ml demineralised water, while the other 50

Petri-dishes were moistened with 4 ml of 2 mg/L GR24 solution. All dishes were sealed with para-film. 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 since 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 randomized design (no blocks). Observation of germinated seeds was done nine times during a period of five 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.

3.2.2 Influence of soil moisture on emergence

In 2011, a greenhouse experiment was conducted to establish suitable soil moisture conditions for emergence of *R. fistulosa* (Experiment 2). The experiment was a two factor, randomized 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 minimize moisture loss through evaporation. Cups were placed on benches in the greenhouse. Twice a week, for a total period of seven weeks, emerged seedlings were counted and removed.

3.2.3 Influence of a host on germination and emergence

In November 2012, the influence of the presence of a host on 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 randomized 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 (WAE). We varied the ages of host plants on the assumption that root exudates and strigolactone production might vary with age of the rice plant. Rice plants were planted in succession in intervals of two weeks, to allow 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 November 26, 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 DAS till 30 DAS, when no newly emerged seedlings had been observed for a period of five 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 were 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) was prepared by dissolving 1 mg of GR24 powder in 1 ml 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, Gottingen, 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 thirty 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, *Striga hermonthica* seeds were used. The *S. hermonthica* seeds had been conditioned for 20 days by placing them between two moistened filter paper in a Petri-dishes at 30°C. Each treatment was replicated three times, resulting in a total of 90 Petri-dishes laid out in a randomized 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 seven days, after which germinated seeds were counted. Two ml of the original root exudate solution from each cultivar was passed through Solid Phase Extraction (SPE) columns (C18-Fast, 500 mg /3 mL; Grace pure) following the method described by Jamil *et al.* (2011), to establish the amount of strigolactones contained in the solutions. This was done to obtain a confirmation on the earlier established differences in strigolactone production between rice cultivars.

3.2.4 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, since 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, randomized complete block design with five replicates. *Rhamphicarpa fistulosa* seed infestation level was the first factor and consisted of 16, 32, 64, 128, 256 seeds/pot. 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). Pre-germinated rice seeds of rice cultivar IR64 were sown in half of the pots on May 28, 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 hours at 70^{o}C. Capsules and flowers were stored in labelled paper 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.

3.3 Statistical analysis

Data was subjected to Analysis of Variance (ANOVA) using statistical software package GenStat for Windows 16th Edition (GenStat, 2000-2013). 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 and Rohlf, 1995b). 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 aboveground biomass dry weight. ANOVA was followed by comparison of means by Least Significant Difference (LSD). All data are presented in original scale with respective standard errors (SE). Means and SE of transformed data were back-transformed from log(x+1), using Delta method for SE (Onofri *et al.*, 2010).

3.4 Results

3.4.1 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 revealed 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 solid phase extraction 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) was significantly higher than that of cultivar IR64 (2.98 pmol/ml) and cultivar Shiokari (1.84 pmol/ml). 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* 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.

3.4.2 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). Though 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

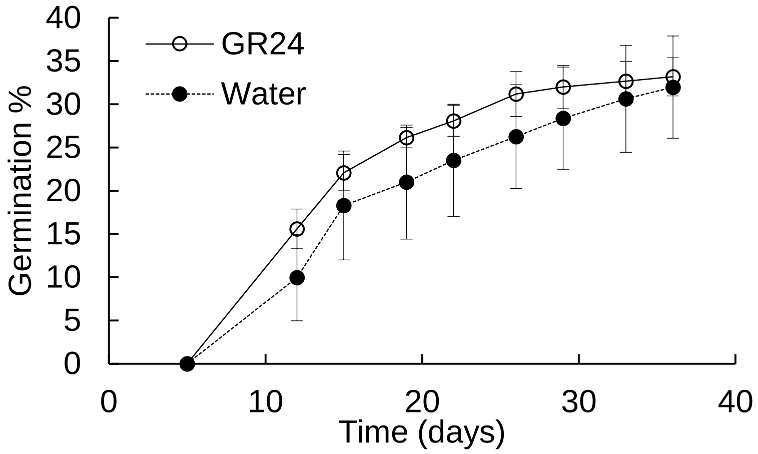

Figure 1. Progression of germination of *R. fistulosa* seeds over time treated with GR24 and water under light conditions in Petri-dishes (n = 5). Vertical bars are standard errors of mean. Seeds treated with GR24 and water exposed to dark conditions are not included, as no germination was observed,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

Table 1. Germination percentage (G%) of *R. 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

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 to 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

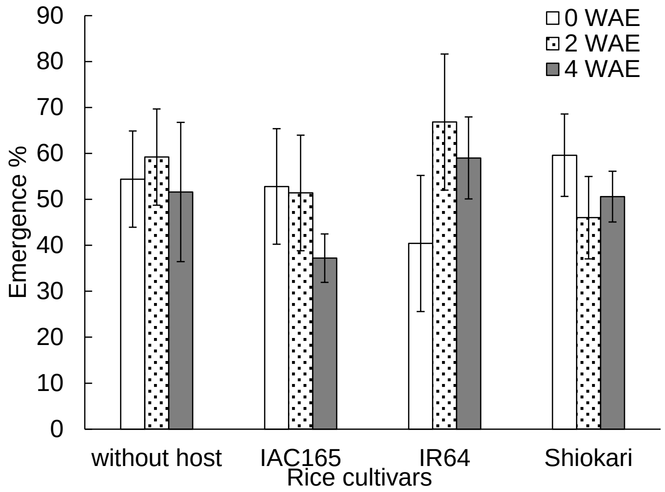

Figure 2. Emergence of *R. fistulosa* at 30 DAS in pots without a host plant and in pots with rice plants of cultivar IAC165, IR64 and Shiokari that were either 0, 2 or 4 weeks old at the introduction time of parasite seeds (n = 5). Vertical bars are standard errors of mean, (Experiment 3).

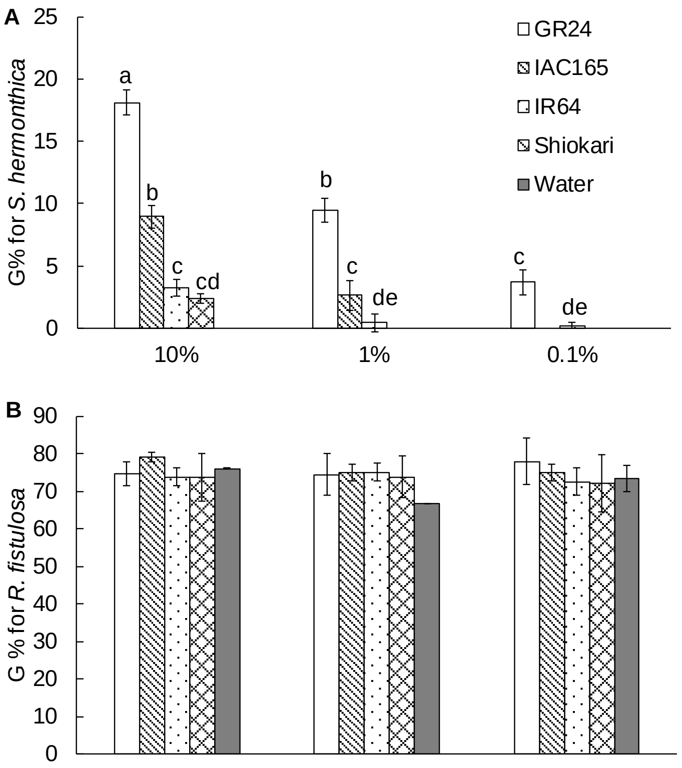

Figure 3. Germination percentage (G%) of *S. hermonthica* (A) and *R. fistulosa* (B) treated with dilutions of artificial germination stimulant GR24 and root exudates extracted from rice cultivars IAC165, IR64 and Shiokari and water (n = 3). Vertical bars are standard errors of mean. LSD-test was used 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).

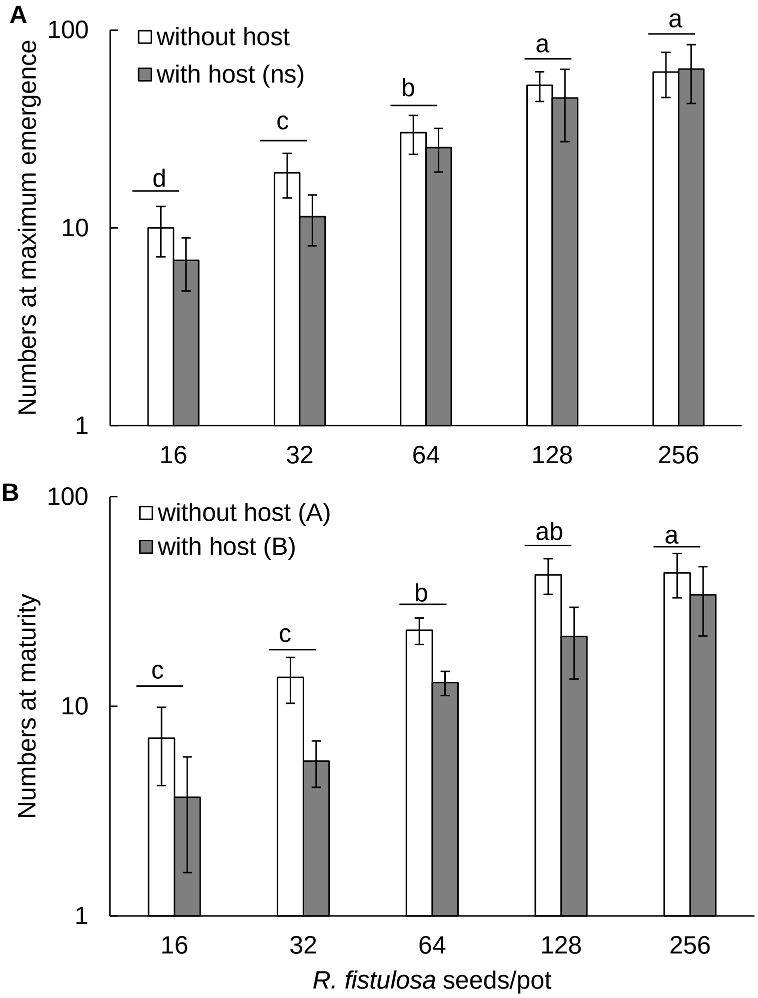

Figure 4. Number of *R. fistulosa* plants in the absence and presence of a rice host plant at a range of *R. fistulosa* seed infestation levels, that emerged (A), (n = 5) and at maturity (B), (n = 5). Vertical bars are standard errors of mean. Different letters on the legend indicate significant differences (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).

(10.7±2.09cm) ($F_{1,33}$ = 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) or 12-18 cm (256 seeds/pot) (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 0-6 cm till 54-60 cm. At high infestation levels the distribution was skewed such that there were more plants in the lower plant height categories.

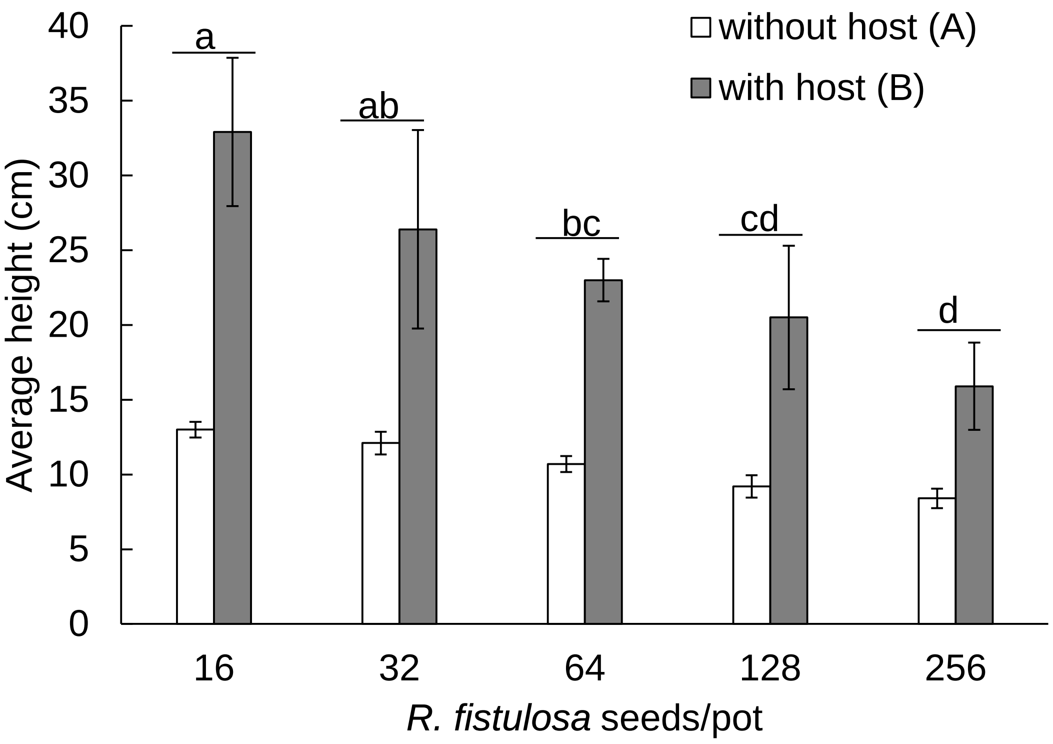

Figure 5. Average height of *R. fistulosa* plants with and without a host at a range of *R. fistulosa* seed infestation levels, at maturity (110 DAS), (n = 5). Vertical bars are standard errors of mean. Different letters on the legend indicate significant differences between plants with and without a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

Both at 60 DAS and at 110 DAS, dry total aboveground 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 DAS ($F_{4,33} = 4.95$; $P < 0.003$) (Fig 7B). This effect of seed infestation level was particularly evident at 60 DAS, with on average 0.5 g of shoot biomass at the lowest infestation level, compared to 2.6 g of shoot biomass at the highest infestation level. At 110 DAS the range was smaller with on average 5.5 g 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 ($F_{1,30} = 139.74$; $P < 0.001$) and 1000-seed weight ($F_{1,31} = 27.89$; $P < 0.001$) were all significantly influenced by the presence

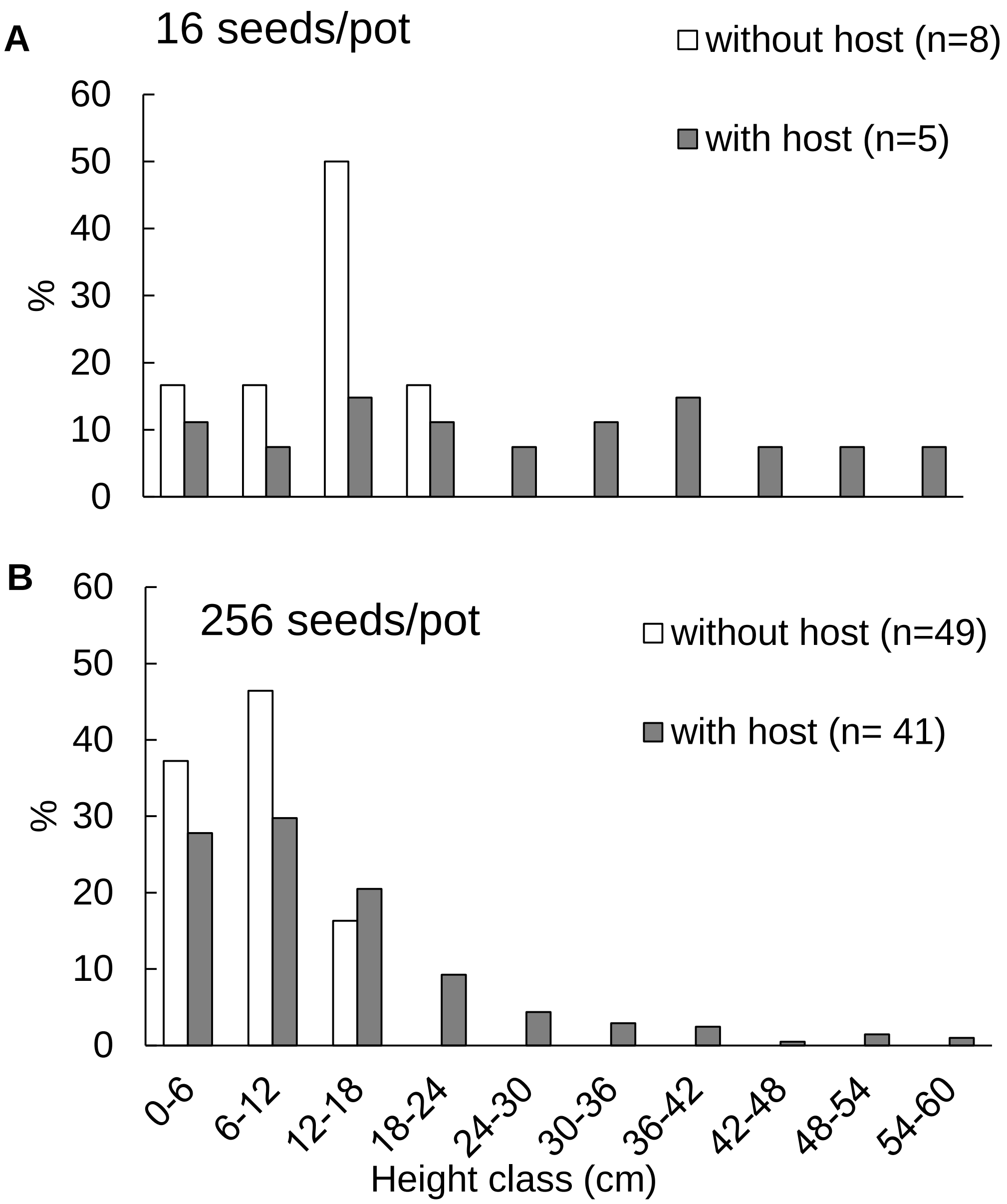

Figure 6. Plant height distribution of *R. fistulosa* plants with and without a host at seed infestation levels of 16 and 256 seeds per pot, using ten height classes with a width of 6 cm. Number of plants is expressed as a percentage to facilitate comparison between treatments. The actual number of established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

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 to 13,000 seeds per pot in the absence of rice. Apart from this 3.7 times 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 number of

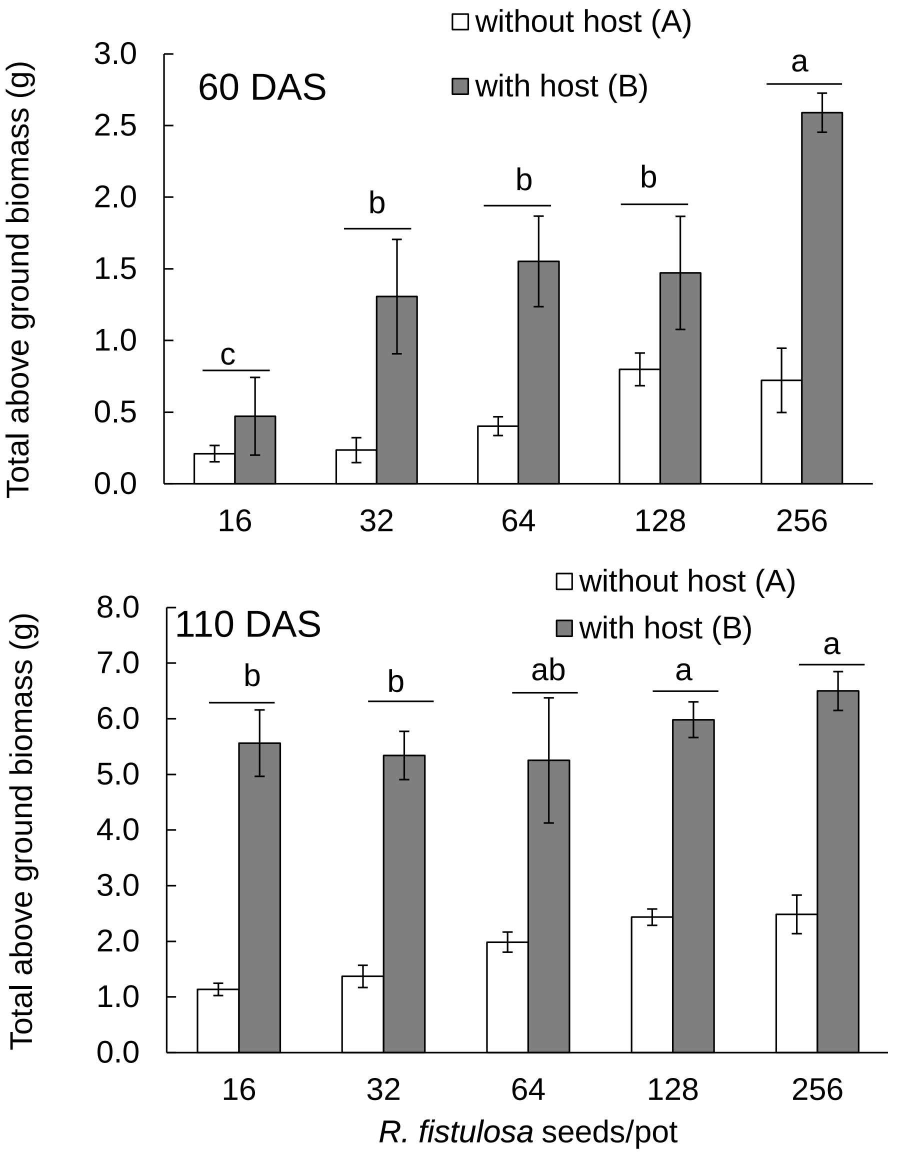

Figure 7. Total above ground biomass of *R. fistulosa* plants with and without a host at a range of *R. fistulosa* seed infestation levels, at 60 DAS and at maturity (110 DAS), (n = 5). Vertical bars are standard errors of mean. Different letters on the legend indicate significant differences ($P<0.05$) 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).

Table 2. Analysis of variance of reproductive characteristics of *R. 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}					

capsules produced and the 1000-seed weight. An illustration of the growth of *R. fistulosa* with and without a host is shown in Figure 8 below.

Figure 8. Growth of *R. fistulosa* in the absence and presence of a host

3.5 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 adapted to 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 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 (e.g. Schu¨tz *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 and 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 attached to the host at a relatively early stage, thereby out-competing the non-attached and later attached individuals due to 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 revealed that intraspecific competition among parasite plants at 60 DAS was far less pronounced than at 110 DAS, irrespective of host plant presence. At 60 DAS, parasite biomass still steadily increased with increasing infestation level, whereas at 110 DAS parasite biomass at higher infestation levels reached a plateau. This plateau represents the carrying capacity and 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 3 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 to 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 with 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 and Press, 1993a).

