Influence of parasitic weeds on rice-weed competition
Influence of parasitic weeds on rice-weed competition

MSc. Thesis Report
ABSTRACT

Over the last decades rice (*Oryza* spp) became more important as a staple food crop for the African continent. In two decades, the locally produced rice in Africa doubled to almost 30 Mt in 2013. Still, the local production is insufficient to meet local demand and this particularly holds for Sub-Saharan Africa (SSA). Rice yields in SSA pertain to the lowest in the world. One of the major constraints accountable for these low yields are weeds. The parasitic weeds *Striga asiatica* and *Rhamphicarpa fistulosa* can cause devastating yield losses in SSA rice production. Recent field observations suggest that the presence of parasitic weeds influences the competitive relations between rice and non-parasitic (or ordinary) weeds. In the presence of *S. asiatica* the competitiveness of ordinary weeds was increased, whereas in the presence of *R. fistulosa* the ordinary weeds were further suppressed. Two pot experiments, carried out under greenhouse conditions, were used to study how the competitive relations between rice and the ordinary weed *Mitracarpus villosus* was affected by the presence of either *S. asiatica* or *R. fistulosa*. Plant dry biomass was used as a measure for the competitive effects. *S. asiatica* caused significant reductions in total pot biomass compared to pots with rice alone. Such a reduction was however not observed if next to rice also the *M. villosus* was present. This absence of a reduction in total pot biomass was not because the ordinary weed filled the gap that was created through the negative effect of the parasite on its rice host, rather rice biomass did not decrease in the rice-ordinary weed mixture. Emergence of *S. asiatica* in this mixture was lower, and this might be accountable for the minor effect of the parasite on the host. It is suggested that the root system of the ordinary weed might have disturbed the establishment of a connection between the host and the parasite, resulting in a reduced emergence of *S. asiatica*. *R. fistulosa* reduced rice biomass much more strongly than *S. asiatica*. In the rice-weed mixture, the ordinary weed was not able to profit from the reduced growth of the rice plant, as *R. fistulosa* grew fiercely and developed into a strong competitor. Consequently, the competitive ability of the ordinary weed, just as that of the rice plant, was strongly decreased. The experiments clearly show that the presence of a parasitic weed affects the growth and competitive relation between rice and ordinary weeds both directly and indirectly. The outcome of this complex multi-species interaction depends a lot on the parasitic weed species. *R. fistulosa* showed to be a damaging pest and utilized its ability to parasitize rice to gain a stronger competitiveness against the developed into the species dominating not only the rice, but also the ordinary weed. In case of *S. asiatica*, the ordinary weed reduced the infestation level of the parasitic weed and consequently the competitive relation between rice and the ordinary weed remained relatively undisturbed.
The first steps of this thesis took place about 10,000 km South from here, at a smallholder field, in a remote and isolated village in central Tanzania. There, in one of the maize fields of my good friend Lazaro, I saw the root parasitic weed *Striga asiatica* ‘stealing’ water and nutrients from the maize, in such amounts that the maize plant was seriously weakened. With the knowledge I gained in previous research with those root parasites I started to explain the villagers how devastating this small plant, with its beautiful red flowers, could be to its host. How the parasite is able to connect its root to those of the maize plant (by biting in them and start sucking the water and food out of the maize). And how the parasite is actually able to take the life of the maize plant and produce thousands of tiny seeds for itself, in an attempt to start the whole process again next season, when there is a suitable host. Once again, by facing the problem in practice, I realised how serious the parasitic weed topic could be for smallholder farmers in Sub-Saharan Africa. At this point I decided that I should contribute in the process to solve this problem. No matter how big my contribution should be, I should work on this topic.

So, I would like to thank my supervisors Aad and Lammert for giving me the opportunity to carry out research with root parasitic weeds of tropical cereals. Lammert, I am grateful for the supervision I received from your side. Not only I gained more inside in competitive and parasitic plant interactions, you gave my also the inside about the importance of ‘time’ in research. Sometimes one should slow down the process, to cogitate some principles once more. And Aad, apart from the interesting and useful discussions we had about my experiments and the results I want to thank you also for the long conversations and discussions we had about the parasitic problem in Africa, smallholder farmers in Africa, and actually the African life and culture in general, I appreciate these moments a lot.