Since 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 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, minimizing the added value of a specific host plant recognition mechanism. The actual host plant range of *R. fistulosa* has yet to be 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. Though Gbéhounou and Assigbé (2004) reported that seeds of *R. fistulosa*, remain viable for approximately one 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 and Fresco, 1991). Such soil conditions are typically found in the rain-fed lowland rice production systems practiced 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 to natural vegetation types that are commonly characterized 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 due to seasonal tillage. Zero- or minimum-tillage crop production systems may form the exception. These systems, observed by Chauhan and 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 and 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, i.e. 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 (e.g. 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 revealed 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. Since 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; Schut, 2015), such insights and ideas are highly relevant for future rice production in SSA, particularly in the rain-fed lowland production systems.

Chapter 4

Impact of the hemi-parasitic facultative weed *Rhamphicarpa fistulosa* (Hochst.) Benth. on photosynthesis of its rice host *Oryza sativa* L.

Stella Kabiri[1], Aad van Ast[1], Jonne Rodenburg[2], Stephanie Pflug[1], Hanna Kool[1] & Lammert Bastiaans[1]

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[1]Centre for Crop Systems Analysis, Wageningen University & Research, the Netherlands

[2]Africa Rice Center (AfricaRice), 01 BP 4029 Abidjan 01, Côte d’Ivoire

ABSTRACT

The impact of *Rhamphicarpa fistulosa* on photosynthesis of rice as a host was examined. We investigated whether *R. fistulosa* affects photosynthesis of rice, identified the period when the effect was first detected and investigated whether the level of impact was dependent on the density of parasite infection. We further explored possible mechanisms underlying the reaction of host photosynthesis following infection. Three pot experiments were conducted using rice, cultivar IR64, as the host plant species. In experiment 1 rice plants infected with 30 *R. fistulosa* plants were used and gas exchange measurements were determined at 30, 37, 44, 51, 58 and 65 DAS. Experiment 2 included rice plants infected with 7, 14, 28, 56 and 112 seedlings of *R. fistulosa* per pot. Gas exchange measurements were conducted at 65 DAS. Experiment 3 included rice in combination with either 6 or 13 plants of *R. fistulosa*, and gas exchange measurements were conducted at 29, 34, 41, 49, 56, 63, 70 and 77 DAS. In Experiment 1, photosynthesis at light saturation of 58 day-old rice plants was suppressed to half that of uninfected plants, though infected rice plants managed to maintain initial light use efficiency. Similarly, in Experiment 3, photosynthesis at light saturation of rice was first suppressed at 56 days, whereas initial light use efficiency was not affected. This clearly demonstrated that photosynthesis of rice is first affected at around eight weeks after sowing. Later on during rice plant development, initial light use efficiency was also reduced, both in Experiment 1 and Experiment 3. In Experiment 3, from 56 DAS on, gas exchange measurements were expanded with fluorescence measurements. Already at 56 DAS, the slope of the linear relationship between light adapted quantum efficiency of PSII electron transport (Φ_{PSII}) and the quantum yield of CO_2 assimilation (Φ_{CO2}) of infected plants was less than those of un-infected plants. This indicates that parasite infection either reduced light absorbance or reduced the efficiency of electrons for fixing CO_2. This might result from a too low concentration of CO_2 at the carboxylation site, following from the lower stomatal conductance. In all three experiments, dark respiration rates of the host were not influenced by parasite infection. In Experiment 2 suppression of host photosynthesis at light saturation and the initial light use efficiency was a function of the degree of parasite infestation. Chlorophyll content, specific leaf area and shoot weight of *R. fistulosa*-infected plants was also dependent on the degree of infestation. The experiments revealed that there was a considerable time lag between the parasite's acquisition of benefits from the association in terms of growth and the reduction of host photosynthesis, while the reduction in host growth coincided with suppression of host photosynthesis. Our findings thus indicate that *R. fistulosa* affects host growth by first extracting assimilates and making considerable gains in growth, before the parasite impacts host photosynthesis.

Key words: Rice VampireWeed, Parasitic plants, Photosynthetic rate, CO_2 assimilation, Stomatal conductance, quantum yield.

4.1 Introduction

In recent years, rice has become important as a food and cash crop in sub-Saharan Africa (SSA). As a result, the area under rice cultivation has expanded and currently the total area is 9.9 million hectares (Seck *et al.*, 2012). However, this expansion has not been sufficient to cover current demand, as SSA still depends on over 30% of global rice imports to feed its growing population (Seck *et al.*, 2012). One of the reasons for this insufficiency is that most of the expansion has been into marginal rain-fed areas (Sakurai, 2006; Balasubramanian *et al.*, 2007). Apart from sub-optimal provision of water and nutrients, these areas are natural habitats of parasitic weed species. In SSA, the obligate *Striga* spp. are well known biotic production constraints, mainly thriving on traditional staple food crops like sorghum, millet and maize. Recently, the facultative parasitic weed *Rhamphicarpa fistulosa* has gradually developed into an important pest of rain-fed rice (Rodenburg *et al.*, 2015a).

Rhamphicarpa fistulosa is a root hemiparasitic annual forb of the family Orobanchaceae that is native to SSA (Kuijt, 1969; Ouédraogo *et al.*, 1999). Its habitat range covers open sunny grasslands such as temporary wetlands or water-logged soils (Hansen, 1975). As a root hemi parasite, *R. fistulosa* possesses haustoria, specialized organs that form the connection with the host enabling the host-parasite transfer of water, nutrients and carbon (Kuijt, 1969; Kabiri *et al.*, 2015). The species is facultative, meaning that it can complete its life cycle as a free living plant (Ouédraogo *et al.*, 1999). However, when in association with a host, the parasite grows taller, produces more biomass and has a higher fecundity (Kabiri *et al.*, 2016). The parasitism has a negative effect on the host plant, resulting is severe yield reductions. It is however not known how exactly *R. fistulosa* affects its host, and how the host responds to infection.

In some host-parasite associations, the negative impact parasitic plants have on their hosts is practically restricted to withdrawal of assimilates. For example, in an association of the facultative parasite *Rhinanthus minor* and barley, the parasite trafficked up to 20% of host solutes from the xylem sap, reducing host growth by 22% (Jiang *et al.*, 2003). Seel and Press (1996) reported that infection of the perennial grass host *Poa alpina* by *R. minor* merely affected flower bud formation and biomass partitioning. Parasitic plants can however also impact their hosts by manipulating host-plant architecture like in the *Striga*-sorghum association. Van Ast *et al.*, (2000) observed that internode lengths of a *Striga* susceptible sorghum cultivar were two folds shorter than those of uninfected plants. This might easily result in an increased level of self-shading which, in turn, negatively impacts the production of assimilates by the host. Assimilate production of host plants is also frequently reported to be inhibited through a direct negative impact of the parasite on the photosynthetic capacity of its host (Watling and Press, 2001; Cameron *et al.*, 2005). For example, with *Striga* spp. the largest effect on host yield was caused by the effect of the parasite on host photosynthesis (Cechin and Press, 1994; Gurney *et al.*, 1995). Graves *et al.*, (1989) observed that in an association of *Striga hermonthica* and sorghum, 80% of the host yield loss was caused by parasite-induced suppression of host photosynthesis. These parasite-induced effects on host photosynthesis are both host and parasite specific (Watling and Press, 2001; Hibberd *et al.*, 1998a). For instance, in an association between broomrape (*Orobanche cernua*) and tobacco no evidence of suppression of host photosynthesis was found, even when parasite densities were increased (Hibberd *et al.*, 1998a). In addition to variability among species, Rodenburg *et al.*, (2008) observed clear differences in photosynthetic response among sorghum genotypes after infection with *S. hermonthica*. Based on their results, they proposed the use of photosynthesis measurements as a basis for screening for host tolerance. This method proved successful for identification of tolerant genotypes of rice (Rodenburg *et al.*, 2017). The degree of impact on host photosynthesis is also influenced by time and level of infection. For example, van Ast (2006) observed that, at high *S. hermonthica* infestation levels, a 40-50% reduction in photosynthetic rate of sorghum leaves was already observed at 19 days after sorghum emergence (DAE), well before the parasite emerged. Gradually, leaf photosynthetic rate at lower *Striga*-infestation levels was also reduced, though initially to a lower extent. From 57 DAE on the reduction in leaf photosynthetic rate following *Striga* infection was independent of *Striga* infestation level.

The mechanisms behind suppression of host photosynthesis are complex but have frequently been associated with lowering of stomatal conductance (Frost *et al.*, 1997; Gurney *et al.*, 1995). It is suspected that abscisic acid (ABA), a plant growth regulator, is involved in controlling stomatal responses (Cechin and Press, 1993; Watling and Press, 2001). Yet, Cechin and Press (1993) proved that photosynthetic suppression following parasite infection could not be sorely attributed to lower stomatal conductance, implying the involvement of other mechanisms. Some known mechanisms include inefficiencies in the photosynthetic apparatus of host leaves. It has been shown that, under suppressed photosynthesis, the photon flux density (PFD) to photosynthesis ratio increases such that excess absorbed light beyond that utilized by photosynthesis damages the photosynthetic apparatus (Demmin-Adams and Adams, 1992). For example, *Striga*-infected plants were found to be more sensitive to light-induced reduction in the photosynthetic capacity (photoinhibition) because of damage to the electron transport system (Ramlan and Graves, 1996). Consequently, the recovery of the apparent quantum yield was slower amongst infected plants. Photoinhibition is particularly likely to occur in regions with high light intensity, precisely where rice is cultivated.

Determining whether *R. fistulosa* impacts photosynthesis of its rice host, quantifying the magnitude of this impact and elucidating the mechanisms behind it, will provide valuable insights in the virulent potential of the parasite and the ability of rice to withstand infection without too much damage. It could be that, similar to what is observed with other host-parasite associations, the reduction in yield might be amplified by a negative effect of the parasite on leaf photosynthetic rate of the host. Thus the objective of this study was to find out

(i) if *R. fistulosa* affects photosynthesis of rice, (ii) if the level of reduction in leaf photosynthetic rate depends on weed infestation level and (iii) which mechanisms underlie a possible reduction in leaf photosynthetic rate of its rice host.

4.2 Materials and Methods

Between 2012 and 2014, three greenhouse pot experiments were conducted at Wageningen University, the Netherlands. Rice, cultivar IR64, was used as the host plant species. Seeds of *R. fistulosa* were from a seed lot collected in 2009 from an infested rice field in Kyela, Tanzania. The germination percentage of these seeds was between 48% and 60%. The pots used were unperforated 6 L capacity pots filled with approximately 7.1 kg of a 1:1 mixture of dry arable soil and sand. The pots were watered daily to maintain saturated soil conditions since wet soils are most suitable for germination, growth and development of *R. fistulosa* . Screens were used to create a day length of 12 h (from 7.00 a.m. to 7.00 p.m.). Growing conditions were set to 26°C/23°C for day/night temperatures, but during warm summer days daytime temperature was regularly higher with a maximum of 32ºC. 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 $\mu Em^{-2}s^{-1}$.

In all experiments, rice seeds were pre-germinated for 48 hours in an incubator at 33°C after which they were planted in the centre of a pot at a depth of 1-2 cm. Only one rice seedling was planted per pot. On the same day, *R. fistulosa* seeds were mixed with about 9 g of dry sand and evenly sprinkled on top of the soil surface. The surface was then moistened with a fine spray of water. After emergence, *R. fistulosa* seedlings were uniformly thinned to a pre-set density.

4.2.1 Experiment 1

Experiment 1 was carried out from May 16 to July 23, 2012, to investigate if *R. fistulosa* affected leaf photosynthetic rate of rice. The experiment had a randomized complete block design with two treatments in four replicates and pots for six gas exchange measurement dates. The treatments included rice plants infected with 30 *R. fistulosa* plants and un-infected control plants. Additionally, pots that contained 30 host-less *R. fistulosa* plants were included as a second control, against which the growth of *R. fistulosa* in the presence of a host was compared. Leaf photosynthetic rate was determined at 30/31, 36/38, 44/45, 51/52, 58/59 and 65/66 days after sowing (presented as 30, 37, 44, 51, 58 and 65 DAS, respectively). The total number of pots was 72. Fertilizers were not applied. Apart from SPAD and SLA of the rice leaves on which the measurements were carried out, total above-ground dry weight of the rice plants was determined. Plant height and above-ground dry weight of *R. fistulosa* plants in pots with and without rice were determined on the same day. To obtain dry weight, rice and *R. fistulosa* plants were cut and dried in the oven at 70°C for 48 hours.

4.2.2 Experiment 2

Experiment 2 investigated the relation between the suppression in leaf photosynthetic rate and weed infestation level. The experiment was conducted from May 5 to July 31, 2012. It was a randomized complete block design with six treatments in four replicates. The treatments included rice plants infected with 7, 14, 28, 56 and 112 seedlings of *R. fistulosa* per pot and an un-infected control plant. Gas exchange measurements were conducted at 65 DAS as it had been noted in Experiment 1 that photosynthesis of the host was clearly influenced by parasite infection at this time. Gas exchange measurements on rice plants were conducted with the same irradiance levels in the same procedure as the first experiment. SPAD and specific leaf area (SLA) of leaves on which gas exchange measurements were done were measured.

4.2.3 Experiment 3

To investigate the mechanisms underlying the reduction in leaf photosynthetic rate of the rice host, a third experiment was installed from April 22 to July 9, 2014. The experiment had a randomized complete block design, with three treatments and four replicates. The three treatments included rice in combination with 13 plants of *R. fistulosa*, rice combined with 6 plants of *R. fistulosa* and uninfected rice plants as a control. The total number of pots was 96. Gas exchange measurement dates were done eight times at 29/30, 34, 40/41, 48/49, 55/56/57, 62/63/64, 69/70/71 and at 76/77 DAS (presented as 30, 34, 40, 48, 56, 63, 70 and 76 DAS, respectively). Irradiance levels were measured in descending order as explained above. At 34 DAS, gas exchange measurements were limited to the three highest light levels (2000, 1500, 1000 µE m^{-2} s^{-1}), due to limited availability of the photosynthesis equipment. At 56, 63 and 70 DAS gas exchange measurements were expanded with fluorescence measurements.

4.2.4 Gas exchange measurements

From around 30 DAS on, leaf photosynthetic rate of infected and non-infected control rice plants were regularly determined. Gas exchange measurements were done on the middle of the adaxial side of the youngest fully developed leaf of the main tiller. The measurements were conducted during the day between 9.30 a.m. and 4.00 p.m. using photosynthesis measurement equipment (LI-COR, Li-6400XT Lincoln, NE, USA). Leaf temperature and the average relative humidity in the leaf chamber were maintained at 29.9°C and 66% respectively. For the first two experiments, leaves were first dark adapted for 15 min, after which light intensity was increased subsequently at irradiance levels (PAR) 0, 20, 50, 100, 500, 1000 and 1500 µE $m^{-2}s\text{-}^{1}$. At each irradiance level, the leaf was exposed for 6 minutes before a measurement was recorded. For the third experiment, additional irradiance levels were introduced and the measurements were taken in descending order (2000, 1500, 1000, 500, 160, 120, 80, 40, 0 µE m^{-2} s^{-1}) to improve the light adaptation time of the leaf. The response of photosynthesis to irradiance was examined by fitting the exponential model of von Caemmerer and Farquhar (1981) for gas exchange in leaves to the measured data.

$$A = A_{max} \times \left(1 - exp\left(-\varepsilon \times \frac{PAR}{A_{max}}\right)\right) - R_d \qquad (1)$$

In this model, A is the net assimilation rate (µmol CO_2 m^{-2} s^{-1}), A_{max} is the maximum gross assimilation rate (µmol CO_2 m^{-2} s^{-1}), ε is initial light use efficiency (µmol CO_2 $µE^{-1}$), R_d is the dark respiration rate (µmol CO_2 m^{-2} s^{-1}) and *PAR* is the photosynthetic active radiation (µE m^{-2} s^{-1}).

After the gas exchange measurements, leaf greenness and specific leaf area (SLA) of the measured leaf were determined. Leaf greenness was measured using a SPAD Chlorophyll Meter (SPAD 502, Spectrum Technologies, Inc., Plainfield, IL, USA), after which a 12-cm-long piece of the leaf was cut and the leaf width was determined. The leaf piece was then placed in an oven for 48 hours at 70°C after which dry weight was determined to derive SLA.

4.2.5 Fluorescence

While all of the above measurements were done in all three experiments, in the third and last experiment, additional observations were made on leaves of 56, 63 and 70 day old rice plants. These measurements were conducted to determine whether kinetics of fluorescence induction was altered in leaves of *R. fistulosa*-infected plants. The protocol followed for fluorescence measurements were done according to methods of Maxwell and Johnson (2000) using the LI-COR, Li-6400XT (Lincoln, NE, USA) equipment. Leaf temperature and the average relative humidity in the leaf chamber were maintained as above.

Dark adapted leaves were exposed continuously to the respective light irradiances outlined above producing a transient closure of the PS II photochemical reaction centres in a light adapted state (F'_m) till the fluorescence steady-state where fluorescence intensity had saturated or was constant (F_t). The fraction of absorbed photons used for photochemistry for a light adapted leaf known as the quantum yield of PS II (*ΦPSII*) was calculated using Equation 2:

$$\Phi PSII = (F'_m - F_t)/F'_m \qquad (2)$$

Quantum yield of CO_2 assimilation was calculated as:

$$\Phi CO_2 = (A - R_d)/ PAR \qquad (3)$$

Where, ΦCO_2 is the quantum yield of CO_2 assimilation based on incident radiation, A is the photosynthetic rate (μmol CO_2 m^{-2} s^{-1}), R_d is the dark respiration rate (μmol CO_2 m^{-2} s^{-1}), *PAR* is the incident photosynthetic active radiation (μE m^{-2} s^{-1}).

4.2.6 Statistical analysis

Curve fitting to obtain the photosynthetic parameters of the light response curve that best fit the model (Eq. 1) were performed using R version 2.1.5. Estimates of the parameters made by the curve, SPAD values, specific leaf area and dry weight data were subjected to Analysis of Variance (ANOVA) using statistical software package GenStat for Windows 17th Edition (GenStat, 2016). ANOVA was preceded by tests for homoscedasticity and normality, as recommended by (Sokal and Rohlf, 1995), and followed by comparison of means by Least Significant Difference (LSD).

4.3 Results

4.3.1 Experiment 1

In Experiment 1, the model (Eq. 1) described the data shown with a good fit of the photosynthetic light response curves as was reflected in R^2 values ranging from 0.941 to 0.999. The photosynthetic light response curves increased quickly at lower irradiances and more slowly at higher irradiances until a plateau was reached (Fig. 1). Compared to infected plants, the maximum gross photosynthetic rate per unit leaf area (A_{max}) for uninfected plants decreased gradually with time from 25.0 (μmol CO_2 m^{-2} s^{-1}) at 30 DAS to 17.2 (μmol CO_2 $m^{-2}s^{-1}$) at 65 DAS. Photosynthesis-light response curves of *R. fistulosa*-infected plants only differed from that of uninfected plants at 58 DAS and at 65 DAS. At these two dates, A_{max} of infected plants was twice (P<0.021) and three times (P<0.008) less than that of uninfected control plants, respectively. The initial slope of the photosynthesis light response curves of control plants, representing the initial light use efficiency (ε), was stable over time, with an average value of 0.069 (μmol CO_2 μE^{-1}) along all gas exchange measurement dates. The initial light use efficiency (ε) only differed from that of uninfected plants at 65 DAS where ε of infected plants was three times less than that of uninfected plants (P< 0.006).

Dark respiration rate, R_d of uninfected plants decreased from 1.12 (μmol CO_2 m^{-2} s^{-1}) at 30 DAS to 0.89 (μmol CO_2 m^{-2} s^{-1}) at 65 DAS. SPAD values were stable with time having an average value of 35.3 (Fig 2A), while specific leaf area (SLA) gradually decreased from 42.2 (cm^2 g^{-1}) at 30 DAS to 22.1 (cm^2 g^{-1}) at 65 DAS (Fig 2B). The maximum photosynthetic rate per gram of leaf dry weight, A_{mass}, decreased gradually from 1.060 (μmol CO_2 g^{-1} s^{-1}) at 30 DAS to 0.381 (μmol CO_2 g^{-1} s^{-1}) at 65 DAS. Dark respiration rate, R_d, did not differ significantly between infected and uninfected plants (P values ranging from 0.198 to 0.703) at any observation date. SPAD values only differed from that of uninfected plants at 65 DAS, where values of infected plants were half those of uninfected plants (P< 0.006) (Fig 2A). The

SLA of *R. fistulosa*-infected plants was 1.5 and 1.7 times more than that of uninfected plants at 58 DAS (*P*<0.006) and at 65 DAS (*P*<0.004), respectively (Fig 2B). A_{mass}, like ε and SPAD, only differed from that of uninfected plants at 65 DAS, where A_{mass} of infected plants was only half that of uninfected plants (*P*< 0.022).

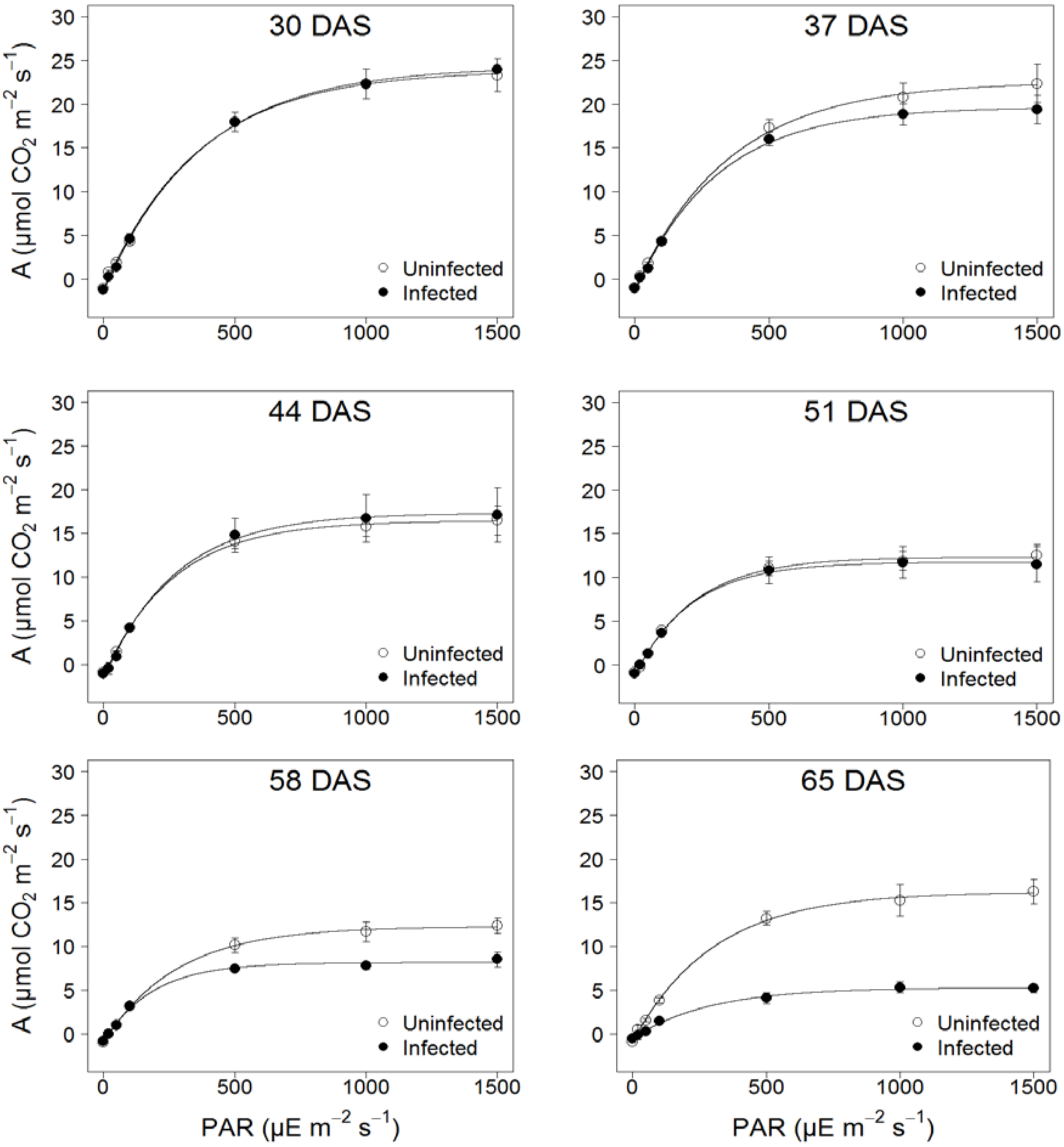

Figure 1. Time course of photosynthesis-light response curves for leaves from rice plants infected by *R. fistulosa* compared to leaves from uninfected rice plants (Experiment 1). Vertical bars are standard errors of mean. *N*=3 for infected plants at 58 DAS, otherwise *N*=4.

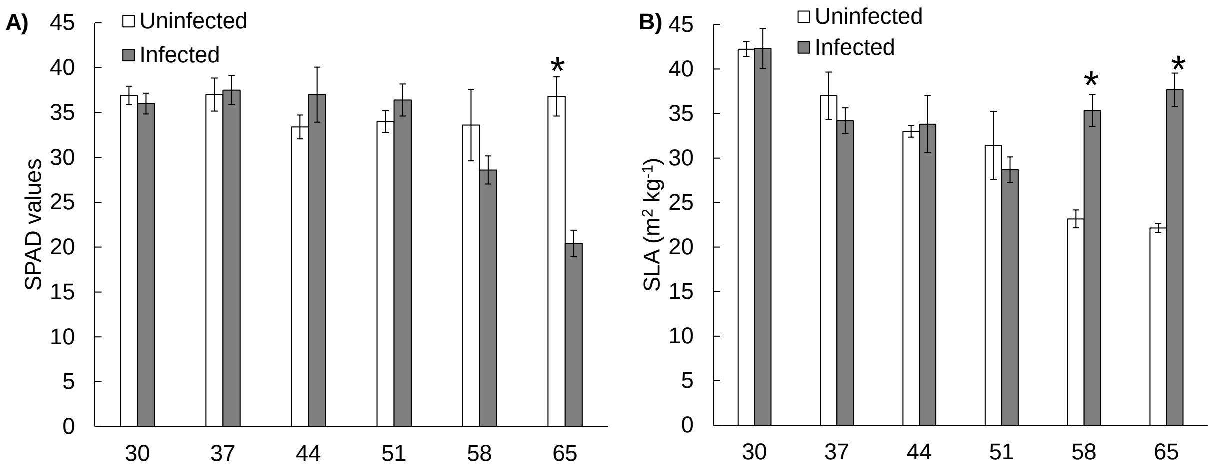

Figure 2. SPAD values (A), specific leaf area, SLA (B) of the youngest fully developed leaf of uninfected and *R. fistulosa* infected rice plants (Experiment 1). Vertical bars are standard errors of mean. * indicates significant differences *($P< 0.05$)* between infected and uninfected rice plants.

Rhamphicarpa fistulosa started benefiting from the host at 51 DAS. Parasite plants in association with a host were one-and-a-half times taller than host-less ones (Fig. 3A), while at 58 and 65 DAS they were on average double the height of host-less ones (P<0.001). Their shoot dry weight was three folds more than host-less plants at 51 and 58 DAS, while at 65 DAS the shoot dry weight was four times more than that of host-less ones. Total above-ground dry weight of uninfected rice plants increased steadily until 58 DAS (Fig. 3B). Infected rice plants followed the same pattern until 51 DAS. At 58 and 65 DAS shoot dry weight was significantly smaller than that of uninfected control plants and had also dropped compared to the shoot dry weight at 51 DAS (Fig. 3B). At these two dates the total above-ground dry weight of infected plants was four times smaller than that of uninfected plants (P<0.001).

4.3.2 Experiment 2

The model (Eq. 1) also described the data in Experiment 2 with a good fit with R^2 values ranging from 0.989 to 0.993. The maximum gross photosynthetic rate per unit leaf area (A_{max}) and the initial light use efficiency (ε) of uninfected plants was 17.5 (μmol CO_2 m^{-2} s^{-1}) and 0.0668 (μmol CO_2 $μE^{-1}$), respectively (Fig 4A and B). The A_{max} and ε of infected plants, was significantly reduced (P<0.001) with increasing *R. fistulosa* infection rates. The A_{max} of rice plants infected with 56 *R. fistulosa* plants did not differ from those infected with 7 *R. fistulosa* plants and were generally healthier in all replications. It was observed that in comparison to the the other treatments 85% of the *R. fistulosa* plants in the 56 *R. fistulosa* treatment were

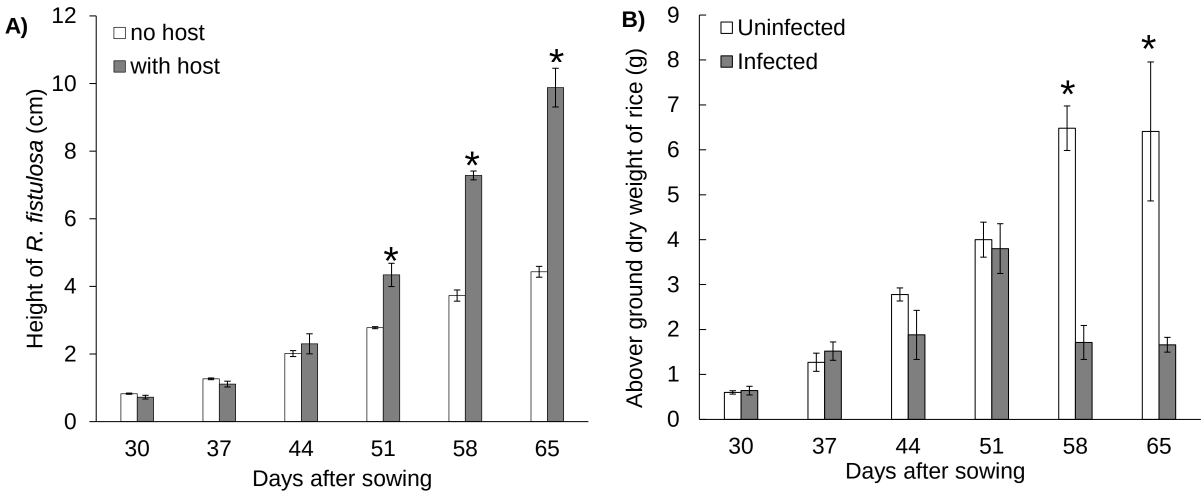

Figure 3. (A) Height of *R.fistulosa* plants that interacted with a rice host compared to *R. fistulosa* plants that did not grow with a host (Experiment 1). (B) Above-ground dry weight of uninfected rice plants and *R. fistulosa*-infected rice plants .Vertical bars are standard errors of mean. * indicates significant differences *($P < 0.05$)* between (A) host-less parasitic plants and plants in the presence ofa host and between (B) infected and uninfected rice plants.

shorter than 2 cm, pointing to late host attachment by *R. fistulosa*.

Dark respiration rate (R_d) (data not shown) was not influenced by increasing infection levels (P=0.641) as it was observed that the R_d of uninfected plants did not differ from that of infected plants. SPAD values and SLA were significantly affected by increasing infection levels (P=0.001 and P<0.001, respectively). With the exception of 56 *R. fistulosa* plants per pot, the SPAD values with an infection level of 14 parasites per pot or more were significantly less than those of the uninfected rice plants. SLA of uninfected rice plants was significantly less than that of the infected rice plants with an infestation level of 14 parasities per pot or more. The host shoot dry weight of infected plants significantly differed from that of uninfected plants (P<0.001) with the lowest shoot dry weight observed at the highest parasite infestation levels (Fig 4E). Shoot dry weight of *R. fistulosa* showed the opposite pattern, with the highest shoot dry weight at the highest infestation level (P<0.001) (Fig 4F).

4.3.3 Experiment 3

4.3.3.1 Photosynthetic parameters

Based on the light response curves (Fig 5) it was determined that amongst uninfected rice plants, the maximum gross photosynthetic rate per unit leaf area (A_{max}), the initial light use efficiency (ε) and dark respiration rate (R_d) decreased with time. The A_{max} for uninfected plants decreased from 32.8 (μmol CO_2 m^{-2} s^{-1}) at 29 DAS to 21.6 (μmol CO_2 m^{-2} s^{-1}) at 77 DAS, the ε also decreased from 0.044 (μmol CO_2 μE^{-1}) at 29 DAS to 0.058 (μmol CO_2 μE^{-1})

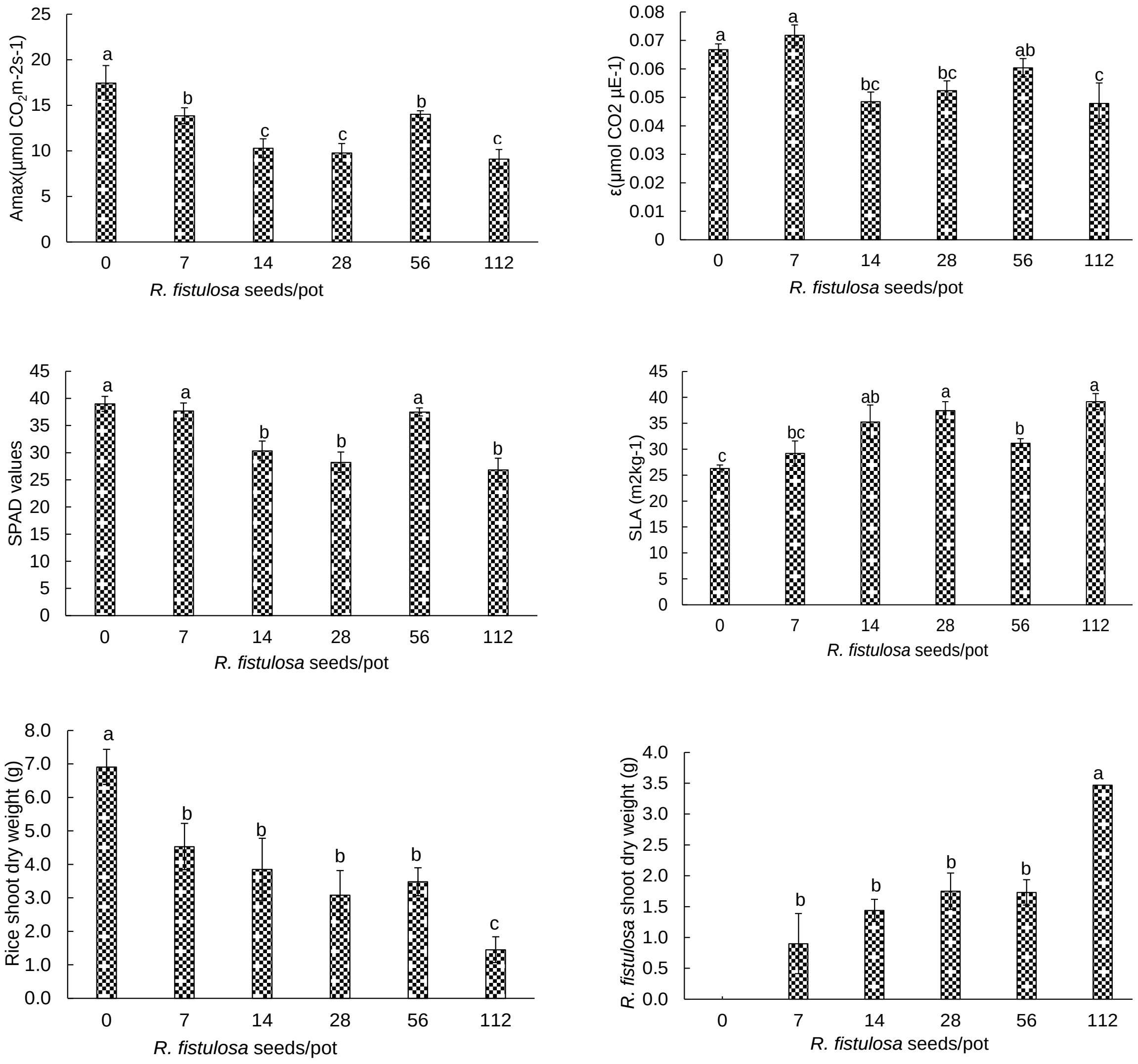

Figure 4. Maximum gross photosynthetic rate per unit leaf area, A_{max} (A), the initial light use efficiency, ε (B), SPAD values (C) and specific leaf area, SLA (D) of rice plants as influenced by infestation levels of *R. fistulosa* (Experiment 2). (E) shows the above-ground dry weight of the host, and (F) is above-ground dry weight of the parasite. Vertical bars are standard errors of mean. Non similar letters indicate significant differences ($P<0.05$).

at 77 DAS and dark respiration rates (R_d) decreased from 0.536 (µmol CO_2 m^{-2} s^{-1}) at 29 DAS to 0.342 (µmol CO_2 m^{-2} s^{-1}) at 77 DAS. Meanwhile SPAD values of uninfected plants were relatively stable over time, having an average value of 33.6 (Fig 6A). The reduction in photosynthetic parameters was consistent with the reduction in specific leaf area (SLA), which reduced from 35.1 (cm^2 g^{-1}) at 29 DAS to 20.9 (cm^2 g^{-1}) at 77 DAS (Fig 6A). Similarly the maximum photosynthetic rate per gram of leaf dry weight, (A_{mass}), of uninfected rice plants decreased from 1.145 (µmol CO_2 g^{-1} s^{-1}) at 29 DAS to 0.452 (µmol CO_2 g^{-1} s^{-1}) at 77 DAS.

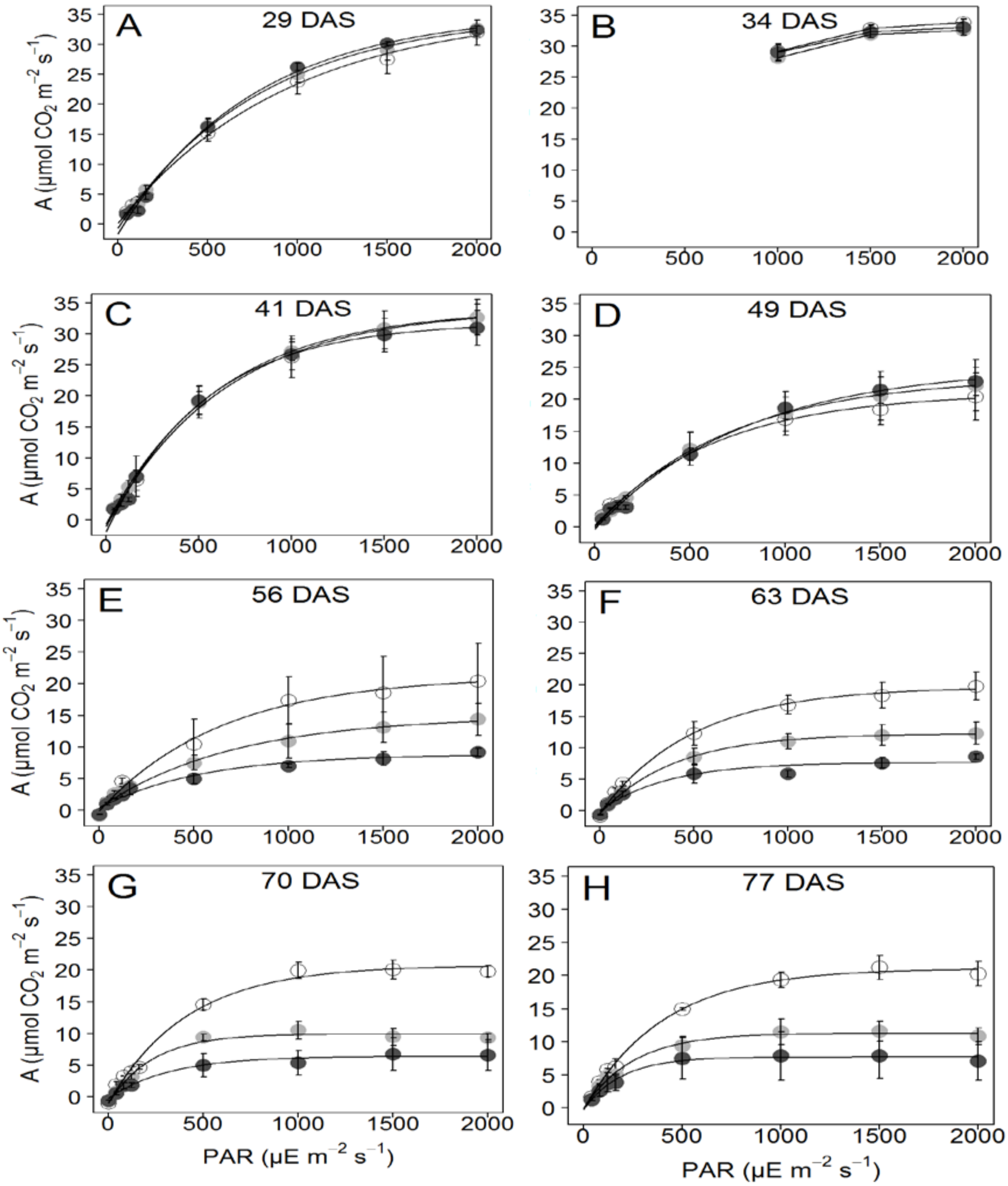

Figure 5. Time course of photosynthesis-light response curves for leaves from rice plants infected by *R. fistulosa* compared to leaves from uninfected rice plants in Experiment 3. Grey circles: 6 *R. fistulosa* plants per pot; black circles: 13 *R. fistulosa* plants per pot; open circles: uninfected plants. Vertical bars represent standard errors of mean.

Irrespective of the parasite infestation level, the photosynthesis-light response curves of *R. fistulosa*-infected plants were similar to those of uninfected plants until 56 DAS and beyond ($P < 0.001$) (Fig 5). The A_{max} of rice plants infected with 6 *R. fistulosa* plants was about 30% less than that of uninfected plants at 56 DAS and 63 DAS which later depreciated to a difference of 45% at 70 DAS and 77 DAS. The A_{max} of rice plants infected with 13 *R.*

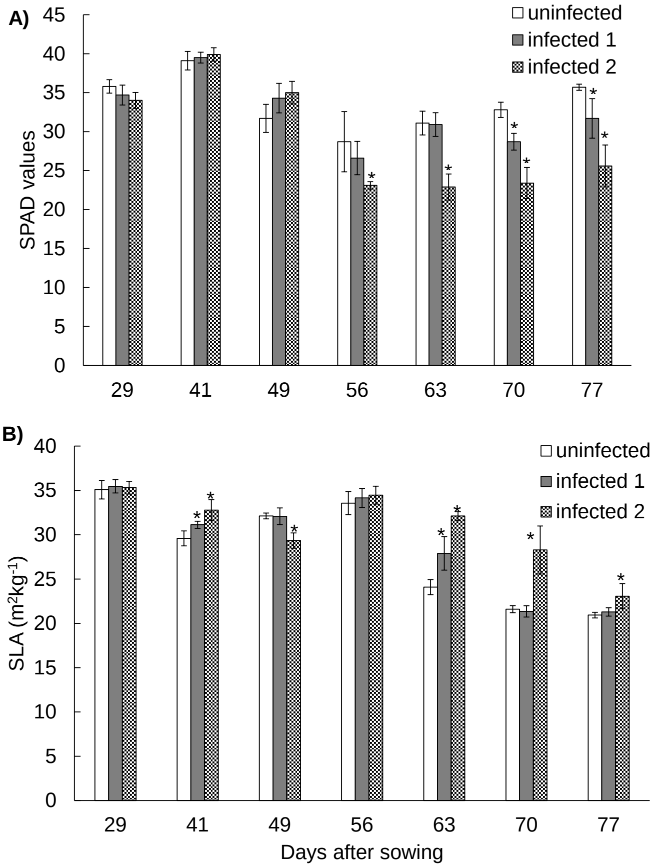

Figure 6: SPAD values (A) and specific leaf area, SLA (B) of the youngest fully developed leaf of uninfected rice plants and rice plants infected by 6 *R. fistulosa* plants per pot (infected 1) and 13 *R. fistulosa* plants per pot (infected 2) in Experiment 3. Vertical bars are standard errors of mean. * indicates significant differences *($P < 0.05$)* between infected and uninfected rice plants.

fistulosa plants was over 60% less than that of uninfected plants at all these dates (56, 63, 70 and 77 DAS). Infected plants at both infestation levels maintained similar levels of initial light use efficiency, ε, as uninfected plants until the last observation date at 77 DAS (Fig 5H). At this date, the ε of infected plants was significantly lower ($P < 0.009$) than uninfected plants by 12 % and 27% at infection rates of 6 and 13 parasites per pot, respectively. The R_d differed significantly between observation dates ($P < 0.002$), but did not differ significantly between infected and uninfected plants ($P < 0.867$) irrespective of parasite infestation level.

SPAD values of infected plants reduced with time but values of plants infected with 13 *R. fistulosa* plants differed significantly from uninfected plants at an earlier date than those infected with 6 *R. fistulosa* plants. By 56 DAS, SPAD values of plants infected with 13 *R. fistulosa* plants were significantly lower ($P < 0.032$) than uninfected plants (Fig 6 A), while values from plants infected with 6 *R. fistulosa* plants only differed significantly from uninfected plants at 70 DAS (*P*<0.004) and 77 DAS (*P*<0.069) (Fig 6A). The SLA of *R. fistulosa*-infected and uninfected plants did not differ significantly at 29 DAS (Fig 6B). Later at 41 DAS the SLA was significantly more (*P*<0.001) than that of uninfected plants by 5 % and 10 % for infestation rates of 6 and 13 *R. fistulosa* plants respectively. At 49 DAS and 56 DAS, there was no significant effect from *R. fistulosa* infection. The SLA of rice plants infected with 6 *R. fistulosa* plants was 16% more than that of uninfected plants at 63 DAS (*P*<0.001) but later at 70 DAS and 77 DAS, the SLA did not differ significantly from that of uninfected plants. The SLA of rice plants infected with 13 *R. fistulosa* plants was 33% more than uninfected plants at 63 DAS (*P*<0.001) and 31% more at 70 DAS. At 77 DAS the difference between infected and uninfected plants was just 6%.