The chair groups of CSA and PPS was a very stimulating working environment for me. I think, due to a special mix of different people working at those two departments, I really felt me welcome for the six months I was amongst you. The same is true for the people at Unifarm, the working environment motivated me a lot to carry out my experiments with a lot of passion.

The team of the PARASITE project I want to thank for their interest in my research. Hanna, Stella and Xinxin, thanks for the ideas I could share with you. And of course Jonne, you showed always a great interest in my study and your enthusiasm about the topic gave me a lot of energy to continue. We will meet again, in Dar es Salaam, or Wageningen, or...

I appreciate the moments I could ventilate my ideas and thoughts with my housemates, and also with my family and friends. Thanks for the discussions, or for just listening to my story.

A special word of gratitude goes to Diana. Thank for all those moments and days you helped me with the (boring) work in my experiments. And I want to thank you for the patience you had during all those moments that my thesis was more important than my accompany to you.

Finally, I want to thank all of them who I forgot to thank in person, and you as reader of this report. Maybe you will get inspired by the topic and contribute also in one or another way in solving this problem with parasitic weeds in tropical cereals. In my case, I am still highly interested in the topic, and I hope my study contributed to come a bit closer to a solution.

Luuk C.M. van Dijk

Wageningen, September 2014
1. INTRODUCTION

Rice

Rice (*Oryza* spp.) is a main staple food for more than half of the world population. In Africa it is, after maize, the most important cereal, with a total production of 29.0 Mt in 2013 (Seck et al., 2010; FAO, 2014). Traditionally, rice was a main staple in West Africa and Madagascar. However, rice consumption is growing with 4.5% yearly throughout Africa and has become one of the main staple food crops in sub-Saharan Africa (SSA). Increasing income per capita, the price relative to that of other staple crops, increasing population and urbanisation and changing diets are the main driving factors for the increase of rice consumption (Balasubramanian et al., 2007; Seck et al., 2010; Seck et al., 2012). The total rice production in Africa doubled in the last two decades (Figure 1). Still, rice production in SSA cannot meet the increased demand for rice. In 2011, the rate of imported rice in SSA was 10.0 Mt, compared to 20.4 Mt of locally produced rice (FAO, 2014).

In SSA, rice is grown in five main agro-ecosystems: 1) rain-fed uplands and hydromorphic slopes (39% of the total cultivated rice area), 2) rain-fed lowlands and floodplains (33%), 3) irrigated lands (19%), 4) deep-water basins in floodplains (9%) and 5) mangrove-swamps (9%) (Balasubramanian et al., 2007; Rodenburg and Johnson, 2009). In these areas, rice is generally produced by smallholder farmers. Due to many constraints, the rice production in SSA reaches a yield of 2.07 t·ha⁻¹, what is the lowest in the world (in 2013, the world average was 4.49 t·ha⁻¹; FAO, 2014). Major constraints in SSA rice production are either social or biophysical. Social constraints, are for example, lack of inputs, labour, extension workers, technology or infrastructure. Biophysical constraints to rice production are both abiotic (e.g. drought, flooding, extreme temperatures, toxicities and poor soils) and biotic (e.g. bird, rodent and insect attacks, diseases and weeds) (Balasubramanian et al., 2007; Rodenburg, 2014). Causing an average loss of 35% worldwide, weeds are the most important constraint in rice production (Oerke and Dehne, 2004; Rodenburg and Johnson, 2009).

![Figure 1. Rice production 1994-2013 in Africa (FAO, 2014).](image)
Weeds

All undesired plants in a cultivated area can be considered weeds. Two types of crop losses are caused by weeds. First, and most damaging, is the direct loss, following from competition for nutrients, water and light with the cultivated crop. Second, weeds can contaminate crop harvests with their seeds. Additionally, weeds can be a host, or provide shelter, for undesired insects, fungi and viruses (Nieto et al., 1968). Competition between plants happens when two or more plants are growing together, so that the available, limited, resources have to be shared. The more competitive a weed is or the higher the density, the more crop yield will be negatively affected.