4.3.3.2 Stomatal conductance

Stomatal conductance of leaves of both uninfected rice plants and plants infected by 6 and 13 *R. fistulosa* plants was measured at 56, 63 and 70 DAS. In all cases, stomatal conductance increased with increasing irradiance from 0 to the highest light level at 2000 µE $m^{-2}s^{-1}$ PAR (Fig 7). Mean stomatal conductance values of uninfected plants at 56 DAS were 0.0366 (mol $m^{-2}s^{-1}$) in the dark and rose to 0.356 (mol $m^{-2}s^{-1}$) at 2000 µE $m^{-2}s^{-1}$ PAR (Fig 7A). A week later (63 DAS), the mean stomatal conductance of uninfected plants was 0.0192 (mol $m^{-2}s^{-1}$) in the dark and increased to 0.0206 (mol $m^{-2}s^{-1}$) at 2000 µE $m^{-2}s^{-1}$ PAR (Fig 7B). After another week (70 DAS), the mean stomatal conductance of uninfected plants were 0.0373 (mol $m^{-2}s^{-1}$) in the dark and increased to 0.345 (mol $m^{-2}s^{-1}$) at 2000 µE $m^{-2}s^{-1}$ PAR (Fig 7C).

At 56 DAS the only significant difference in mean stomatal conductance of infected and uninfected plants was in the dark and at 120 µE $m^{-2}s^{-1}$ PAR. In the dark, values of infected plants were 0.63 and 0.61 times less than that of uninfected plants at infection rates of 6 and 13 *R. fistulosa* plants, respectively (*P*=0.025). At 120 µE $m^{-2}s^{-1}$ PAR, the values of infected plants were 0.46 and 0.36 times less than that of uninfected plants at 6 and 13 *R. fistulosa* infection rates respectively, (*P*=0.002). At 63 DAS, significant differences in mean values of infected and uninfected plants were only observed at a much higher irradiance level of 1000 µE $m^{-2}s^{-1}$ PAR. The values of infected plants were 0.66 and 0.35 times less than that of uninfected plants at infection rates of 6 and 13 *R. fistulosa* plants, respectively (*P*=0.002).

At 70 DAS, significant differences in stomatal conductance of infected and uninfected plants were first observed at lower irradiance levels of 40 and 80 µE $m^{-2}s^{-1}$ PAR and at all the highest irradiance levels from 500 to 2000 µE $m^{-2}s^{-1}$ PAR. At 40 µE $m^{-2}s^{-1}$ PAR stomatal conductance of uninfected rice plants were twice as high than that of infected plants at both infection rates (*P*=0.003). At 80 µE $m^{-2}s^{-1}$ PAR, the values of uninfected rice plants were 3 times and 2 times more than uninfected plants at infection rates of 6 and 13 *R. fistulosa* plants, respectively (*P*<0.001). At the high irradiances, stomatal conductance of rice plants infected

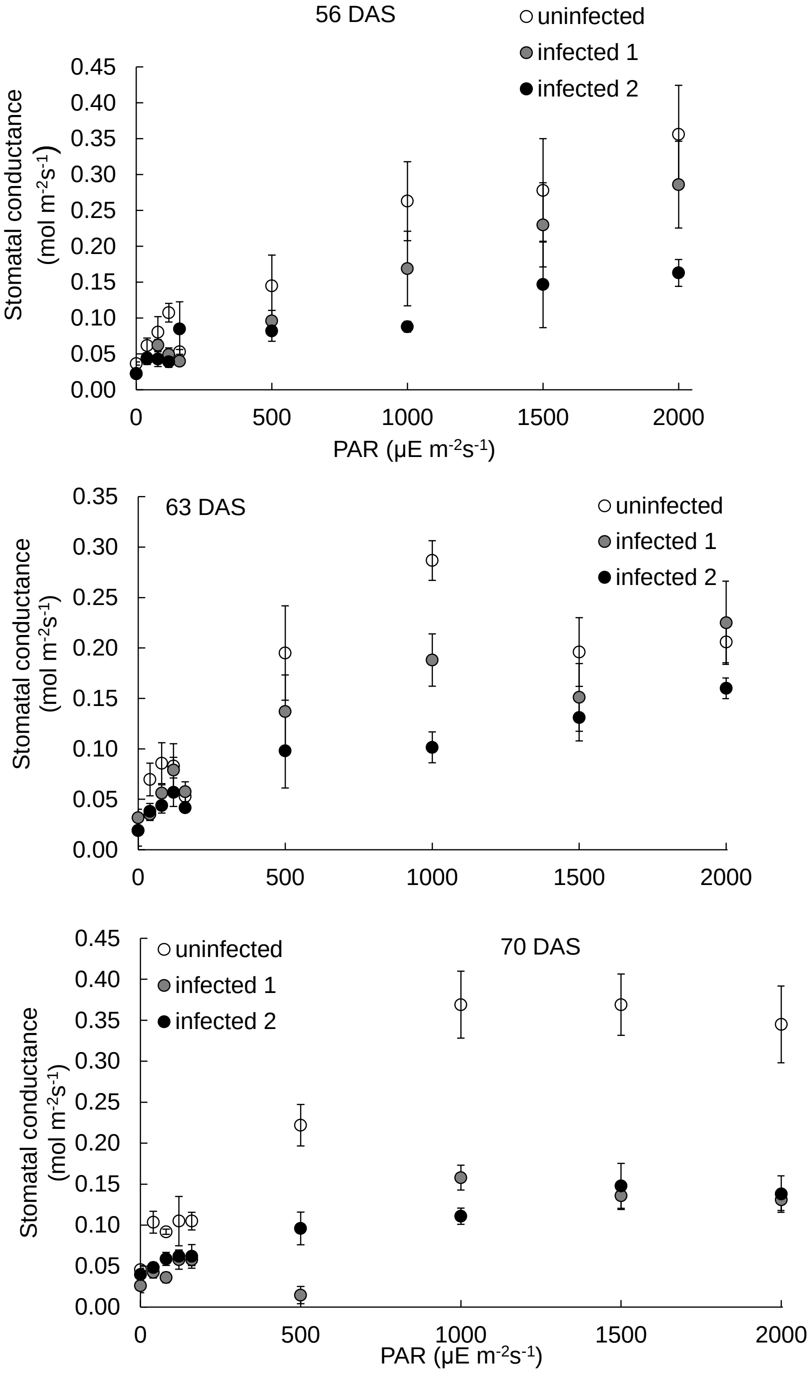

Figure 7. Stomatal conductance of uninfected rice plants and rice plants infected by 6 *R. fistulosa* plants per pot (infected 1) and 13 *R. fistulosa* plants per pot (infected 2) in Experiment 3 at 56, 63 and 70 DAS. Vertical bars are standard errors of mean.

by 6 *R. fistulosa* plants were 34%, 57%, 63% and 62% less than that of uninfected plants at 500, 1000, 1500 and 2000 µE $m^{-2}s^{-1}$ PAR, respectively. Stomatal conductance of rice plants infected by 13 *R. fistulosa* plants were 57% and 70% less than that of uninfected plants at 500

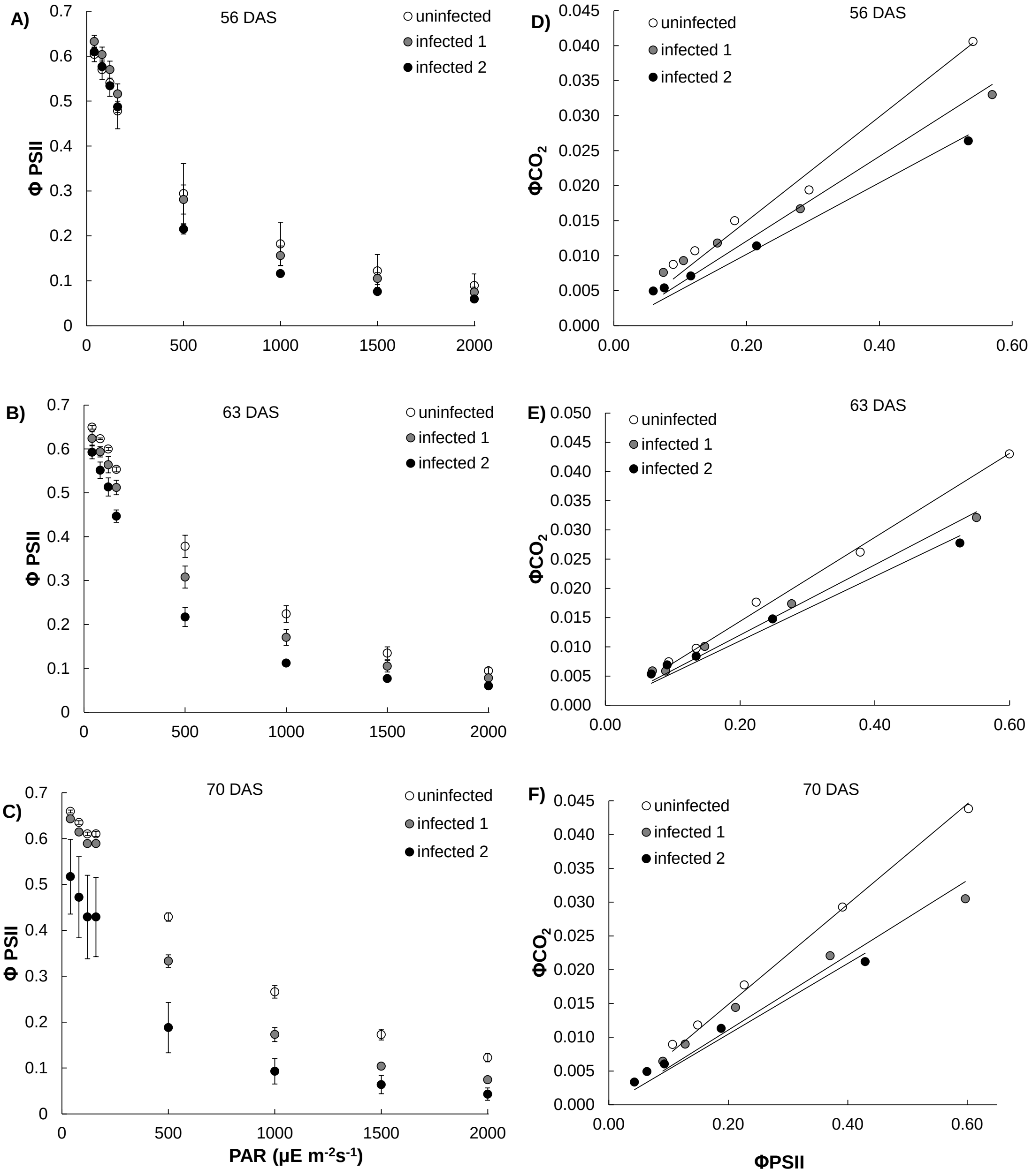

Figure 8. The light response of Φ_{PSII} (A, B, C) and the relationship between Φ_{CO2}, and Φ_{PSII} (E, F, G) of leaves of uninfected rice plants and *R. fistulosa*-infected plants at infection rates of 6 plants (infected 1) and 13 plants (infected 2).

(*P*=0.008), 1000 µE m^{-2}s^{-1} PAR (*P*=0.001), respectively and 60% less than uninfected plants at both 1500 (*P*=0.002) and 2000 µE m^{-2}s^{-1} PAR (*P*=0.007).

4.3.3.3 Light adapted quantum efficiency of PSII electron transport and quantum yield of CO_2 assimilation

The light adapted quantum efficiency of PSII electron transport, Φ_{PSII}, indicates the efficiency for light use or the electron transport by PSII. The control rice plants showed, more or less, the same values of Φ_{PSII} over time (Fig 8A, B and C). At all three observation dates, the trend was found that Φ_{PSII} of rice with low *R. fistulosa* density was reduced at middle and high light levels, while at low light levels, below 160 µE $m^{-2}s^{-1}$ PAR, no change compared to the control plants was observed. The Φ_{PSII} of rice with high *R. fistulosa* density was even strongly reduced compared to rice with low *R. fistulosa* density. Besides a reduction of Φ_{PSII} at middle and high light levels, also Φ_{PSII} at low light levels was reduced, particularly at 70 DAS.

Quantum yield of CO_2 assimilation, Φ_{CO2}, was correlated with Φ_{PSII} of both uninfected rice plants and plants infected by 6 and 13 *R. fistulosa* plants at 56, 63 and 70 DAS (Fig 8D, E and F respectively). In all treatments, a linear relationship was observed between Φ_{CO2} and Φ_{PSII} (R^2 values ranging from 0.938 to 0.996). The slopes of the linear relation between Φ_{CO2} and Φ_{PSII}, for infected plants deviated from that of uninfected plants. The reduced slopes of infected plants indicate that with the same amount of available electrons less CO_2 was fixed, indicating that CO_2 fixation was relatively inefficient. At 56 DAS the slopes deviated from that of uninfected plants by 19% and 32% for infection rates of 6 and 13 *R. fistulosa* plants, respectively (Fig 8D). At 63 DAS the slopes deviated by 16% and 23% (Fig 8E) and at 70 DAS, the slopes further deviated by 28% and 30% for rice plants infected by 6 and 13 *R. fistulosa* plants, respectively (Fig 8F).

4.4 Discussion

All three experiments clearly showed that infection with *R. fistulosa* had a negative effect on leaf photosynthetic rate of rice. In Experiment 1 and 3, where leaf photosynthetic rate was regularly observed with intervals of about one week, it was found that the first reductions in leaf photosynthetic rate appeared at around eight weeks after rice sowing. Infection initially only resulted in a reduced A_{max}, whereas initial light use efficiency, ε, was not affected. At 65 DAS, also ε was reduced. Only in the third experiment, where parasite infestation level was relatively mild, such a reduction was only observed at 77 DAS. Also for the frequently studied host plant-parasite association sorghum – *S. hermonthica* it was observed that both A_{max} and ε of the host plant were reduced after infection by the parasite (e.g. van Ast, 2006). Such a reduction was however already observed at 19 DAE of the sorghum host. The relatively late reduction in leaf photosynthetic rate following the presence of *R. fistulosa*, is likely due to a much later time of parasite infection. In the study of van Ast (2006), the first attachments of *S. hermonthica* to the roots of the parasite were already observed at 10 DAE. Germination of the obligate parasite is triggered by root exudates of the host plant and any presence of a parasite seed to the close proximity of a host root might soon result in infection. *Rhamphicarpa fistulosa* is a facultative parasite, of which the seeds only germinate close to the soil surface (Kabiri *et al.*, 2015). Germination of the tiny seed results in the establishment of a tiny seedling. For connection to the root system of the host, the roots of the parasite need to grow towards the roots of their host in the shortest possible time. In the current experiments the time to first attachment of the parasite was not directly established. However, in

Experiment 1 plant height of the parasite was determined and it was shown that a significant increase in plant height compared to the control was first observed at 51 DAS, indicating that by that time at least some of the parasites had managed to establish a connection with their host. One week later these connections were confirmed by a reduced leaf photosynthetic rate of the host.

The time gap of one week between a significant increase in plant height of the parasite and the reduction in leaf photosynthetic rate of the host might suggest that only taller parasites are able to physiologically disturb a host plant. On the other hand, it might also simply mean that the parasite needs a certain amount of time before it is able to deregulate its host plant. At 58 DAS, simultaneous with the first reduction in leaf photosynthetic rate, the dry weight of the host plant was significantly reduced. Next to a significant reduction compared to the control plant, the dry weight of the host was also about 50% less than the dry weight of the host plant one week earlier. This suggests that between 51 and 58 DAS, the parasite managed to withdraw a considerable amount of biomass from its host. It is exactly in this period of time that also the leaf photosynthetic rate of infected host plants started to drop. This strongly suggests that the physiological deregulation of the host plant necessary to withdraw water, solutes and assimilates from the host plant is connected to the negative influence on the leaf photosynthetic rate of the host. Whether the reduction in leaf photosynthetic rate of the host is functionally related to the stealing of nutrients and assimilates, or is just an unwanted side-effect, cannot be resolved based on the results of the current experiments.

Reductions in photosynthetic rates can be caused by different factors, and these include thinner leaves and reduced chlorophyll content. Therefore, after photosynthesis measurement, leaf thickness and SPAD of the measured leaves were determined. In all three experiments, an increase in SLA (thinner leaves) and a reduction in SPAD of infected leaves, relative to that of leaves of uninfected plants, were observed (Fig. 2, 4 and 6). These deviations were first observed at the time that leaf photosynthetic rate of infected plants started to reduce compared to the photosynthetic rate of uninfected plants. These deviations were in general larger with rice plants that suffered from a higher parasite infestation level. Thinner leaves might easily result in a lower chlorophyll content per unit leaf area, as confirmed by the reduction in SPAD-values. As chlorophyll allows plants to absorb energy from light, the observed reduction in leaf greenness following parasite infection has likely contributed to the reduction in leaf photosynthetic rate. The reductions in leaf photosynthetic rate however frequently surpassed the deviations in SLA and SPAD, strongly suggesting that also other parasite-induced effects were responsible for a reduction in leaf photosynthetic rate.

The reduction in A_{max} of infected rice plants increased with parasite infestation level. The maximum reduction was about 60% (Fig. 4), which is comparable to the maximum reduction obtained at 70 and 77 DAS in Experiment 3 (Fig. 5). Also with the sorghum - *S. hermonthica* association it was observed that the reduction in leaf photosynthetic rate was stronger at higher parasite infestation level. In this case, the maximum reduction in leaf photosynthetic rate was about 50%. In a study with broomrape (*Orobanche ramosa*)-tomato association (Mauromicale *et al.*, 2008) photosynthesis of tomato was suppressed by 39-50%.

The chlorophyll fluorescence data on PSII measured at 56, 63 and 70 DAS do provide at least some clues on how photosynthesis was affected after infection with *R. fistulosa*. Data regularly show a significant reduction in Φ_{PSII}, referring to a reduced electron transport rate (Fig 8A-C). On top of that, the slope that represents the relation between Φ_{PSII} and Φ_{CO2} was significantly reduced, meaning that part of the electrons were not used for fixation of CO_2 (Fig 8D-F). A reduction in the ratio Φ_{CO2}/Φ_{PSII} could mean that there were more electrons passing through PSII than were required to sustain the observed CO_2 assimilation. Possibly, alternative electron sinks other than CO_2 assimilation were operating. Fryer *et al*., (1998) suggested several alternative sinks to cause low Φ_{CO2}/Φ_{PSII} values, including photorespiration. Another reason for such a reduction in efficiency is that at the carboxylation site, the concentration of CO_2 is simply too low. Such a limitation of CO_2 might be due to an increased stomatal resistance, or due to an increased mesophyll resistance. Measurements presented in Fig. 7 indeed confirm that the stomatal conductance of infected rice plants was reduced. Lower stomatal conductance reduces the diffusion of CO_2 into the leaves (Farquhar and Sharkey, 1982), and this feature was found in *Striga*-infected hosts (Gurney *et al.*, 1995; Frost *et al.*, 1997). Parasitic plants have in general high transpiration rates, which may result in insufficient water for the host, resulting in a closure of stomata (Stewart and Press, 1990). Some root parasites, such as nematodes, are known to reduce transpiration rates of their host (Schans and Arntzen, 1991) and it has been suggested that this is a result of increased levels of abscisic acid (ABA). Abscisic acid is known as a stress hormone and it can cause stomata closure also in the absence of water shortage. Also other symptoms in *Striga*-infected plants are known to be sensitive to changes in ABA-concentrations. Abscisic acid can be involved in reducing leaf area expansion and stem extension, and in increasing the root:shoot ratio (Taylor *et al.*, 1996; Frost *et al.*, 1997; Watling and Press, 2001). These phenomena are also observed in rice plants after infection with *R. fistulosa* (Chapter 5), suggesting that also in this host-parasite association ABA concentrations might be affected.

Yet another explanation for the reduced Φ_{CO2}/Φ_{PSII} ratio of infected plants is that absorption of PAR was reduced, as our Φ_{CO2} was based on incident radiation. Absorption was not directly measured. If infection resulted in a lowered irradiance absorption, it means that the actual efficiency of CO_2-fixation was underestimated. This in turn will contribute to a reduction in slope as observed in Fig 8D-F. The reduced SPAD-values of infected leaves (Fig. 6) indeed provide support for the suggestion that a reduced absorption of PAR contributed, at least in part, to the reduced efficiency of electrons for fixing CO_2. The large impact of *R. fistulosa* infection on both stomatal conductance and photosynthetic metabolism of rice is similar to the effect that other parasitic plant species have on C_3 hosts. Also in the *Striga*-C_3

host associations (Watling and Press, 2000) and in *Cuscuta*-host associations (Shen *et al.*, 2007) the parasite impaired both stomatal conductance and host photosynthetic metabolism.

Our results clearly show that the facultative parasitic plant *R. fistulosa* has an influence on the photochemical process and resistance to gas exchange of its host. Infection led to significant reductions in photosynthetic rate, stomatal conductance, the quantum efficiency of PSII e^- transport (Φ_{PSII}) and chlorophyll content. Reductions were observed starting from eight weeks after sowing, whereby the level of reduction depended on parasite infestation level. In addition, parasite infection led to a reduced slope in the linear relationship between quantum yield of CO_2 assimilation (Φ_{CO2}) and quantum efficiency of PSII (Φ_{PSII}). These results shed some light on the metabolic host-parasite interactions between rice and *R. fistulosa.*

Chapter 5

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

Stella Kabiri[1], Jonne Rodenburg[2], Aad van Ast[1]& Lammert Bastiaans[1]

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[1]Centre for Crop Systems Analysis, Wageningen University & Research, the Netherlands
[2]Africa Rice Center (AfricaRice), 01 BP 4029 Abidjan 01, Côte d'Ivoire

ABSTRACT

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

Key words: Rice Vampireweed, Oryza sativa, parasitic weed, rain-fed lowland rice.

5.1 Introduction

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

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

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

fistulosa were included to obtain a more complete comprehension of the host plant–parasite interaction.

5.2 Materials and Methods

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

5.2.1 Experiment 1

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

The experiment followed a randomized complete block design with six replicates and two sampling times, at 60 DAS (Days after sowing of both host and parasite) and at maturity (110 DAS), resulting in a total of 96 pots. Imperforated pots with 5 L capacity were filled with 6 kg of a mixture of coarse sand and arable soil (1:1 v/v). The pots were watered daily to maintain saturated conditions. Rice seeds were pre-germinated for 48 hours in an incubator at 33^0C, after which one pre-germinated seed was planted in the centre of each pot at a depth of 1-2 cm. On the same day, *R. fistulosa* seeds were mixed with about 9 g of dry sand and evenly sprinkled on top of the soil surface. Fertilizers were applied in two split applications, one at 18 DAS and another at 56 DAS (maximum tillering). At each application, 624 mg of NPK (12:10:18) fertilizer per pot was provided, equivalent to a total of 50 kg N ha^{-1}.