Problems with weeds can be caused by poor land preparation before sowing, crop seed contamination with weed seeds, low quality of sowing material, inadequate field management, lack of crop rotation and labour (Becker and Johnson, 1999; 2001). The seriousness of weed problems in the field is influenced by the presence and impact of other constraints (Rodenburg and Johnson, 2009). As weed management is adequate (e.g. due to availability of knowledge, labour for weeding or herbicides), rice yield losses will be correspondingly lower. However, in most cases in SSA, weed management is poor (Balasubramanian et al., 2007). Enhancement of farmer’s weed management may result in a yield gain between 15-23% in SSA. The total yield loss due to weeds in SSA rice production in 2008 was estimated of at least 2.2 Mt (Rodenburg and Johnson, 2009). In the different agro-ecosystems and due to differences in rice cultivation, yield losses caused by weeds can vary. In a lowland ecosystem, rice yield reduction can vary between 28-74% when transplanted, and from 28-89% when direct seeded. In an upland rice ecosystem, yield losses are estimated to range from 48-100% (Rodenburg et al., 2011a).

Depending on agro-ecosystem, most important weeds in African rice cultivation are the perennials *Imperata cylindrica, Cyperus rotundus, Chromolaena odorata, Oryza longistaminata* and *Cyperus* spp., and the annuals *Digitaria horizontalis, Euphorbia heterophylla, Echinochloa* spp., *Oryza barthii, Ischaemum rugosum, Cyperus difformis, Cyperus iria, Fimbristylis littoralis and Sphenoclea zeylanica*. Besides, there is the group of the root-parasitic weeds; *Striga hermonthica, Striga asiatica* and *Rhamphicarpa fistulosa*. They are considered as the most damaging group of weeds regarding rice cultivation in SSA (Raynal-Roques, 1994; Rodenburg and Johnson, 2009; Rodenburg et al., 2010).

As it is in the name, parasitic weeds are able to parasitize other plants. By forming haustoria, root-parasitic weeds are able to connect to the roots of the host plant and to withdraw nutrients, water and assimilates from its host (Kuijt, 1969; Musselman, 1980; Parker and Riches, 1993). However, for both *Striga* spp. as well as for *R. fistulosa*, the gained biomass of the parasite, due to production of its own assimilates and parasitization of its host, is lower than the reduction in biomass of the host plant. In case of *Striga*, it was observed that the parasite is able to reduce host’s photosynthesis (Press et al., 1987; Rodenburg et al., 2008). *Striga* spp. and *R. fistulosa* are much more abundant in fields with low soil fertility, degrading soils or suboptimal water control (Raynal-Roques, 1994; Joel, 2000; Parker, 2009; Rodenburg et al., 2010: Parker, 2012).
*Striga* spp.

*Striga* spp. (witchweeds) are parasitic weeds belonging to the Orobanchaceae family and cause serious damage to its host crops. In SSA *S. hermonthica* (Del.) Benth. (Purple or Giant witchweed) and *S. asiatica* (L.) Kuntze (Asiatic or Red witchweed) create great yield losses to tropical cereals as rice, sorghum and maize in upland environments (Musselman, 1980; Mohamed et al., 2006; Parker, 2009; Rodenburg and Johnson, 2009). *Striga* spp. are obligate hemiparasitic weeds that parasitize the roots of their hosts via xylem-to-xylem connections in the haustoria (Press et al., 1991; Parker and Riches, 1993; Mohamed et al., 2001). Before being able to germinate, the small (0.2-0.35 mm, 3.7·10^{-6} g) seeds of *Striga* need a period of preconditioning (warm and moist conditions) and after that secondary metabolites secreted from host roots (Figure 2). Since the seeds are so small and contain only little reserves, the radicle has to invade the host root within a few days, if not the germinated seeds will not survive (Parker and Riches, 1993; Bouwmeester et al., 2003; Mohamed et al., 2006). Damage to the host plant occurs shortly after *Striga* is connected to the hosts vascular system, so even before the parasite is emerged above the soil.

A sensitive host plant shows minor growth, low biomass and no or underdeveloped flowers (Rodenburg and Johnson, 2009). Heavy infested rice fields can show damages from 60% up to total crop failure (Sauerborn, 1991). Nowadays, more than 40% of the area cultivated with cereals in SSA is infested with *Striga*. Mostly, poor smallholders with degraded soils are the victim of these parasitic weeds. It is expected that in the near future the *Striga* problem will only increase since poor farmers are economically limited in control options and the cultivated area of rice will keep growing due to population growth, urbanisation and chancing diets (Mohamed et al., 2006; Rodenburg et al., 2010).