5.2.2 Experiment 2

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

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

5.2.3 Destructive samplings

At the sampling dates outlined above, rice tillers were counted and rice plant height was measured from the base of the stem to the youngest ligule of the main shoot. After that, rice plants were separated into leaves, stems and roots. At final harvest, panicles were included. Rice plant leaf area was measured with a desk-top leaf area meter, model LI-3100C (LI-COR, Lincoln, Nebraska, USA). Roots were cleaned by carefully washing with water. In pots containing rice and *R. fistulosa* plants, the mixed root system was put in a plastic bag after washing, and frozen overnight at -20°C. The freezing process turned the roots of *R. fistulosa* into a dark purple colour, facilitating separation from host plant roots. All above-ground biomass of all parasites were also harvested to account for the parasite biomass in each pot. All plant parts of both host and parasite were dried in an oven at 70^{0}C for 48 hours to determine dry weight.

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

5.2.4 Statistical analysis

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

5.3 Results

5.3.1 Influence of parasite density (Experiment 1)

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

At 60 DAS the biomass accumulated by the parasite, steadily increased with density (Fig. 1a; $F_{6,29} = 28.15$; $P < 0.001$). Also, the fraction that *R. fistulosa* accumulated from the total biomass (host + parasite) increased with density level (Fig. 1a; $F_{6,35} = 23.31$; $P< 0.001$) such that at high infestation levels the parasite had accumulated more than half of the total biomass. While the biomass of the parasite increased with density, there was a decline in the biomass of infested rice plants with increasing parasite density (Fig. 1a; $F_{7,35} = 25.15$; $P< 0.001$). At all densities, the total biomass at 60 DAS was significantly lower than the sole biomass of non-infested rice plants (Fig. 1a; $F_{5,35} = 5.53$; $P< 0.001$).

Compared to the *R. fistulosa*-free rice plants, a reduction in dry matter allocated to the stems was observed at densities of 31 seeds per pot and higher (Fig. 1b; $F_{7,35}= 13.51$; $P< 0.001$), whereas an increased fraction dry matter allocated to the roots started at 62 seeds pot (Fig. 1b; $F_{7,35}= 13.51$; $P< 0.001$). Dry matter allocation to the leaves was not affected by *R. fistulosa* but rice plant height was. From the lowest density of 15 seeds per pot onwards, infested plants were stunted. At 60 DAS, height reductions compared to *R. fistulosa*-free rice plants ranged from 3 cm, at a density of 15 seeds per pot, to nearly 8 cm at 1000 seeds per pot (Table 1). At the same time, rice leaf area was reduced at densities ≥ 62 seeds per pot, while tiller production was reduced at densities ≥ 125 seeds per pot (Table 1).

At 110 DAS, at densities ≥ 250 seeds per pot, the number of *R. fistulosa* plants was markedly lower than that at 60 DAS (Table 1). This decrease was not observed at lower *R. fistulosa* infestation levels, suggesting that the decrease at higher infestation levels resulted from density dependent mortality. Between 60 DAS and 110 DAS the parasite biomass at all.

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

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

Values (means) followed by a different letter are significantly different ($P<0.05$). S. E. D. = standard error of differences of means; L.S.D. = least significant difference at the 95% confidence level.

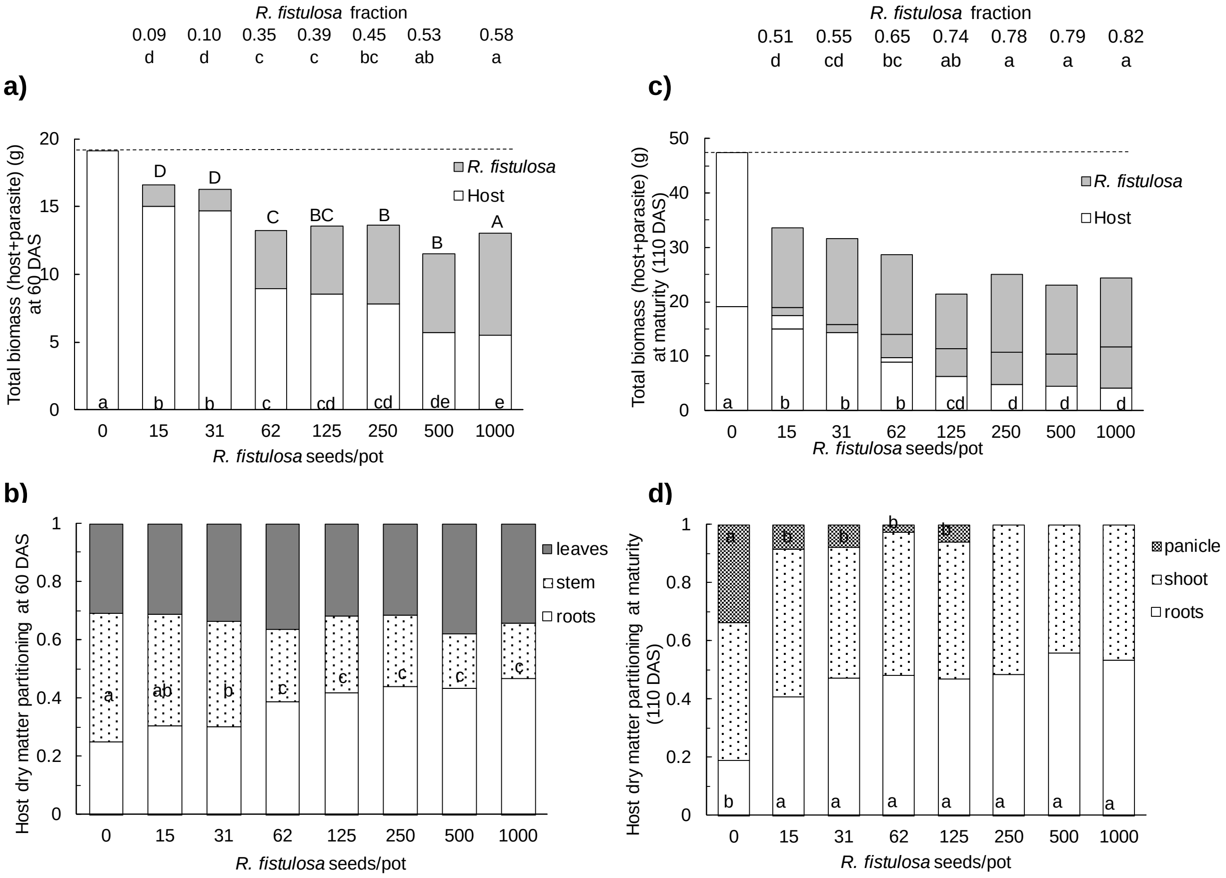

Figure 1. Total biomass of rice and *R. fistulosa* (g/pot) at 60 (A) and 110 DAS (C) and the fraction of biomass partitioned to host plant parts at 60 (B) and 110 DAS (D) as a function of *R. fistulosa* infestation level (*R. fistulosa* seeds/pot). The dotted lines above A and C indicate the average dry weight of *R. fistulosa-free* rice plants. Division marks between the bars in C indicate the amount of biomass accrued by either host or parasite between 60 and 110 DAS. Above A and C the fraction of *R. fistulosa* dry weight to the total biomass (host and parasite) is presented. Bars indicated with different letters are significantly different (P<005).

densities increased considerably. At 110 DAS, there were no significant differences between the absolute biomass of *R. fistulosa* plants across parasite densities (Fig. 1c; $F_{7,27} = 1.87$; $P = 0.122$), but the fraction of total biomass (host and parasite) accumulated by the parasite increased with density (Fig. 1c; $F_{7,27} = 28.15$; $P < 0.001$). At the highest parasite infestation level this fraction was even higher than 80%. Between 60 and 110 DAS, there was a 2.5 fold increase in the biomass of non-infested rice plants (Fig. 1c). In contrast, biomass of infested rice plants increased only marginally (at the lowest infestation levels) or not at all (at the highest infestation levels). The significant differences in rice biomass between infestation levels thus remained (Fig. 1c; $F_{5,34} = 28.15$; $P < 0.001$). At all *R. fistulosa* infestation levels, the total biomass of host and parasite at 110 DAS was significantly lower than the biomass of non-infested rice plants (Fig. 1c; $F_{5,34}$= 8.32; P< 0.001).

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

5.3.2 Time course of parasitism (Experiment 2)

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

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

Steady parasite-inflicted host plant biomass reductions were also first observed at 71 DAS, and increased thereafter (Fig. 3). These reductions started at the density of 13 parasite plants ($F_{2,6}$ = 14.18, P = 0.005). At 99 DAS and 111 DAS both infestation levels resulted in significantly reduced host plant biomass compared to the non-infested plants (99 DAS; $F_{2,6}$ = 163.7, P< 0.001 and 111 DAS; $F_{2,6}$ = 98.24, P< 0.001). The host plant biomass at the two infestation densities remained significantly different from one another (Fig. 3B). Biomass accumulation of the rice plants in the second half of the growing season was again minimal, at

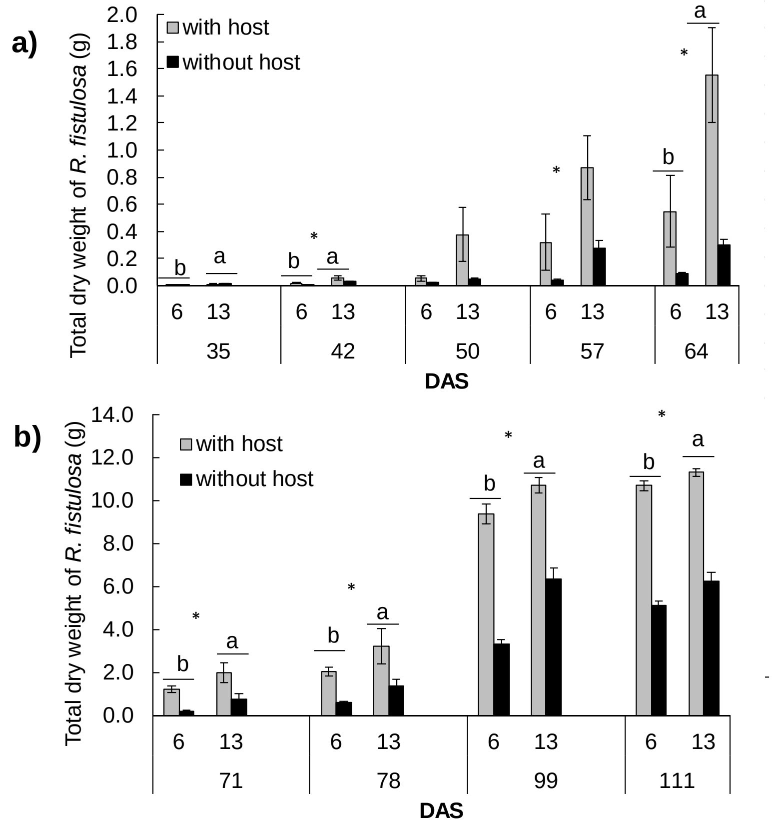

Figure 2. Development of *R. fistulosa* biomass (g/pot) in association with a host (grey bars) or without a host (black bars) at 35, 42, 50, 57 and 64 DAS (A) and at 71, 78, 99 and 111 DAS (B). Bars with different letters show significant differences ($P<0.05$) between infestation of 6 and 13 *R. fistulosa*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

both 6 and 13 *R. fistulosa* plants per pot, resulting in significant reductions in kernel yield. *Rhamphicarpa fistulosa*-free rice plants produced 11.3 g grains (data not shown) while infested plants produced only 0.88 g at 6 and 0.22 g at 13 *R. fistulosa* plants per pot ($F_{3,6}$ = 80.62, $P< 0.001$).

Starting at 42 DAS, infested rice plants distributed relatively more dry matter to the roots at the expense of the stems, compared to parasite-free plants (Fig. 4; $F_{2,6}$ = 16.41, P= 0.004). At 78 DAS there was also a clearer difference between the two densities ($F_{2,6}$ = 25.77, P = 0.001). At maturity (111 DAS), while in *R. fistulosa*-free rice plants about 5% of total dry matter was present in the leaves, in the parasite infested plants this was 17% ($F_{3,6}$ = 131.66, $P< 0.001$). More prominently, whereas 34% of the total dry matter of non-infested rice plants was found in the roots, 50% and 59% of the dry matter of infested plants was found in this part ($F_{2,6}$ = 60.57, $P< 0.001$). In the infested plants only 11% (at 6 parasites per pot) and 5%

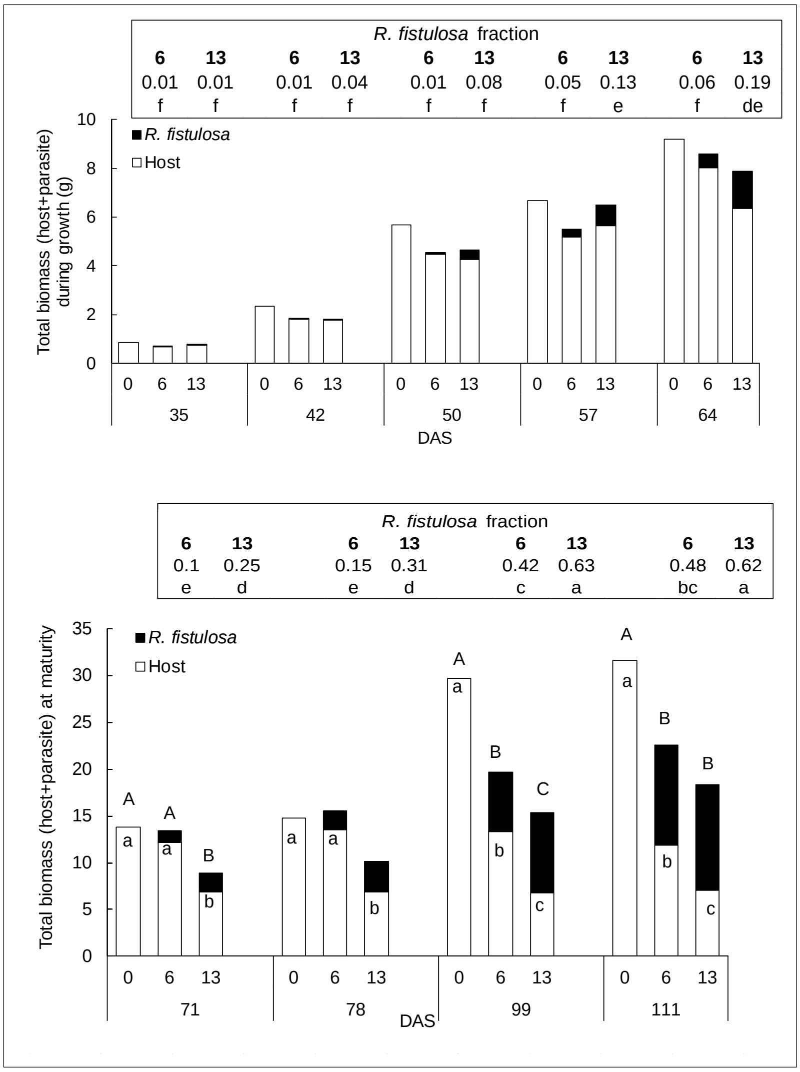

Figure 3. Development of total biomass (g/pot) of rice (white bars) and *R. fistulosa* (black bars) at different sampling dates in response to infestations of 0, 6 and 13 *R. fistulosa* plants per pot. For each sampling date, bars indicated with different lower-case letters indicate significant differences (P<005) in host biomass, whereas bars indicated with different upper-case letters specify significant differences (P<005) in total biomass (host and parasite). Above each figure, the fraction of *R. fistulosa* dry weight to the total biomass is shown.

(at 13 parasites) of total dry matter was found in the panicles, compared to 33% of total dry matter of the uninfected plants (Fig. 4; $F_{2,6} = 111.05$, $P < 0.001$).

Significant parasite-inflicted height reductions were first observed at 64 DAS at both parasite densities. Height differences between these densities occurred only at 99 and 111 DAS (Table 2). Steady parasite-inflicted leaf area reductions were observed from 71 DAS onwards at an infestation of 13 *R. fistulosa* plants per pot. At the lower infestation level leaf area reductions were only significant at 99 and 111 DAS. Steady reductions in tiller number were observed from 71 DAS onwards at an infestation of 13 *R. fistulosa* plants per pot. At 6 parasites per pot, tiller reductions compared to the parasite-free rice plants were only

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

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

Values (means) followed by a different letter are significantly different ($P<0.05$). Bolded figures show F x,y indicates F values (F), degrees of freedom of treatments (x) and degrees of freedom of residuals (y).

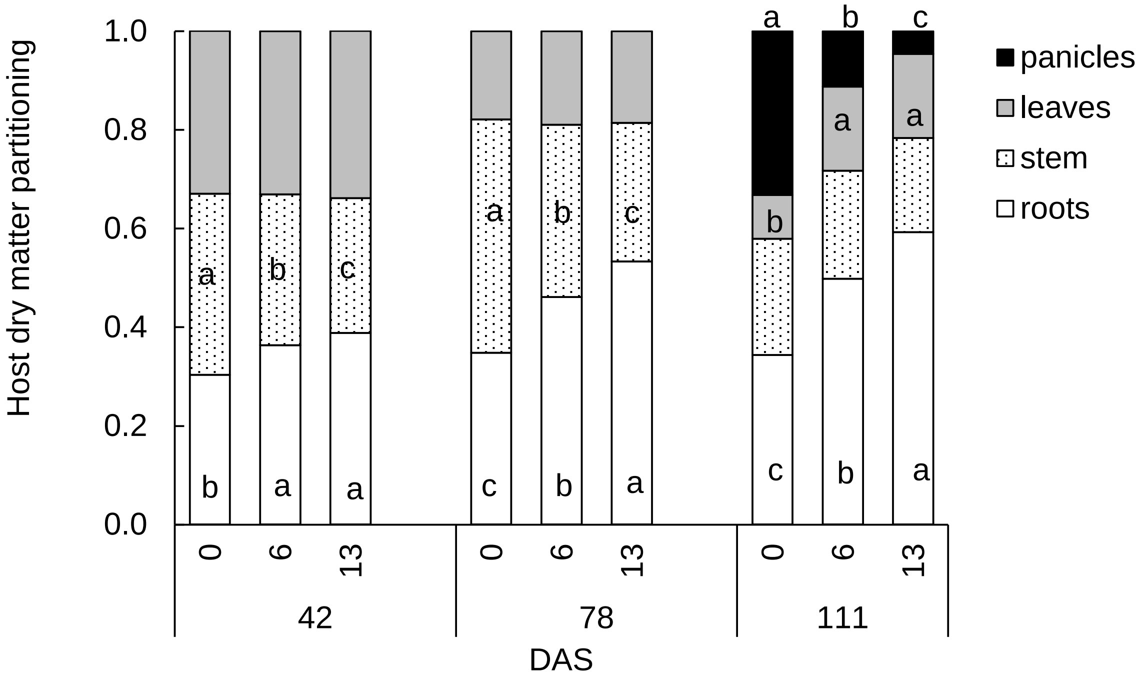

Figure 4. The fraction biomass partitioned to panicles, leaves, stems and roots in response to infestations of 0, 6 and 13 *R. fistulosa* plants per pot at 42, 78 and 111 days after sowing (DAS). Bars with different letters are significantly different (P<005) at the corresponding sampling date.

significant at 99 and 111 DAS. Starting at 42 DAS, infested rice plants distributed relatively more dry matter to the roots at the expense of the stems, compared to parasite-free plants (Fig. 4; $F_{2,6}$ = 16.41, P= 0.004). At 78 DAS there was also a clearer difference between the two densities ($F_{2,6}$ = 25.77, P = 0.001). At maturity (111 DAS), while in *R. fistulosa*-free rice plants about 5% of total dry matter was present in the leaves, in the parasite infested plants this was 17% ($F_{3,6}$ = 131.66, P< 0.001). More prominently, whereas 34% of the total dry matter of non-infested rice plants was found in the roots, 50% and 59% of the dry matter of infested plants was found in this part ($F_{2,6}$ = 60.57, P< 0.001). In the infested plants only 11% (at 6 parasites per pot) and 5% (at 13 parasites) of total dry matter was found in the panicles, compared to 33% of total dry matter of the uninfected plants (Fig. 4; $F_{2,6}$ = 111.05, P< 0.001).

Significant parasite-inflicted height reductions were first observed at 64 DAS at both parasite densities. Height differences between these densities occurred only at 99 and 111 DAS (Table 2). Steady parasite-inflicted leaf area reductions were observed from 71 DAS onwards at an infestation of 13 *R. fistulosa* plants per pot. At the lower infestation level leaf area reductions were only significant at 99 and 111 DAS. Steady reductions in tiller number were observed from 71 DAS onwards at an infestation of 13 *R. fistulosa* plants per pot. At 6

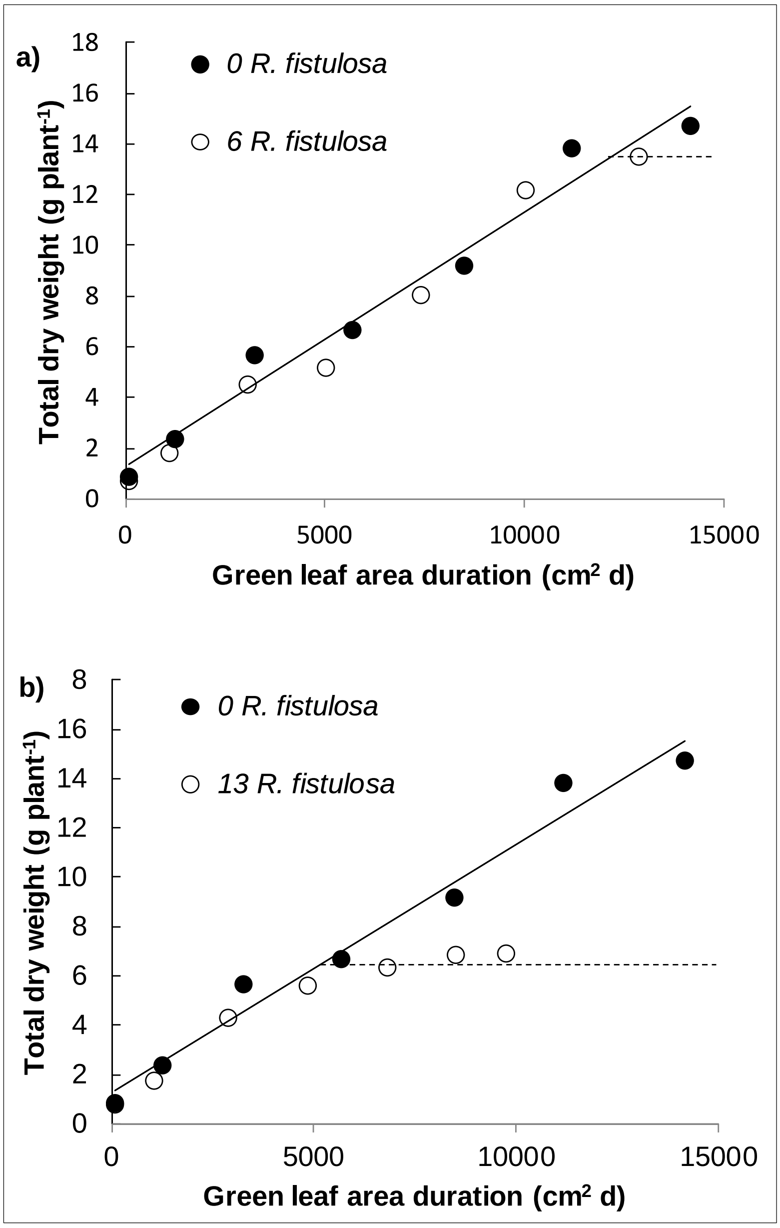

Figure 5. Relationship between rice plant dry weight (g/plant) and green leaf area duration (cm^2d) for uninfested rice plants and for rice plants infested with 6 *R. fistulosa* (A) and 13 *R. fistulosa* plants per pot (B). The solid line represents the result of linear regression ($y = 0.001x + 1.292$;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

parasites per pot, tiller reductions compared to the parasite-free rice plants were only significant at 99 and 111 DAS.

A linear relationship was found between total per plant dry weight and green leaf area duration (LAD) of control plants. From 35 to 78 DAS, LAD increased to around 14.000 cm^2d and total rice plant dry weight increase per unit LAD —hence the conversion of intercepted radiation in rice plant dry weight, i.e. the light use efficiency (LUE)— was about 1.0 g per 1000 cm^2d (Fig 5a, b). At the high infestation level (Fig 5b), total LAD was reduced to about

10.000 cm^2d, implying that infested rice plants only captured about two thirds of the light of a control plant. This reduction in light interception was obvious from 57 DAS onwards. From this sampling time on, also the LUE dropped dramatically and reached a value of around 0.2 g per 1000 cm^2d. At the low infestation level (Fig 5b) between 35-78 DAS, only a reduction in light interception was observed while no clear reduction in LUE was noticed. Light interception of parasite-infested plants lagged behind from 57 DAS onwards and was about 1250 cm^2d lower than the control at 78 DAS. This corresponds to a reduction of about 1.25 g in rice plant dry weight. At the low parasite density, the accumulated rice plant dry weight at 99 and 111 DAS was comparable to the dry weight observed at 78 DAS (Fig. 3). Dry matter accumulation during these last growth stages had come to a standstill.

At 64 DAS, the rice plants at the highest infestation level (13 plants per pot) reached a total plant dry weight of around 6.5 g. From then on, hardly any further increase in plant dry weight was observed, whereas green leaf area was still present showing that the production of assimilates only benefited the parasite. This is clearly illustrated in Fig. 5a: whereas LAD was still progressing while total plant dry weight had come to a standstill, indicating a LUE close to nil. For the low infestation level (6 plants per pot) the reduction in biomass up to 78 DAS could still be explained by a reduced light interception, implying that till that moment LUE was hardly affected (Fig. 5).

5.4 Discussion

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

Many studies show that the ultimate effects of hemiparasites may be difficult to predict, because even closely-related species can have very different effects on the same host species as was observed by Matthies (1997). The present results, for the first time demonstrate that a facultative parasitic plant can completely enslave its host. Towards maturity of both host and parasite, the biomass of the infected host plant appeared to remain constant while the green leaf area still continued to be present and the biomass of the parasite kept on increasing. This indicated that any assimilates produced by the host were being taken up by the parasite. The host seemed to be led to produce assimilates solely for the benefit of the parasite. This is a kind of parasite-induced manipulation that has not been described before with parasitic plants.

Hemi-parasitic plants have been shown to manipulate the host plant in such way that the advantages from parasitism are higher than the disadvantages from host competition. Studies with other facultative parasites (i.e. *Castilleja integra, C. miniata* and *C. chromosa*) have shown a reduction of total host and parasite biomass but the biomass accumulated by the hemi-parasitic plants was lower than the reduction in biomass of the host plants. This is in conformity with our results. Whereas *R. fistulosa* accumulated most of the biomass of the total host-parasite association, the combined sum of host and parasite biomass was always lower than that of *R. fistulosa*-free rice plants alone. A similar dominance of the parasite in the total (host + parasite) biomass was observed with the facultative hemi-parasitic plant *Rhinanthus minor* on the grass species *Phleum bertolonii* (Cameron *et al.*, 2008) and also with *Castilleja integra, C. miniata* and *C. chromosa,* and the annual hemiparasite *Orthocarpus purpurascens* on *Lolium perenne* (Matthies, 1997). Matthies (1997) also found that facultative parasites had a lower efficiency of resource utilization than the host. While this implies that the parasite will do better when environmental resource availability increases, Fibich et al., (2010) also demonstrated that, because hosts are better competitors for light, concomitant increases in host performance will outcompete hemiparasites once the resource availability reaches a certain level. Yet, in the current study, the observed reduction of the above-ground host biomass at the expense of the host root biomass implies that *R. fistulosa* is indeed quite successful in manipulating the host to optimize the balance between resource acquisition and competition. In further studies with *R. fistulosa*, the trade-off between parasitism and competition with its host should be investigated.

A reduction of productivity in the presence of hemi-parasites may be a common occurrence, indicating that the negative impacts some facultative parasites have on their hosts is attributed to withdrawal of assimilates. For example, *R. minor* on barley, trafficked up to 20% of host solutes from the xylem sap, reducing host growth by a similar magnitude of 22% (Jiang *et al.*, 2003). Seel and Press (1996) reported that infection of the perennial grass host *Poa alpina* by *R. minor* affected flower bud formation and biomass partitioning. The present results show that even relatively low infestation levels of *R. fistulosa* already resulted in reductions in kernel weight of the host plant of up to 79-92%. *Rhamphicarpa*-induced suppression of host biomass resulted in the parasite accumulating more than 50-80% of total biomass (host and parasite), depending on the level of infection. Yet, the degree of *Rhamphicarpa*-induced suppression of host biomass could not be fully accounted for by the biomass obtained by the parasite as uninfected rice plants had more biomass than the combined sum of the biomass of the parasite and the infected host. Since the loss in host biomass cannot be fully explained by a gain in parasite biomass, the relation between the host and parasite cannot be not purely characterized as a source-sink relation but entails a pathological component. Such a pathological effect can be caused by (i) poor resource use efficiency by the parasite such that the gain in the parasite is less than the reduction in host biomass (Matthies, 1995; Matthies, 1996; Matthies, 1997). It has been shown in may studies that the resource efficiency of hemi-parasites is generally less than that of their hosts (Bloom *et al.*, 1985; Aflakpui *et al.*, 1998; Hao *et al.*, 2005; Hibberd *et al.*, 1998b; Matthies, 1995;

Matthies, 1997). A pathological effect can also be caused by (ii) disruption of host water and nutrient balances (Watling and Press, 2001), (iii) alteration of host plant growth regulators causing changes in host plant allometry, (iv) negative feedback effects of disrupted host water, nutrient balances and host plant regulators on host plant photosynthesis through self-shading, drought stress or malnutrition effects (Parker and Riches, 1993). For instance, *Cuscuta* (dodder) species have been observed to create stronger sinks for assimilates than any of the hosts organs (Parker and Riches, 1993). With *Striga*, a pathological effect caused by an imbalance in growth regulators in the host has been reported. The growth regulator imbalance includes an increase in abscisic acid levels and a reduction in the levels of cytokinins and gibberellic acid that in turn negatively affect host photosynthesis and host growth (Gurney *et al.*, 1995; Taylor *et al.*, 1996; Watling and Press, 2001).

The missing biomass in the rice-*R. fistulosa* interaction could also be a result of a more direct host photosynthetic repression by *R. fistulosa*. Negative effects on host leaf photosynthetic rate are commonly observed in a variety of interactions with obligate parasitic plant species (e.g. Rodenburg *et al.*, 2008; Cechin and Press, 1993; Watling and Press, 2001; Rodenburg *et al.*, 2017b) and with other facultative parasitic plants (Cameron *et al.*, 2008; Hwangbo *et al.*, 2003). Specifically in a study with *R. minor*, Hwangbo *et al.*, (2003) observed that the parasite reduced host photosynthesis, leaf area and biomass which is in conformity with our findings. However, despite the negative effects of *R. minor* on its host, the total biomass of the association was not less than that of the uninfected host. This contradicts our findings, pointing to a parasite species-specific, a host-species specific effect or a combination of these. It could be that *R. minor* is more ‘autotrophic’ than *R. fistulosa* implying that the latter, has a higher degree of host dependence. Hwangbo *et al.,* (2003) also showed that a parasite-inflicted reduction in host-plant leaf area will result in reduced light interception, followed by a reduced production of biomass. It is this feedback mechanism that also contributed to the gap between the biomass accumulated by healthy and parasite-infected rice plants in the current study.

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

In this study we clearly demonstrate that an observed parasite induced reduction of host plant biomass (22-71%) and host plant kernel production (78-100%) is originating first from reductions in host-plant light interception, followed by reductions in host-plant light use efficiency. Reductions in light interception are caused by reduced green leaf area and this is probably the result of the common source-sink relations previously observed in host-parasite interactions, whereby the parasitic plant is the external sink that draws assimilates, minerals, and water from the host, and whereby the parasite consumes more resources than any organ of the host itself (Shen *et al.*, 2006). The reductions in light use efficiency can stem from reduced functionality of the photosynthetic apparatus of the host plant which in turn can have several causes, as previously mentioned. In further studies with *R. fistulosa*, the assimilate flow from host to parasite and the pathological effects should be investigated, both quantitatively —to see how important resource extraction is— as well as qualitatively —to understand which nutrients and growth regulators are primarily involved.

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

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

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Chapter 6

General Discussion

6.1 Introduction

In sub-Saharan Africa (SSA) large gaps still exist between potential and actual yield of staple food crops. To feed the growing population of SSA, these gaps need to be closed and overall production needs to be enhanced by increasing cropping intensity and the sustainable expansion of irrigated production area (van Ittersum *et al.*, 2016). Biotic production constraints such as weeds, pests and diseases are one of the major causes for existing yield gaps of food crops (Evans and Fischer, 1999). Among the most important biotic constraints to production of traditional staple crops like maize, sorghum and millet are parasitic weeds, particularly *Striga* spp. (Gressel and Joel, 2013). These parasitic weeds are typically a problem of low fertility soils. Large areas in SSA are infested and it has become nearly impossible to eradicate these parasitic weed infestations (Gressel and Joel, 2013).

In the last decade, rice has become an increasingly important food crop in Africa, where consumption of rice has grown faster than any other major staple crop (Seck *et al.*, 2013). Due to the fast growth in rice consumption, the current production is insufficient to cover local consumption. Rice in SSA is primarily grown in rain-fed production environments, comprising 70% of the area under rice production in this region (Diagne et al., 2013). In these production environments rice farmers often encounter parasitic weeds. The infestations are estimated to cover 1.34 million ha of SSA rice area, causing an estimated annual production loss of up to 829,000 tons of paddy (Rodenburg *et al.*, 2016b). Contributing to this high infestation level is a relatively new parasitic weed, *Rhamphicarpa fistulosa*, which is particularly found in the rain-fed lowlands. The parasite has gained in importance after rice production area was expanded towards these wetter areas, which are the natural habitat of this parasite. *Rhamphicarpa fistulosa* has been found to occur in at least 36 countries in SSA, and in 28 of these, rice is cultivated in rain-fed lowlands (Rodenburg *et al.*, 2016b; Rodenburg *et al.*, 2015a). Despite its occurrence in many of the rice growing countries in SSA, there is very little information regarding the biology and ecology of this parasitic weed species.

Therefore the aim of the work described in this thesis was to reveal the biology and ecology of the little known, yet devastating, facultative parasitic weed *R. fistulosa* and to assess the factors that cause this weed to thrive. Such knowledge would lay the foundation for further investigations, for instance on likely effects of global changes such as climate variabilities and extremes on future distribution of the weed, and on the design of sustainable management strategies to control *R. fistulosa*. Both field surveys and green house experiments were carried out to investigate environmental conditions suitable for the parasite to flourish at each stage of its life cycle. Investigations also addressed the role of a host in the life cycle of the parasite and the kind of relationship *R. fistulosa* develops with its host once it is attached to the roots of its host plant

6.2 Ecological niche of *Rhamphicarpa fistulosa* and its future distribution.

In SSA, *Striga* spp. *and R. fistulosa* are distributed in similar regions, but it was not known whether their habitats overlap. In the current study it was established that the parasitic weeds *S. asiatica* and *R. fistulosa* have distinctly different ecological niches, in which soil moisture is a discerning factor (Chapter 2). We found that while *S. asiatica* did not germinate in saturated soil moisture conditions at all, *R. fistulosa* not only germinated but also produced biomass under a wide range of soil moisture conditions, including highly saturated soils. This implies that *R. fistulosa* has a broader environmental range than *S. asiatica*. The natural environment of *R. fistulosa*, which are the wetlands, are increasingly turned into agricultural areas for rice production (Rodenburg *et al.*, 2014a). Moreover, rice is a suitable host and susceptible to *R. fistulosa* infestation (Chapter 5).

Based on these insights, we postulate that continued rice cultivation in the parasite's natural environment is the main factor responsible for the increased importance of this parasitic plant species as an agricultural weed. Area expansion and increased rice seed exchanges on local markets may further spread *R. fistulosa* to new areas. The parasite has very small seeds that can easily be dispersed through transportation of crop seeds, agricultural implements or movements of cattle and people. Secondly, in the face of climate change, where rainfall has been predicted to be erratic and irregular, new areas might appear that suddenly are well suited for a species like *R. fistulosa* with its high affinity for wetter conditions. With its broad ecological range, prolific production of tiny seed and opportunistic parasitic nature, the weed could become potentially invasive by colonizing these new areas. It has been observed before that weed species with such characteristics can be potentially invasive as they can easily spread and adapt to ecosystems beyond the original or most favorable ones (Booth *et al.*, 2010). The current lack of knowledge on control among farmers and extension as recently observed in affected areas (Schut, 2015; Rodenburg *et al.*, 2015b) provides an even grimmer outlook. However, unlike most species of invasive potential that are introduced into an exotic environment, that renders them free from competitors and predators, *R. fistulosa* is a native species. Therefore its invasion could be contained by competitors and predators that are already present. On the other hand, the continued cultivation of a suitable host such as rice in *R. fistulosa*'s natural environment can increase the parasites dominance over other species in its natural niche and thus intensify its success. One facultative parasite that is known to have an enormous impact on its natural niche is *Rhinanthus minor*. *Rhinanthus* plants have been known to change community diversity of other species by differential resistance to infection between potential host plants (Cameron *et al.*, 2006; Cameron and Seel, 2007) thereby causing a shift in the competitive balance between species within the community (Gibson and Watkinson, 1991).

6.3 Advantages and disadvantages of the opportunistic strategy of *R. fistulosa*

In the evolution of parasitism three stages are recognized: (i) the ability to develop a haustorium and connect to a host plant (ii) the ability to recognize the root of a host plant and only germinate when triggered by host root exudates and (iii) the absence of chlorophyll and thus the complete reliance on a host plant (Westwood *et al.*, 2010). Being facultative and holding the ability to germinate without being triggered by a host (Chapter 3), clearly indicates that *R. fistulosa* is still at the earliest evolutionary stages of parasitism. This factor markedly distinguishes *R. fistulosa* from other important parasitic weed species in Africa. For instance, for *Striga* spp. to germinate, the seeds rely on germination stimulants from root exudates secreted from a host (Yoneyama *et al.*, 2013). To germinate only when there is a high likelihood to find a host root to attach to seems a sensible strategy, as it secures the parasite's ability to successfully complete its life cycle. Such a strategy also prevents extravagant depletion of the seed bank when growing conditions are unsuitable. *Striga* spp. clearly made evolutionary advances by developing mechanisms to synchronize germination with the life cycle of its host (Berner *et al.*, 1995; Ejeta and Gressel, 2007; Mourik *et al.*, 2011). Considering the advantages such a strategy has, it might seem logical to expect that in the long run a facultative parasite like *R. fistulosa*, will also evolve into an obligate parasite. Still one might also argue that a more opportunistic strategy, characteristic for *R. fistulosa*, in which the presence of a host plant only becomes apparent after germination, has its own advantages.

For instance, if the longevity of the seed bank of *R. fistulosa* is relatively short, as was observed by Gbéhounou and Assigbé (2004), the species can simply not afford to postpone germination until a host plant is present. It is not unlikely, that the short longevity of the seeds of *R. fistulosa* is caused by intermittent wet and dry soil conditions of its ephemeral habitat (Chapter 2). This suggests that the strategy of *R. fistulosa* to germinate when conditions are suitable (high moisture and light conditions; Chapter 2) and produce either a high (when a host plant is present) or a relatively small (in the absence of a host) amount of seed, is the best possible strategy, given these circumstances. In Chapter 3, we found the confirmation that *R. fistulosa* plants are able to complete their life cycle both in association and without a host. In both situations, physiological maturity was reached in a similar period of time. Differences in seed production rate also became evident however, as *R. fistulosa* plants that associated with a host produced an average of 48,000 seeds per pot compared to only 13,000 seeds per pot in the absence of a host. Similar reproductive advantages of interaction with a host have also been reported for other facultative hemiparasites, like *R. minor*, *Euphrasia frigida* and *Melampyrum sylvaticum* (Seel and Press, 1993a). For *R. fistulosa*, this lower seed number would still be more than enough to compensate for the seeds lost through germination, predation and decay, an thereby to replenish the seedbank. At the same time, the results obtained in the study presented in this thesis represent a situation where, in the absence of a suitable host, *R. fistulosa* did not have to compete with any other plant. In the experimental set-up, the parasite was grown all alone. Under natural conditions, the parasite is likely to grow in the presence of other plants species. If those other plants are no suitable hosts, they will act as pure competitors to the parasite, suppressing the parasite's growth and reproductive output. The difference between seed production in the presence and absence of a suitable host might therefore, in reality, well be higher than the factor 3.7 found in this experiment. Next to a higher seed number, connection with a host plant also resulted in an increased seed size. This observation points at another possible fitness advantage related to parasitism.

6.4 Magnitude of the parasitic effects on the host plant

The negative impacts some facultative parasites have on their hosts is attributed to withdrawal of assimilates. For example, *R. minor* on barley, trafficked up to 20% of host solutes from the xylem sap, reducing host growth by a similar magnitude of 22% (Jiang *et al.,* 2003b). Seel and Press (1996) reported that infection of the perennial grass host *Poa alpina* by *R. minor* merely affected flower bud formation and biomass partitioning. The experiments described in this thesis showed that even relatively low infestation levels of *R. fistulosa* already resulted in reductions in kernel weight of the host plant of up to 79-92% (Chapter 5). *Rhamphicarpa*-induced suppression of host biomass resulted in the parasite accumulating more than 50-80% of total biomass (host and parasite), depending on the level of infection (Chapter 5). Yet, the degree of *Rhamphicarpa*-induced suppression of host biomass could not be fully accounted for from the biomass achieved by the parasite as uninfected plants had more biomass than the combined sum of host and parasite biomass in infected plants. The missing biomass can be a result of several factors but for the first time with *R. fistulosa* we show that part of the missing component could be a result of host photosynthetic repression by *R. fistulosa*.

The reduction in biomass or yield of rice could not just be the result of a withdrawal of assimilates by *R. fistulosa*, but also of significant reductions in the host photosynthetic rates inflicted by the parasite (Chapter 4). The reduction in photosynthesis, which ranged from 24% up to 65% was accompanied by reductions in stomatal conductance, the quantum efficiency of PSII (Φ_{PSII}), chlorophyll content and eventually reductions in accumulated plant biomass. A similar effect has been observed in *Striga*-infected sorghum where the reduction in photosynthetic rate following parasite infection increased sensitivity of sorghum to photo inhibition (Graves *et al.*, 1989; Ramlan and Graves, 1996; Press and Stewart, 1987), which had an eventual strong effect on yield. Yield can be affected due to the limitation of carbon supply (Graves *et al.*, 1989) as one of the most important determining factors of the carbon cost of photo inhibition is the rate of recovery (Ramlan and Graves, 1996). The results strongly suggested a highly specific parasite-induced dysfunction of photosynthesis associated with the electron transport system. Despite these physiological effects on the host, *R. fistulosa* did not kill the host even in situations with a considerable density of parasites connected to one rice plant. *R. fistulosa* was able to completely dominate its host and to manipulate the host to produce assimilates entirely for its own benefit, while keeping the host plant alive (Chapter 5). This again shows that *R. fistulosa* is an opportunistic and harmful parasitic species.

6.5 Implications for control of *Rhamphicarpa fistulosa*

Weed eradiation is generally an expensive venture. In SSA weed management is not a priority among the majority of governments and yet weed eradication programmes require a backing from political actors and, most importantly, adequate funding (Panetta, 2009). Furthermore for African agriculture which is usually on small-holder basis, ambitious eradication plans are less likely to deliver economic returns (Lindgren *et al.*, 2010; Panetta, 2015). This raises a question as to whether to consider eradication or maintenance management.

Eradication efforts of *S. asiatica* in the USA were based on a combination of effective control —implementing innovations causing suicidal seed germination— and prevention of new invasions through implementation of strict phytosanitary regulations. These efforts span for more than 50 years and were successful in reducing seed bank persistence of this parasitic weed (Simberloff, 2014). In Africa, due to limited resources and low political will, the control of parasitic weeds is by targeting management rather than eradication. Integrated control packages for *Striga* spp. have been developed over the years. Some of these packages include agronomic management practices packaged with soil moisture conservation and soil fertility management schemes. In Ethiopia, an integrated approach that involved short fallows and crop rotation reduced emergence of *S. hermonthica* in pearl millet and marginally increased yield of pearl millet (Ejeta and Gressel, 2007). Another integrated package in West Africa, consisting of crop rotation, resistant varieties, trap crops and intercrops reduced *S. hermonthica* infection in maize by 35-46% and increased yield by 76-100% (Ejenta, 2007). Likewise, with *R. fistulosa* there is probably no silver bullet solution to its control, but, like for *Striga* spp., a successful strategy should be constructed on a number of integrated measures. Simple copycats of these integrated packages are not likely to be successful for controlling *R. fistulosa*, due to the differences between the two parasitic weed species, both in terms of their biology and in terms of their environment. Modifications of *Striga* control strategies should thus be made for the control of *R. fistulosa*.