![Figure 2](image-url)

**Figure 2.** Graphic life cycle of a parasitic weed like *Striga*. (i) Seeds of *Striga* need a period of preconditioning in the soil to become sensitive for secondary metabolites of host roots to induce the germination. (ii) The roots of the host plant can produce germination stimulants for the parasitic weed seeds. (iii) The parasites radicle grows to the host roots to form a haustorium and set the xylem-to-xylem connection to start feeding from the host. (iv) When the parasite matures it will produce flowers. (v) Last step is seed production and dispersal to the soil. *Striga* is able to produce 5,000-85,000 seeds per plant. (vi) After dispersal, seeds are cumulated to the seed bank in the soil and become sensitive to germination stimulants (i). Adapted from Bouwmeester et al. (2004).
R. fistulosa

R. fistulosa (Hochst.) Benth is another species of the Orobanchaceae that is able to parasitize rice. It is a facultative hemiparasite, widely spread in Africa and also in India with preference for hydromorphic environments (Raynal-Roques, 1994; Dembélé et al., 1994; Neumann et al., 1998), but it can also survive in dryer environments when parasitizing a host (Ouédraogo et al., 1999), or even without host plant (personal observation). Apart from rice, it is able to infect cowpea, maize, millet and sorghum by forming xylem-to-xylem connections at the host roots to withdraw water, nutrients and assimilates (Kuijt, 1969; Ouédraogo et al., 1999).

Since it is a facultative parasite, R. fistulosa is also able to complete its lifecycle without being connected to a host. Rhamphicarpa does not need host root exudates to germinate, but it was found that light is a requirement for the small (11.3·10^{-6} g, Rodenburg et al., 2011a) seeds to germinate (Ouédraogo et al., 1999). Without a host, R. fistulosa plants stay small (about 15 cm) and pale green. The white flowers as well as the seed capsules also remain small. In contrast, when R. fistulosa attends its lifecycle while connected to a host, it shows a large (>50 cm) and bushy habit, a dark green to purple colour and the white flowers and the seed capsules are big and numerous.

In the field, R. fistulosa can cause crop losses up to complete crop failure. It is reported to be more damaging as Striga spp. (Raynal-Roques, 1994; Ouédraogo et al., 1999; Rodenburg et al., 2011a). Surveys carried out in Benin and Tanzania, reported cases of farmers who abandon their R. fistulosa infested fields (Kayeke et al., 2010; Rodenburg et al., 2011a). Abandoning a field does not signify a stop to the further spread of R. fistulosa, since the weed can grow independently and has a broader (unknown) host range (Ouédraogo et al., 1999; J. Rodenburg personal communication).

Observations in research

Research may provide more insight in the interaction between rice and the parasitic weeds and it can provide clues to farmers on how to deal with the parasitic weed problem. Within the framework of the PARASITE-program (Preparing African Rice Farmers Against Parasitic Weeds in a Changing Environment), recently a lot of rice-parasitic weed research was carried out.

In field trials in Kyela, Tanzania, the rice-parasitic weed interaction (S. asiatica, R. fistulosa) was studied under different fertilizer regimes. As both, Striga spp. as well as Rhamphicarpa, favour a low fertile environment, the reverse can be used as method of control (N’cho et al., 2014). It is well-known that an improved level of soil fertility has a positive effect on crop biomass production as well as on the biomass and appearance of undesired ordinary (= non-parasitic) weeds (Blackshaw et al., 2004; Sweeney et al., 2008). So, in plots with an improved soil fertility, higher numbers of ordinary weeds are obvious.

In the S. asiatica trial, it was observed that in the parasite infested plots the amount of ordinary weed biomass was higher. Conversely, in the R. fistulosa trial, a suppressed growth of ‘ordinary’ weeds was observed when R. fistulosa occurred in the field. (L. Bastiaans, personal communication).