Through the experiments in this thesis, we used a wide range of the parasite seed infestation levels of *R. fistulosa* that interacted with a single rice plant per pot. We found that total seed production rate was hardly influenced by the parasite’s infestation level, except for the seed production rate at the lowest seed infestation levels (Chapter 3). Moreover, the emergence percentage of *R. fistulosa* was higher at lower seed infestation levels. Density dependence was thus found during the emergence stage, while plant survival was considerably influenced by host presence during the growing stage. The implication is that since the survival and seed production of *R. fistulosa* is greatly enhanced by the presence of a host, long-term management of this weed is problematic in areas where rice is continuously grown. Based on our current knowledge of the biology and ecology of this parasite explored in this thesis, the likelihood of effectiveness of *Striga* control measures against *R. fistulosa* are discussed below.

6.5.1 Chemical control

In *Striga,* ethylene, ethephon, strigol and strigol analogues can induce suicidal germination of *Striga* seeds in the absence of a suitable host (Esilaba and Ransom, 1997). These artificial germination stimulants would not be effective with *R. fistulosa* but chemical control through herbicides could be used. A technology that complements *Striga* resistance in maize known as imazapyr resistant maize (IRM) was found to be effective against *Striga*. Coating the herbicide Imazapyr on maize and sorghum seeds was reported to reduce *Striga* infestation, *Striga* flowering and *Striga* seed set (Kanampiu *et al.*, 2003; Groote *et al.*, 2008; Ndung'u, 2009). However this technology has been found to be toxic to other crops that do not have Imazapyr resistance and is therefore not suitable for mixed cropping (Kanampiu *et al.*, 2003). Among the herbicides tested, 2,4-D has been the most selective and cheapest, but had only a narrow window of application, such that the only safe treatment for the crop was targeted spray of 2,4-D (Fasil, 2004). Yet, the existence of underground stages in the lifecycle of *Striga* means that these may not be easily targeted. For *R. fistulosa* we observed that there is some delay between the emergence of the parasite and the onset of visible damage to the host. This time lag can be a window of opportunity that can be used for herbicide interventions. It was reported by Gbéhounou and Assigbé (2004) that 2,4-D was effective against *R. fistulosa*. Herbicides are however not always a suitable technology for small-holder farmers, because they are often costly, and more importantly, they require specific knowledge, application equipment and functional input markets (Rodenburg and Johnson, 2009). The lack of knowledge on the use of these chemicals can result in pollution of the lowlands that, apart from agriculture, also provide other important ecosystem services.

6.5.2 Trap crops

Leguminous plants such as cowpea (*Vigna unguiculata*), groundnut (*Arachis hypogaea*), soybean (*Glycine max*) and sesame (*Sesamum indicum*) have been used as trap crops to induce suicidal germination in *Striga* (Schulz *et al.*, 2003; Carsky *et al.*, 2000). This innovation is based on the fact that these trap crops produce strigolactones that stimulate the germination of *Striga,* but after germination the parasite is not able to attach to the roots of these crops (Umba *et al.*, 1999). The result is that the parasite does not develop to flowering and seed production. In return, the *Striga* seed bank in the soil is reduced. The use of trap crops is entirely based on the stimulation of seed germination combined with the inability to attach to these plants. As clearly demonstrated, germination of *R. fistulosa* is not triggered by root exudates, implying that the use of trap crops will not contribute to depletion of its seed bank to the same extent as with *Striga* spp.

6.5.3 Crop rotation

For *Striga*, crop rotation of infested fields with non-susceptible crops is ideally the simplest solution. Crop rotation with crops that are not suitable hosts interrupts further addition of *Striga* seed into the soil seed bank. Crop rotations have been found to be effective mostly when followed during several years (Parker and Riches, 1993). For instance in Ethiopia two years of crop rotation with a non-host reduced *Striga* infestation by 50% (Shank, 2002). In Mali, a cowpea rotation increased subsequent millet grain yields by 37% in the following three seasons (Samake, 2003). It has been recommended that the choice of rotational crops should be based on local conditions and on its potential as a trap crop (Parker and Riches, 1993). Crop rotating cereals, combined with trap crops could eventually reduce *Striga* seed present in the soil. For *R. fistulosa* the host plant range has not been conclusively established. In a recent study, *R. fistulosa* was reported to parasitize Groundnut (*Arachis hypogaea*), Soybean (*Glycine max*), and Jute Mallow (*Corchorus olitorius*) (Houngbédji and Gibot-Leclerc, 2015). Non-host crops, used in crop rotation could perhaps prevent an exponential increase of the seed bank of *R. fistulosa*, since the parasite will be less productive when it cannot parasitize (Chapter 3). Moreover a non-host crop species could compete with the parasite for resources, which would further suppress its reproduction. However, one shortfall of using crop rotation is that there are few, if any, food crops other than rice, which can thrive in high soil moisture environments. This suggests that crop rotation with food crops is not likely to develop into an important component of any integrated control strategy against *R. fistulosa*. Improved fallows, with adapted leguminous fodder crop species, could be an alternative approach.

6.5.4 Intercrops

Some successes of intercropping cereals with legumes has been reported to be effective against *S. hermonthica* (Fasil, 2002). Yet, farming systems in East Africa, still have large infestations in the field despite intercropping cereals with legumes. An intercropping, innovation named as the “push-pull strategy” where, insect pests (stemborers) are deterred away from the target crop (push) while the pests are simultaneously attracted (pull) to a trap crop in the field margin (Cook *et al.*, 2007; Hassanali *et al.*, 2008) was found (by serendipity) to also reduce infection levels of *Striga* spp. (Khan *et al.*, 2002). When a leguminous species such as *Desmodium* spp. was used in the “push-pull strategy” it was found to have allelopathic effects of inhibiting the development of haustoria in *Striga* (Khan *et al.*, 2002; Khan *et al.*, 2008). With *R. fistulosa*, the applicability of the “push-pull strategy” would not be possibly enticing, as intercropping is risky for rice since rice is a weak competitor. This means that an intercrop often exerts too much competition to the rice crop. For that reason there are not so many examples of successful intercropping strategies for rice. Also the lack of suitable species that are adapted to the rain-fed lowland environments would hamper intercropping as a viable control option.

6.5.5 Catch crops

Another strategy is the use of catch crops. These crops — for instance forage grasses in crop-livestock farming systems —stimulate high germination rates of parasite seeds (Parker and Riches, 1993). The parasite germinates and connects to the roots of the catch crop. However, before the parasite has reached its seed production stage the catch crop is harvested along with the parasite. With this strategy, the catch crops are efficient in reducing the seed bank of the parasite after at least three sequential years of this practice (Esilaba and Ransom, 1997). Such a practice is not relevant for *R. fistulosa* as no crop is needed to initiate the germination of *R. fistulosa*. Field preparation and providing good conditions for germination of the parasite is sufficient to obtain seedlings of the parasite. As long as these seedlings are killed before seed set, it would help to reduce the seed bank. In agriculture this strategy is known as the “stale (or false) seed bed technique”. This is considered one of the most effective control strategies of *R. fistulosa*. Just before rice sowing, the weed can be removed by hoes or killed using herbicides based on 2,-4 D amine (Gbehounou & Assigbe, 2003). This can reduce the seed bank at an earlier stage. The only limitation of this technique would be that rice is often planted shortly after the onset of the first rains to escape drought stress during kernel filling. If seeding is postponed, to be able to kill the flush of *R. fistulosa* seedlings, it might result in less optimal rice yields. Furthermore, in most rice-based systems in SSA, rice cultivation is very laborious in terms of weeding and chasing birds (Kijima *et al.*, 2008; Rodenburg *et al.*, 2014b). Therefore management strategies that require additional labor would stand a lower chance of adoption by farmers.

6.5.6 Breeding for Resistance and Tolerance

The use of resistant and tolerant rice cultivars has recently been proven to be effective for *Striga*. Cultivars that are resistant to *Striga* are those that support few or no *Striga* attachments when parasitized (Rodenburg *et al.*, 2005). These exist in two forms, pre and post-attachment resistance. Pre-attachment resistant varieties reduce *Striga* germination by producing low amounts of germination stimulants in their root exudates thus reducing the number of parasite attachments (Jamil *et al.*, 2011). Post-attachment resistant varieties exhibit a hypersensitive response when infected by *Striga* by developing a necrotic tissue around the parasite attachment area which prevents host root cortex invasion (Cissoko *et al.*, 2011). The lack of responsiveness to root exudates of *R. fistulosa*, means that for this weed species the search for resistant varieties should focus on post-attachment resistance. In this thesis we used rice cultivar IR64 as the study host. The considerable parasite-inflicted yield losses depicted by this cultivar showed that IR64 is considerably susceptible to *R. fistulosa* infection, whereas *R. fistulosa* had a high propensity to virulence on its host. By definition, parasite virulence is the rate at which a host becomes infected and the level of damage inflicted to the host by the parasite (Gandon *et al.*, 2002). A recent study by Rodenburg *et al.*, (2016a) confirmed varietal differences of rice cultivars in infection level and yields in fields infested by *R. fistulosa*. A number of useful rice varieties were identified. It was also suggested that varietal differences in *R. fistulosa* biomass could be used to select for resistance, while low *R. fistulosa*-inflicted yield loss could be the basis for selection of tolerant lines. *Striga* tolerance is based on reduced negative impacts on host photosynthesis in some host (like rice) varieties. Whether this is also true for *R. fistulosa* could be a subject to further studies. The use of resistant and tolerant cultivars could be an effective and inexpensive component of an integrated *R. fistulosa* management strategy. The only problem sometimes encountered is that both farmers and consumers usually have their varietal preferences regarding rice grain characteristics like taste and size. Secondly, seed systems in rural Africa are weak, which hampers the availability of certified seed of improved varieties on local markets.

6.7 Epilogue

Rhamphicarpa fistulosa was predicted by Raynal Roques (1994), a couple of decades ago, to develop from a minor to a major parasitic weed in SSA's agricultural systems (Raynal Roques, 1994). True to the prediction, *R. fistulosa* became a major problem especially in rain-fed rice systems (Rodenburg *et al.*, 2011; Rodenburg *et al.*, 2010 ; Kabiri *et al.*, 2015), specifically, because there was a delay in the acknowledgement of this problem. The parasite was relatively unknown and farmers and extension workers did not know that it was a parasitic weed and treated it like a normal weed. The information about the ecology and biology of *R. fistulosa* generated from the work in this thesis provides clues on what factors might contribute to the future development of the problem. These include clues under scenarios of changing climates and land use, as well as to the development of possible management strategies. Research gaps still exist. More studies are required to investigate the role that genetic variation of rice genotypes and *R. fistulosa* play to determine the outcome of the interaction between rice and *R. fistulosa*. There is a need to determine resistant and/or tolerant rice cultivars to *R. fistulosa,* and, ideally, the molecular genetic basis of resistance to this parasite. Just as the research efforts put in management of *Striga* spp., more than the current attention needs to be paid to *R. fistulosa* specifically, to solve the problems this parasitic weed causes to the rice sector in sub-Saharan Africa.

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Summary

Rice is an increasingly important crop in Africa where consumption has grown faster than any other major staple food crop. The increasing scarcity of good arable land necessary to produce food for the growing population of this continent has driven rice growers to expand into marginal areas. Newly opened fields in marginal areas are often characterized by low or heterogeneous soil fertility, escalating the problem of weeds and parasitic weeds that thrive under such conditions. These weeds become particularly problematic in subsistence farming systems characterized by low-input production methods. One of the most investigated genera of parasitic weed species, is *Striga* of the family Orobanchaceae. *Striga* spp. are obligate root hemi-parasitic weeds. Obligate indicates that these species can only complete their life-cycle when connected to a host plant. The *Striga* species causing the most severe problems in African agriculture are *S. hermonthica* and *S. asiatica*. Heavy infestation of *Striga* spp. can be found throughout sub-Saharan Africa. In contrast with *Striga* spp., we know much less of another parasitic weed that constrains cereal production, *Rhamphicarpa fistulosa*. This parasitic weed has become a more serious problem to rice production in Africa in recent years. *Rhamphicarpa fistulosa*, is a facultative root hemi-parasite of the family Orobanchaceae that is widely distributed over the continent. This species is able to parasitize a host plant, but also able to complete its life-cycle without being connected to a host. The species most likely has a wide host range but is most often found parasitizing rice, a crop that is ecologically well adapted to the water-logged environments where *R. fistulosa* thrives. Since rice cultivation is expanding into lowlands where *R. fistulosa* naturally occurs, this parasitic weed has the potential to become more problematic in the near future. At the onset of the research presented in this thesis, the understanding of the ecology and biology of the species and its dependence and effects on a host, was rather limited, in particular when compared to the widely studied *Striga* species. A more profound understanding would be instrumental to assess the extent of the problem and the directions it can evolve into, and would lay the necessary foundation for the development of effective management strategies. Therefore the study of this thesis focused on unraveling the ecology, biology and host-interactions of the facultative parasitic weed *Rhamphicarpa fistulosa*.

In the first investigation (Chapter 2), in Kyela —a rice growing area in south Tanzania where both parasites are present— *Striga asiatica* was observed in higher lying and drier fields, while *R. fistulosa* was observed in the lower lying wetter fields. Furthermore, non-parasitic weed species that were exclusive to *S. asiatica*-infested fields are adapted to open well-drained soils, while species that were exclusive to *R. fistulosa* fields are typical for water-logged soils. Controlled experiments confirmed these observations on species-specific hydrological preferences of these parasitic weeds. These results imply that changes in climate, specifically moisture regimes, will be crucial for its future prevalence.

In a second investigation (Chapter 3), we hypothesized that, being a wetland species, germination of *R. fistulosa* should be stimulated by daylight and high soil moisture levels.

Second, we hypothesized that if host plant presence increases the parasite's reproductive output then a germination stimulatory effect from host presence is likely to have developed. A Petri-dish and pot experiment revealed that daylight and completely saturated soil conditions were indeed 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, differing in strigolactone quality and quantity, revealed that host plant presence, regardless of 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 a synthetic germination stimulant (GR24) were 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, while germination of *R. fistulosa* was similar in all treatments. 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.

In a third investigation (Chapter 4) we investigated how *R. fistulosa* affects its host, and how the host responds to infection. This study focused on elucidating the impacts of infection by *R. fistulosa* on host photosynthesis. Using three greenhouse pot experiments we investigated if *R. fistulosa* affects photosynthesis of rice, if the level of reduction in leaf photosynthetic rate depends on weed infestation level and which mechanisms underlie a possible reduction in leaf photosynthetic rate of its rice host. Reduction in the photosynthetic rate of rice was first observed at around 55 days after infestation. Suppression of host photosynthesis and the initial light use efficiency was a function of the level of parasite infestation levels. Chlorophyll content, specific leaf area and shoot weight of *R. fistulosa*-infected plants was also dependent of the level of infestation. The linear relationship of light adapted quantum efficiency (Φ_{PSII}) and the mean quantum yield of CO_2 assimilation (Φ_{CO2}), of infected plants deviated negatively by 19-32% from that of un-infected plants. Dark respiration rates of the host were not influenced by parasite infection. There was a considerable time lag between the parasite's acquisition of benefits from the association, in terms of growth, and the reduction of host photosynthesis. The reduction in host growth on the other hand coincided with suppression of host photosynthesis. Growth of infected plants was reduced by about 75% compared to uninfected plants. This indicates that *R. fistulosa* affects host growth by first extracting assimilates and making considerable gains in growth, before the parasite exerts negative effects on host photosynthesis. In the final investigation (Chapter 5) we examined the growth and reproduction of *R. fistulosa* and how it benefits considerably from parasitism. We determined accumulation and partitioning of rice-parasite biomass in two pot experiments. Infection increased host root:shoot ratios and decreased host plant height, leaf area and tiller number. Reductions in light interception were followed by reductions in light use efficiency, causing 22-71% losses in host plant biomass and 78-100% losses in host kernel production. Parasitism eventually caused a complete standstill of host plant growth, while the parasite managed to gradually increase its share of the total host plant-parasite biomass up to 50-82%. This implies that ultimately the host plant was producing solely for the sake of the parasite.

In conclusion, *Rhamphicarpa fistulosa* is clearly an opportunistic parasite that shares its ecological niche with rice, which as a host is very susceptible to *R. fistulosa* infection. When attached to a rice plant, the parasite gradually starts dominating its host. *Rhamphicarpa fistulosa* systematically alters the photosynthetic capacity and morphology of the host plant to the point where rice produces assimilates only for its own benefit, whereby the parasite literally turns its host into a slave. New information about the ecology and biology of *Rhamphicarpa* generated from the work of this thesis, provide leads for possible management strategies. We recommend that agronomic management packages for *Rhamphicarpa* should follow a false seedbed technique, whereby the first flush of *R. fistulosa* plants is killed before the actual crop establishment. The technique can be combined with improved water and soil fertility management, if farmers have the necessary means to do so. An integrated approach that targets the reduction of number of germinated seeds, reduction of attached parasites and lastly the reduction of established parasites can lead to effective control of the weed. Further research needs to be focused on the determination of *R. fistulosa* resistant and/or tolerant rice varieties and the molecular genetic basis of resistance to this parasite. The role that genetic variations of rice genotypes and *R. fistulosa* play to determine the outcome of the interaction between these rice-parasite associations should be investigated as well. Just, as the efforts put in studies on management of *Striga* spp, more than the current attention needs to be paid to *R. fistulosa*, specifically for problems it causes to the rice sector in sub-Saharan Africa.

Samenvatting

Rijst is een steeds belangrijker wordend gewas in Afrika, waarvan de consumptie de laatste decennia sterker in omvang is toegenomen dan van enig ander voedselgewas. De toenemende schaarste aan goed akkerland, dat nodig is voor de voedselproductie voor de snel groeiende bevolking op dit continent, heeft de boeren ertoe gedwongen hun areaal uit te breiden naar de meer marginale gronden. Deze recentelijk in gebruik genomen velden worden vaak gekenmerkt door een lage of zeer heterogene bodemvruchtbaarheid. Dit leidt er uiteindelijk toe dat onder deze omstandigheden de onkruidproblematiek, waaronder die van de parasitaire onkruiden, sterk toeneemt. Deze parasitaire onkruiden vormen met name een probleem voor de vele zelfvoorzienende boeren, waarvan de productiesystemen gekenmerkt worden door de minimale inzet van meststoffen en herbiciden. Een van de meest onderzochte geslachten van parasitaire onkruidsoorten is *Striga*, uit de familie van de Orobanchaceae. *Striga* soorten zijn obligate, met de wortels van de gastheerplant verbonden, halfparasieten. Het woord obligaat duidt er op dat deze soorten hun levenscyclus slechts kunnen voltooien wanneer ze vastgehecht zijn aan een waardplant. De *Striga* soorten die de grootste problemen in de Afrikaanse landbouw veroorzaken zijn *S. hermonthica* en *S. asiatica*. Zware besmettingen van graangewassen, zoals sorghum, gierst, mais en rijst, met deze *Striga* soorten worden in de gehele semi-aride en sub-humide zone van sub-Sahara Afrika aangetroffen.

In tegenstelling tot de *Striga* soorten is de kennis van een ander onkruid, dat het gewas rijst parasiteert, *Rhamphicarpa fistulosa*, veel geringer. Deze plantensoort, die zich eveneens aan de wortels van een waardplant hecht, heeft zich recent tot een omvangrijk probleem in de rijstteelt in Afrika ontwikkeld. Anders dan *Striga* is *R. fistulosa* een facultatieve parasiet. Dit betekent dat deze soort zijn levenscyclus ook kan voltooien zonder verbonden te zijn met een gastheer. Deze soort parasiteert een reeks van grasachtige gewassoorten, maar wordt het vaakst op rijst aangetroffen, een gewas dat zeer goed aangepast is aan waterverzadigde bodems. Doordat de teelt van rijst zich steeds meer uitbreidt naar de laaggelegen, natte gebieden, waar *R. fistulosa* van nature voorkomt, is het te voorspellen dat deze soort de potentie heeft om in de toekomst voor steeds grotere problemen in de rijstteelt te gaan zorgen. Aan het begin van het onderzoek dat in het voorliggende proefschrift wordt gepresenteerd, was er slechts weinig kennis van de ecologie en biologie van *R. fistulosa*, de relatie met de waardplant en van de negatieve effecten van de parasiet op de gastheer. Het vergaren van een veel uitgebreidere kennis van de parasiet-gastheer associatie is van wezenlijk belang om de omvang van het probleem in de rijstteelt vast te stellen. Daarnaast kan deze kennis de basis leggen voor de ontwikkeling van effectieve beheersmaatregelen. Om deze redenen ligt de nadruk van het hier gepresenteerde onderzoek op het ontrafelen van de ecologie en de biologie van de facultatieve parasiet *R. fistulosa* en op de interactie van de parasiet met zijn waardplant, de rijstIn het eerste onderzoek (Hoofdstuk 2), in Kyela - een streek in zuidwest Tanzania waar beide parasitaire plantensoorten voorkomen – werd *S. asiatica* aangetroffen in de hoger gelegen gebieden en op de drogere velden, terwijl *R. fistulosa* werd gevonden in de lager gelegen, nattere velden. Verder viel op dat de niet-parasitaire onkruiden die gevonden werden op de met *S. asiatica* besmette velden, soorten zijn die aangepast zijn aan open, goed ontwaterd terrein, terwijl de soorten die exclusief gevonden werden op de velden waar *R. fistulosa* voorkwam, typisch soorten bleken te zijn van waterverzadigde bodems. Experimenten met beide soorten bevestigden dat de groei van *S. asiatica* wordt bevorderd wanneer de soort samen met de rijst opgekweekt wordt in potten met relatief droge grond en dat *R. fistulosa* alleen goed gedijt op waterverzadigde grond. Deze resultaten wijzen er tevens op dat de gevolgen van de klimaatverandering in Afrika, met name voor wat betreft de veranderde neerslagpatronen in verschillende gebieden, cruciaal zijn voor de toekomstige ontwikkeling van beide parasitaire onkruidsoorten.