More biomass accumulation for ordinary weeds in S. asiatica infested plots can possibly be credited to the parasitic effect of S. asiatica on rice. Already, in an early stage S. asiatica starts
forming xylem-to-xylem connections at the host roots. This is the start of the disturbance of the rice’s photosynthetic system, and the withdrawal of nutrients, water and assimilates (Kuijt, 1969; Musselman, 1980; Press et al., 1987; Parker and Riches, 1993). As a result, growth and development of rice is constrained (Rodenburg and Johnson, 2009). It might be speculated that an inhibited growth of the rice plant in an early stage may lead to a disturbance of the common rice-ordinary weed competition, in advantage of the weed. As long as S. asiatica does not parasitize on the weed and S. asiatica is not yet emerged, the ordinary weed is not hampered by the parasite, and may take an advantage of the reduced ability of rice to compete for resources. Even after emergence of S. asiatica, the ordinary weed may undergo little hinder from the parasite, since the parasite does not gain much biomass to compete with (Press et al., 1987; Rodenburg et al., 2008).

The facultative root parasite R. fistulosa is able to complete its lifecycle without a host plant (Kuijt, 1969). Thus, in the field, the parasite is able to start developing before the rice is emerged. For competition between rice and an ordinary weed, R. fistulosa may be a third party in this competition. In case R. fistulosa is not parasitizing, it will not have a large influence, since a single, non-parasitizing R. fistulosa plant does not gain that much biomass (Ouédraogo et al., 1999). However, when R. fistulosa starts parasitizing its host (rice), it is able to withdraw nutrients, water and assimilates, and gain much more biomass. So in this case it can be speculated that in the field, R. fistulosa may become, next to a parasite of rice, an extra competitor of the ordinary weed. Ultimately, this may result in suppressed growth of the ordinary weed.

**Objective**

The objective of this study was to understand how the competitive relations between rice and an ordinary weed species is affected by the presence of a root parasitic weed. As there was an observed, contrasted, change in the biomass accumulation of the ordinary weeds community in rice fields infected with S. asiatica and R. fistulosa, we want to gain insight in how and to what extent crop–weed competitive relations are influenced by the presence of these two parasitic weed species. Therefore the following research question was formulated:

To what extent does the presence of a root-parasitic weed influence the competitive relations between the rice host plant and an ordinary non-parasitic weed species?

And, is the influence of S. asiatica different from the effect of R. fistulosa?

To achieve the objective of this study, two parallel pot experiments were set up to study rice-weed competition under different parasitic weed infestation levels. One experiment was set up with S. asiatica, and one with R. fistulosa. An upland, and lowland rice cultivar were used for respectively the Striga and Rhampicarpa experiment. In both experiments, Mitracarpus villosus, a common weed in upland rice environments in Tanzania, was used as the ordinary weed species.
2. MATERIAL and METHODS

Two parallel pot experiments with rice were carried out in the greenhouse of Wageningen University (compartment 5.4 of Radix Serre, Unifarm Wageningen UR) in the Netherlands. The experimental period was from April to July 2014. In both experiments rice was grown in competition with the ordinary weed *M. villosus*. In one pot experiment the parasitic weed *S. asiatica* was added, whereas in the second experiment *R. fistulosa* was introduced.

Design

A randomized block design, with two factors (plant combination and parasitic weed infestation level) was used for both experiments. Competition amongst rice and an ordinary weed was studied by using replacement series, as formalized by de Wit (1960). In a replacement series two plant species are grown together in a constant total density of plants, while the ratio in which the two species are grown is changed. In both pot experiments rice : ordinary weed ratios of 2:0, 1:1 and 0:2 were used. The assumption was made that three ordinary weed (*Mitracarpus*) plants will crowd for the same space as one rice plant. These three weed plants will be regarded as one ‘plant unit’. An additional monoculture of both rice and ordinary weed, existing of one solitary plant unit per pot, was added to gain insight into intraspecific plant competition (Figure 3).

Three different densities of parasitic weeds were used in both experiments, control (parasite-free), low and high density. In the *Striga* experiment these densities were aimed at about 0, 5 and 30 emerged *S. asiatica* plants per pot. For the *R. fistulosa* experiment 0, 10 and 30 plants per pot were used. Extra pots with *R. fistulosa* on low and high density (without any plant combinations) were added as control. Since *S. asiatica* needs host root exudates to germinate, the *Striga* experiment was without control pots. Both experiments were replicated six times (block factor). An overview is shown in Table 1.

![Figure 3](image)

Figure 3. Schematic overview of five different plant combinations used in the experiments. From left to right; Monoculture rice, mixture rice-weed (1:1), monoculture weed, solitary rice, solitary weed (unit). Over the factor plant combination the three different densities of parasitic infestation was laid out.

Layout

Pots were placed on tables with a height of 0.8 m from ground level. Table length was 9.00 m and width 1.20 m. Distance between the tables and objects was at least 0.60 m to allow access to the pots and avoid border influences. Per table, pots of one experiment were placed. For the *Striga* experiment this resulted in six blocks (replicates) of 15 pots. Per replicate the pots were arranged in three rows of five pots with a distance of 5 cm between pots (outside
diameter of 24 cm). Between replicates there was a space of 10 cm. For the *Rhamphicarpa* experiment, the same layout was used. As the experiment contained two extra pots per replicate (control; parasite only), two pots were placed either left or right of the 3*5 pots unit, at a random position. Both layouts can be found in Appendix 1.

Table 1. Experimental details (both experiments contain 6 replicates).

<table>
<thead>
<tr>
<th>Factor</th>
<th>Levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td><em>S. asiatica</em></td>
</tr>
<tr>
<td></td>
<td><em>R. fistulosa</em></td>
</tr>
<tr>
<td>Plant combination</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Monoculture rice (2 plants)</td>
</tr>
<tr>
<td></td>
<td>Mixture rice:weed (1:1)</td>
</tr>
<tr>
<td></td>
<td>Monoculture weed (2 plant units)</td>
</tr>
<tr>
<td></td>
<td>Solitary rice (1 plant)</td>
</tr>
<tr>
<td></td>
<td>Solitary weed (1 plant unit)</td>
</tr>
<tr>
<td></td>
<td>(control in <em>Rhamphicarpa</em> experiment)</td>
</tr>
<tr>
<td>Parasitic weed density</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td><em>S. asiatica</em> 0 - 0.0009 - 0.0054 g seeds-pot$^{-1}$</td>
</tr>
<tr>
<td></td>
<td><em>R. fistulosa</em> 0 – 10 – 30 plants-pot$^{-1}$</td>
</tr>
</tbody>
</table>

**Germplasm**

Seeds of both parasitic weed species were collected in Kyela, Mbeya district in the Southern highlands of Tanzania. The seeds of *R. fistulosa* (Hochst.) Benth. were collected in 2010 and the seeds of *S. asiatica* (L.) Kuntze in 2012.

As ordinary weed, *M. villosus* (Sw) DC. was used. *M. villosus* is a widespread weed in tropical Africa and also occurs in South America and the West Indies. It is an erect, broadleaved annual weed, growing in upland agricultural fields, young fallows and other soils disturbed by human activity (Verdcourt 1976; Neumann et al., 1989). Seeds of *M. villosus* were collected in Tanzania in 2014.

IAC165 is the rice cultivar that was used in the experiment with *S. asiatica*. IAC165 is a variety that is grown under upland conditions and that is highly susceptible to *Striga* spp. infestations (Johnson et al., 2007).

In the experiment with *R. fistulosa*, the lowland rice variety IR64 was used. IR64 is known as a semi dwarf lowland rice cultivar with a weak competitiveness towards weeds (Haden et al., 2007; Rodenburg et al., 2009). The cultivar is susceptible to *R. fistulosa* infection (Rodenburg et al., 2011b).

**Conditions**

In the greenhouse compartment used for the experiments a tropical environment was mimicked. Temperature was set to 26°C as day and 23°C as night temperature. Day length was kept at 12 h (started 08.00 am) by using non-transparent screens. Artificial light (two rows of four 400 W lamps SON-T Argo, Philips) automatically switched on when outside photosynthetically active radiation (PAR) dropped below 910 μE m$^{-2}$s$^{-1}$ during day conditions. Mean relative humidity was kept between 50 and 70%.

The pots used had a volume of 6 L and a closed bottom. Bottom diameter was 17.0 cm and pot height was 20 cm. The upper diameter was 21.5 cm, consequently the soil surface was 291.0 cm$^2$. The pots were filled until 2 cm from the rim of the pot with 8 kg of a moist mixture of
arable soil and sand at a 1:1 ratio. The soil was collected from a field of the Droevendaal experimental farm in Wageningen (Bio5 soil) and the sand used was commercial, clean masonry sand.