Bij een tweede reeks experimenten (Hoofdstuk 3) werd vooraf verondersteld dat, vanwege het feit dat *R. fistulosa* van origine een soort is van open, moerassige gebieden, de kieming van deze soort gestimuleerd zou worden door licht en door een hoge bodemvochtigheid. Verder werd aangenomen dat als aanwezigheid van een waardplant een sterk positief effect heeft op de zaadproductie van de parasiet, een kieming-stimulerend effect vanuit de gastheer, zoals aanwezig bij *Striga*, ook bij *R. fistulosa* aangetroffen zou worden. Een petrischaal-experiment en een potproef toonden aan dat licht en een waterverzadigde grond inderdaad een vereiste waren voor de kieming. Hiermee werd tevens aangetoond dat de kiemingsvoorwaarden voor *R. fistulosa* typisch zijn voor soorten die voorkomen in gebieden met fluctuerende waterniveaus. Een potproef waarin vijf besmettingsniveaus met *R. fistulosa* zaden waren aangebracht, in combinatie met de al of niet aanwezigheid van een rijstplant, toonde aan dat de aanhechting aan een waardplant voor *R. fistulosa* resulteerde in een 3.7 keer hogere zaadproductie en een 15% hogere gemiddelde zaadgrootte. Ondanks dit reproductieve voordeel wees een volgende potproef met drie rijstvariëteiten, die verschilden in kwaliteit en kwantiteit van kieming-stimulerende stoffen, de zogenaamde strigolactonen, uit dat de aanwezigheid van een waardplant, onafhankelijk van het ontwikkelingsstadium hiervan, de opkomst van *R. fistulosa* kiemplanten niet beïnvloedde. In een vervolgstudie werd het kieming-stimulerende effect van wortelexudaten van dezelfde drie rijstvariëteiten, inclusief een synthetische kiemingsstimulant (GR24), vergeleken met de kieming van *R. fistulosa* zaden in afwezigheid van deze wortelexudaten. In deze studie werd de kieming van de zaden van *R. fistulosa* tevens vergeleken met de kieming van *S. hermonthica*. De kieming van de obligate parasiet *S. hermonthica* bleek volledig afhankelijk van de aanwezigheid van wortelexudaten gewonnen uit de waardplant of van GR24. Kieming vond niet plaats bij zaden die louter bevochtigd werden met water. Dit alles in tegenstelling tot de kieming van de zaden van *R. fistulosa*, de facultatieve parasiet, waarbij kieming bij alle behandelingen in dezelfde mate plaats vond. De afwezigheid bij *R. fistulosa* van een mechanisme dat de aanwezigheid van een potentiële gastheer aantoont, suggereert dat de regulatie van de kieming door licht en bodemvocht bijkans optimaal is. Onze bevindingen lijken ook aan te tonen dat, voor deze facultatieve parasiet, een meer opportunistische kiemingsstrategie superieur is.

In een derde set proeven (Hoofdstuk 4) onderzochten we op welke wijze *R. fistulosa* haar waardplant beïnvloedt en daarnaast hoe de waardplant reageert op infectie met de parasiet. Deze studie was met name gericht op het ontrafelen van de effecten, veroorzaakt door de parasiet, op de fotosynthese van de waardplant. In een drietal potproeven in de kas onderzochten we i) óf *R. fistulosa* een effect heeft op de fotosynthese van de rijst, ii) zo ja, of de mate van de reductie van de fotosynthese bepaald wordt door het niveau van de infectie met de parasiet en iii) welke mechanismen verantwoordelijk zijn voor de reductie in bladfotosynthese van de gastheerplant. Reductie van de maximale fotosynthesesnelheid van rijst werd het eerst gemeten op ongeveer 55 dagen na inoculatie van de potten met *R. fistulosa* zaden. Tevens werd vastgesteld dat de onderdrukking van de fotosynthese-snelheid bij lichtverzadiging en van de initiële lichtbenuttings-efficiëntie van de rijstplant een functie was van het infectieniveau van de parasiet. Het chlorofylgehalte, het specifiek bladoppervlak en de bovengronds biomassa van de rijstplant werd eveneens bepaald door de mate van infectie. Het lineaire verband tussen licht geadapteerde quantum efficiëntie (Φ_{PSII}) en de gemiddelde quantum opbrengst van de CO_2 assimilatie (Φ_{CO2}) van de geïnfecteerde planten was 19-32% lager dan dat van de niet-geïnfecteerde planten. Een aanzienlijk tijdsverschil werd gevonden tussen het moment dat de parasiet begon te profiteren van de verbinding met de gastheer en het moment dat er een reductie in fotosynthsesnelheid van de rijst kon worden vastgesteld. De reductie in groei van de rijst viel echter precies samen met de reductie van de fotosynthse van de rijst. Tevens werd gevonden dat de biomassa-productie van geïnfecteerde rijstplanten met circa 75% gereduceerd was ten op zichte van de niet-geinfecteerde planten. Dit alles duidt er op dat infectie met *R. fistulosa* zich in eerste instantie uit in het onttrekken van assimilaten aan de rijst gevolgd door een aanzienlijke toename in groei, alvorens de parasiet in staat is de fotosynthese van de rijst negatief te beïnvloeden.

Doelstelling van het in Hoofdstuk 5 beschreven onderzoek was de groei en reproductie van de *Rhamphicarpa* te kwantificeren en een verklaring zien te vinden voor het aanzienlijke profijt dat de parasiet heeft van het aangehecht zijn aan een waardplant. In twee potproeven werd de toename van de biomassa en de verdeling van deze biomassa over de twee partners van de associatie, de parasiet en de rijst, bestudeerd. Infectie met de parasiet zorgde voor een toename van de wortel:spruit verhouding en verlaagde de lengte van de rijstplant, het bladoppervlak en het aantal zijspruiten. Een afname in lichtonderschepping werd gevolgd door een reductie van de lichtbenuttings-efficiëntie, hetgeen resulteerde in 22-71% verlies in biomassaproductie van de rijst en 78-100% verlies van de korrelopbrengst. Uiteindelijk veroorzaakte de parasiet een complete stilstand van de groei van de rijst, terwijl de parasiet zelf in staat bleek haar aandeel van de totaal geproduceerde hoeveelheid biomassa van rijst en parasiet met 50-82% te verhogen. Dit impliceert dat uiteindelijk de gastheer alleen nog maar assimilaten produceerde ten behoeve van de parasiet. Concluderend kunnen we stellen dat *R. fistulosa* overduidelijk een opportunistische parasiet is, die zijn ecologische niche deelt met de rijstplant en dat deze laatste als waardplant erg gevoelig blijkt voor infectie met *R. fistulosa*. Na aanhechting aan de wortels van de rijst begint de parasiet meer en meer de gastheer te domineren. Hierbij verandert *R. fistulosa* systematisch de fotosynthesecapaciteit en de morfologie van zijn gastheer tot het moment dat de rijst alleen nog maar assimilaten produceert die ten gunste komen aan de parasiet, waarbij de parasiet letterlijk zijn gastheer dus tot slaaf maakt.

De nieuwe kennis van de ecologie en biologie van *R. fistulosa*, die het onderzoek beschreven in dit proefschrift heeft opgeleverd, biedt aanknopingspunten voor nieuw te ontwikkelen beheersmaatregelen ten behoeve van dit parasitaire onkruid. Het verdient aanbeveling dat agronomische maatregelen voor het reguleren van een *R. fistulosa* besmetting vooraf gegaan worden door het creëren van een vals zaaibed. Deze techniek, die een bijdrage levert aan het reduceren van het aantal zaden van de parasiet in de zaadbank, kan gecombineerd worden met maatregelen die gericht zijn op het verbeteren van de waterhuishouding op veld niveau en van de bodemvruchtbaarheid. Als voorbeeld kan een geïntegreerd pakket maatregelen dienen, dat de reductie van het aantal gekiemde zaden van de parasiet combineert met een reductie van het aantal aangehechte parasieten en uiteindelijk met een reductie van het aantal gevestigde parasieten. Dit pakket maatregelen kan vervolgens een startpunt zijn bij de bestrijding van het parasitaire onkruid. Vervolgonderzoek dient gericht te zijn op het ontwikkelen van *R. fistulosa*-resistente en/of -tolerante rijstcultivars en op de moleculair-genetische basis van deze resistentie. Ook zou de rol die genetische variatie van rijstgenotypen en die van *R. fistulosa* speelt bij de uitkomst van de interactie onderzocht moeten worden. Net zoals er veel energie is gestoken in onderzoek ten behoeve van de beheersing en de bestrijding van *Striga* soorten, zal meer dan de huidige aandacht nodig zijn voor *R. fistulosa*, speciaal voor wat betreft de problemen die deze soort veroorzaakt in de rijstteelt in Afrika ten zuiden van de Sahara.

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I am thankful to the Christian community of Amazing Grace Parish church, Wageningen, who shepherded students and still do during their stay in Wageningen. I looked forward to choir practice on Fridays and singing, dancing and jubilation on Sundays. It was so refreshing after a long hard study week. The smiles and happy moments will always stay in my heart. I am grateful to Pastor Dr Farai Maphosa and Pastor Mrs. Busi Maphosa. Despite your busy work week, you still found time to encourage us with consistency and commitment. I am humbled by your commitment and generosity, a virtue that has taught me about consistency and love. Thank you.

The long stay abroad was crowned with wonderful friends I met in the Netherlands from so many countries who enriched my life. I will mention only the first names for lack of space. Marcia, André, Catarina, Giovani, Marcelo, Goufang, Hiroe, Niel, Dennis Tippe, Yewubnesh, Asfaw, Ibsa, Stephanie, Hanna, Ceser, Adugna, Uta, Nduta and Menza to mention but a few. I remember the dinners we shared and even if we came from all corners of the world, as students, our problems and joys were similar. I am grateful to the Ugandan community at Wageningen for the laughs that we shared. You made my PhD journey memorable.

I am thankful to Mr. Peter Lusembo of NARO-Uganda who gave me my first job in 2004 and mentored me through my carrier to this PhD level. *“You have a very powerful signature sir, please sign me this application”*, I would ask. Almost as surely, I would qualify for the scholarship. I am grateful to the staff at Mukono Zonal Agricultural Research and Development Institute (MUZARDI) for carrying on my duties while I was on study and the warm tidings after I shared a paper.

I am forever grateful to the Dutch government for sponsoring both my MSc and PhD education. Without you, I never would have afforded it. The Netherlands is my favorite country, where people *‘say what they mean and mean what they say’*. It is only in the Netherlands that I met friends from over 150 nationalities of the world. The spirit of inclusion has been influential for the foreign policy the Netherlands shares with many countries. I am almost sure that in the near future, I will still have more partnerships with the Netherlands.

I am grateful to my parents Mr. Fred Kabiri and Mrs. Alice Kabiri for your inspiration for my education since kindergarten when I used to think I was going to school for you. To my sisters, Lydia and Christine and brothers, Peter, and Charles who demanded that I acknowledge him only for the reason of being born 30 mins before me. Last but most important to my beloved husband, Rev. Samuel Galuak Marial for standing by me during this journey when we lived long months apart, for offering a shoulder to cry on, when times were hard, for encouraging me to follow my dreams. For this reason, I dedicate this thesis to you.

List of Publications

1. Kabiri S., Rodenburg J A. van Ast and Bastiaans L. (2017). Slavery in Plants: How the facultative hemi-parasitic plant *Rhamphicarpa fistulosa* can completely dominate its host. *Annals of Applied Biology* DOI: 10.1111/aab.12378

2. Kabiri S, B. Akello, J. Oneka and K. Igbokwe (2016). Diversity in small holder farms in the central cattle corridor of Uganda and the implication for Technology Adoption. Accepted in *Uganda Journal of Agricultural Sciences*.

3. Kabiri S, A. van Ast, J. Rodenburg and L. Bastiaans (2016). Host influence on germination and reproduction of the facultative hemi-parasitic weed *Rhamphicarpa* fistulosa. *Annals of Applied Biology* 169: 144-154.

4. Kabiri S, Rodenburg J, Kayeke J, Ast Av, Makokha DW, Msangi SH, Irakiza R, Bastiaans L. (2015). Can the parasitic weeds *Striga asiatica* and *Rhamphicarpa fistulosa* co-occur in rain-fed rice? *Weed Research* 55, 145-154.

5. Kabiri S, C Ebong, P. Kudsk, S.K. Mathiassen, P. Lusembo, G.S. Byenkya, H. Kasigwa and R. Kabanyoro. (2015). Landscape-scale management of invasive *Cymbopogon afronardus* (stapf) in the rangelands of Uganda. *Agricultural Innovations for Sustainable Development* 3, 270-278.

6. B Fungo, R Kabanyoro, I Mugisa and Kabiri S. (2013). Narrowing yield-gap of rice through soil fertility management in the Lake Victoria Crescent agroecological zone, Uganda. *African Journal of Agricultural Research* 8, 2988-2999.

7. R Kabanyoro, Kabiri S, I Mugisa, W Nakyagaba, L Nasirumbi, G Kituuka, B Kyampeire, M Nampera, T Namirimu, B Fungo (2013). Willingness of farmers to adopt rice intercrops in the Lake Victoria Crescent Agro-ecological Zone of Uganda. *Biology, Agriculture and Healthcare* 3.

Curriculum Vitae

Stella Kabiri

Email: kabiri.marial@gmail.com
LinkedIn: https://nl.linkedin.com/pub/stella-kabiri-marial/79/5aa/a56.
ORCID: Stella Kabiri 0000-0002-7174-6374
Website: http://www.naro.go.ug

Stella Kabiri was born in May 17, 1979 in Mbale, Uganda. She pursued her primary education first from Temple Road Primary School in Nyeri, Kenya and later at Nabuyonga Primary School in Mbale, Uganda. Her secondary education was at Tororo Girl's School, Tororo, and Caltec Academy, Kampala for Advanced levels in 1999 where she studied Physics, Chemistry, Biology and Mathematics. She graduated with honours from Makerere University, Kampala with a Bachelor of Science in Agriculture in 2004, majoring in Crop Science. Her BSc. dissertation under the supervion of Prof. Samuel Kyamanywa was titled "Assessment of yield loss due to thrips (*Megalurothrips sjostedti*) on common Ground nut in Uganda". In 2004 she joined the National Agricultural Research Organization (NARO-Uganda) as a Research Assistant. From 2005 to 2007 she worked for the International Institute of Tropical Agriculture (IITA) as a Research Assistant under the supervision of Dr. Thomas Dubois on Management of micro-organisms to enhance plant health against banana weevil and banana nematodes. In 2007, she received a scholarship from the Dutch government to study a Master's of science in Geo-information science and Earth Observation in the domain of Natural Resources Management, from the International Institute of Geo-information science and Earth Observation (ITC) of the University of Twente, the Netherlands. Under the supervision of Dr. Kees de Bie and Prof. Eric Smaling, she graduated in 2009. The Master's thesis was entitled "Enhancing a remote sensing based crop growth model to include genetic parameters that capture dynamic site specific management aspects. *A case study of rice production in the Mekong delta, Vietnam"*. In 2009, she rejoined NARO as a Crop Entomologist. In 2011, she joined Wageningen University as a PhD fellow under the NWO-WOTRO funded PARASITE project. She was based in Wageningen under the supervision of Dr. Lammert Bastiaans with intermittent periods at Africa Rice in Dar es Salam, Tanzania under the supervision of Dr. Jonne Rodenburg for field work. In 2015, she returned to NARO where she currently works on several research, development and environmental projects. Stella is married to Rev. Samuel Galuak Marial of Juba, South Sudan.

Oral presentations at international conferences

1. Kabiri S, B. Akello, J. Oneka and K. Igbokwe (2016). Diversity in small holder farms in the central cattle corridor of Uganda and the implication for Technology Adoption. NARO-Makerere joint dissemination conference. Speke resort Munyonyo, Kampala 21st-24th November 2016.
2. Kabiri S, A. van Ast, J. Rodenburg and L. Bastiaans (2016). Exploring the life-history strategy of the root parasitic weed *Rhamphicarpa fistulosa*. European Weed Research Society conference, Montpellier, France, June 22-26, 2015.
3. Kabiri S C Ebong, P. Kudsk, S.K. Mathiassen, P. Lusembo, G.S. Byenkya, H. Kasigwa and R. Kabanyoro. (2015). Landscape-scale management of invasive *Cymbopogon afronardus* (stapf) in the rangelands of Uganda. Feeding 1 Billion in Africa in a changing world. 6th African Agricultural science week and Forum for Agricultural Research in Africa (FARA) Accra, Ghana, June 2013.
4. Kabiri S (2009). Enhancing a Remote Sensing Based Crop Growth Model to Include Genetic Parameters That Capture Dynamic Site Specific Management Aspects. A case study of rice production in the Mekong Delta, Vietnam.Geo- Spatial Information and Sustainable Development in Africa. Facing Challenges of Global Changes. Proceedings of AFRICA GIS 2009 26 th-30th October International conference Kampala Uganda.
5. Bastiaans, L, Akanvou L, van Ast A, Gbehounou G, Kabiri S, Kayeke J, Klerkx L, Mourits M, N'cho S, Oude Lansink A, Rodenburg J, Schut M (2013). PARASITE: An integrated research programme on parasitic weeds of rice in sub-Saharan Africa.16th EuropeanWeed Research Society Symposium, 24-27 June 2013, Samsun, Turkey. P. 92.

Poster presentations at international conferences

6. Kabiri S., A. van Ast, J. Rodenburg and L. Bastiaans (2015). Exploring the life-history strategy of the root parasitic weed *Rhamphicarpa fistulosa*. European Weed Research Society conference, Montpellier, France, June 22-26, 2015.
7. Kabiri S, Rodenburg J, Bastiaans L, Kayeke J, van Ast A, Sibuga K, Makokha D, andMsangi SH (2013). Ecological niche differences between *Rhamphicarpa fistulosa* and*Striga asiatica* in rainfed rice. 3rd Africa Rice Congress, 21-24 October, 2013, Yaoundé, Cameroon.
8. Kabiri S, Rodenburg J, Bastiaans L, Kayeke J, van Ast A, Makokha D, Msangi SH,Kallunde PS. (2013). Ecological niche differences between *Rhamphicarpa fistulosa* and *Strigaasiatica* in rain-fed rice. 12th World Congress on Parasitic Plants, 15-19 July, 2013, Sheffield, UK.

Awards

2015	Best Poster Award for the presentation, 'Exploring the life-history strategy of the root parasitic weed *Rhamphicarpa fistulosa*.	European Weed Research Society conference, Montpellier, France, June 22-26, 2015.
2013	2nd Prize, Africa-wide Young Professionals in Science Competition for the paper, Landscape-scale management of the invasive *Cymbopogon afronardus* (stapf) in the rangelands of Uganda.	6th African Agricultural science week and Forum for Agricultural Research in Africa (FARA) Accra, Ghana, June 2013.
2013	Outstanding Young Scientist	National Agricultural Research Organization (Uganda).

PE&RC Training and Education Statement

PE&RC Training and Education Statement

With the training and education activities listed below the PhD candidate has complied with the requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)

Review of literature (6 ECTS)

- Understanding how host-parasite interactions for economically important parasitic weeds in rain-fed rice systems of sub-Saharan Africa are differentially affected by environmental conditions (2012)

Writing of project proposal (4 ECTS)

- Understanding how host-parasite interactions for economically important parasitic weeds in rain-fed rice systems of sub-Saharan Africa are differentially affected by environmental conditions (2011)

Post-graduate courses (3.2 ECTS)

- Generalized linear models; PE&RC (2011)
- Introduction to R for statistical analysis; PE&RC (2013)
- 6th African agricultural science week and Forum for Agricultural Research in Africa (FARA); Accra, Ghana (2013)

Laboratory training and working visits (3 ECTS)

- Consequences of *R. fistulosa* infection on growth, biomass allocation and productivity of rice. (raising *R. fistulosa* in pots); Africa Rice Center, Tanzania (2012, 2013)
- Host parasite interactions of *Striga asiatica* with rice (raising *S. asiatica* in pots); Africa Rice Center and Mikocheni Agricultural Research Institute, Tanzania
- Ecological niche differences between *Striga asiatica* and *Rhamphicarpa fistulosa* in parasitism of rain-fed rice in Sub-saharan Africa (transects and weed identification); Africa Rice Center, Mikocheni Agricultural Research Institute and Sokoine University of Agriculture, Tanzania

Invited review of (unpublished) journal manuscript (5 ECTS)

- African Journal of Microbiology Research: *Penicillium citrinum* VFI-51 as biocontrol agent to control charcoal rot of sorghum (*Sorghum bicolor* (L.) Moench) (2015)
- Journal of Experimental Agriculture: factors influencing the agronomic performance of the adapted yam minisett technique in Nigeria – planting date and gender of the farmer (2015)
- Plant disease: first report of *Rhamphicarpa fistulosa* on *Acroceras zizanioides*, *Cyperus difformis*, *Echinochloa colona*, *Paspalum scrobiculatum* and *Setaria pumila* in Togo (2015)
- Annals of Applied Biology: density-independent reproductive success of the hemiparasitic plant *Striga hermonthica*, despite positively and negatively density-dependent phases (2017)
- Plant disease: the first report of dodder (*Cuscuta epithymum*) as a *Epiparasite* on *Hemiparasitic* Species of *Santalaceae* and *Orobanchaceae* in Poland (2017)

Deficiency, refresh, brush-up courses (1.6 ECTS)

- Wageningen UR digital library introduction; PE&RC (2011)
- Basic statistics; PE&RC (2011)

Competence strengthening / skills courses (13.65 ECTS)

- Career Orientation; PE&RC (2013)
- Voice matters and presentation skills training; PE&RC (2013)
- Writing grant proposals; PE&RC (2013)
- Data management; PE&RC (2013)
- Project and time management; PE&RC (2013)
- Techniques of writing and presenting a scientific paper; PE&RC (2013)
- PhD Competence assessment; PE&RC (2013)
- Communication with the media and the general public; PE&RC (2014)
- Peer consultation; PE&RC (2014)
- Effective behaviour in your professional surrounding; PE&RC (2014)
- Entrepreneurship in and outside science; PE&RC (2014)
- Science for impact: focus on enabling conditions; PE&RC (2014)
- Last stretch of your PhD; PE&RC (2015)

PE&RC Annual meetings, seminars and the PE&RC weekend (2.4 ECTS)

- PE&RC Weekend (2011)
- Symposium of soils (2011)
- PE&RC Day (2011-2014)

Discussion groups / local seminars / other scientific meetings (4.5 ECTS)

- Lunch seminars; PPS and CSA (2011-2015)

- AfricaRice Centre seminars (2012-2014)

International symposia, workshops and conferences (4.7 ECTS)

- 3rd Africa Rice congress; poster presentation; Yaoundé; Cameroon (2013)
- 12th World Congress on Parasitic Plants; poster presentation; Sheffield, UK (2013)
- First international conference on global food security; the Netherlands (2013)
- European Weed Research Society conference; poster presentation, best poster award; Montpellier, France (2015)

Supervision of MSc students (5 ECTS)

- John Constantine: effects of *Striga asiatica* and *Rhamphicarpa fistulosa* densities on yielding abilities of rice (2012)
- Musangi H: ecological differences between *Striga asiatica* and *Rhamphicarpa fistulosa* in rice
- Djoeke Langeloo: *Rhamphicarpa fistulosa*, a noxious parasitic weed in rice (2012)
- Yuusuf Hasan Mahamed: investigating the effect of rice (*Oryza sativa* L.) on the germination of the facultative parasitic weed *Rhamphicarpa fistulosa (Hochst.)Benth* (2012)
- Yewubnesh Seifu: studying the mutual interaction between rice and the parasitic weed *Rhamphicarpa fistulosa*: the role of host size at the moment of parasite infection (2013)

Funding

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