In the first period, directly after sowing, watering was done every day, by misting with a fine spray of water to keep the soil moist enough without disturbing the seedbed. At 7 days after sowing (DAS) seedlings were robust enough to spray with a larger sprayer at three times a week (Monday, Wednesday, Friday). Pots were kept sufficiently moist to avoid water limitation. It was attempted to mimic upland water conditions in the Striga experiment, and lowland water conditions in the Rhamphicarpa experiment, during the entire experimental period.

Once a week weeding was done to remove voluntary weeds and to thin late emerging seedlings of R. fistulosa and M. villosus to maintain the preferred plant numbers per pot.

Infestation and sowing

Infestation of the pots with S. asiatica was done at 11 days before sowing of the rice, to allow pre-conditioning of the parasitic weed seeds. The aim was to obtain about 5 and 30 (low and high density) S. asiatica plants per pot. Based on unpublished experimental data on different S. asiatica densities of Kabiri (2013) pots were infested with 0.075 and 0.45 seeds per cm$^3$ of soil. Since Striga seeds were mixed through the upper 10 cm of soil in the pots, 242 and 1,449 seeds, were needed for the low and high infestation level, respectively. According to Parker and Riches (1993) the weight of one seed of S. asiatica is 3.7·10$^{-6}$ g. Hence pots were infested with 0.0009 and 0.0054 g Striga seeds per pot. After infestation, pots received water until field capacity was reached.

Infestation of the pots with R. fistulosa was done 3 days before sowing of the rice. A total of 70 and 200 seeds per pot were used in the low and high infestation level treatment, respectively. A small pre-test showed that the R. fistulosa seedling emergence fraction was about 40%. According to Rodenburg et al. (2011b), the weight of a R. fistulosa seed is about 11.3 $10^{-6}$ g, hence pots were infested with 0.791 (low) and 2.26 mg (high) of seeds per pot. Seeds were mixed with a small volume of dry sand (about 2 mL) to be able to spread the tiny seeds equally over the pot surface. After infestation, the seeds were carefully incorporated in the top 0.5 cm of the soil. After emergence, R. fistulosa was thinned to 10 plants and 30 plants per pot in the low and high density treatment, respectively. The pots of the R. fistulosa experiment were watered until soil saturation was reached, to create a balance between the waterlogged wetland conditions where R. fistulosa naturally occurs and the upland conditions of M. villosus.

The ordinary weed M. villosus was sown 3 days before sowing of the rice, in both experiments. Germination tests, carried out in advance, showed first emergence about 5 DAS with a total emergence percentage of about 20%. Therefore, seed rates used were 10 times as high as the desired number of plants, to guarantee a sufficiently high number of emerged plants. Sowing pattern was according to Figure 3. For M. villosus, emerged seedlings were thinned to three or six plants per pot, depending on treatment.

Both IR64 and IAC165 rice seeds were sown at a depth of 1-1.5 cm. Seeds were sown in the middle of the pot or equally allocated over the pot surface, according to Figure 3.
Observations
Most observations were done at a weekly basis in both experiments. Rice plant height was taken from soil level up to the youngest emerged ligule on the stem. Ordinary weed height was assessed from soil up to highest point in the plant’s canopy. Weekly (green) leaf counts were carried out for rice and ordinary weed plants. In case of ordinary weed, only those leaves larger than 1 cm were counted. Rice tillers (including the main stem) and the number of emerged *S. asiatica* plants were counted weekly.

Harvest
Harvest of the *Rhamphicarpa* experiment was conducted at 63 DAS, the *Striga* experiment was harvested at 78 DAS. In case of rice, the numbers of leaves, tillers and panicles were counted per pot. Rice in the *Rhamphicarpa* experiment did not have panicles at the moment of harvest. Fresh weights of these plant parts were taken and leaf area was measured (LI-3100 leaf area meter, LI-COR, Lincoln, NE, USA).

*M. villosus* leaves and stems were counted per pot. The many side-branches of *M. villosus* in the *Striga* experiment were also counted. Fresh weights of leaves and stems (+ side-branches) were assessed and leaf area was measured.

In both experiments, fresh weights of the parasitic weeds were determined per pot. For *Striga*, total number of emerged plants per pot were determined and length of all Striga plants was individually measured. All harvested (above soil) plant material was dried at 70°C for at least 48 h and subsequently dry weights were determined.

Data analyses
Biomass used in the data analyses were above ground plant dry weight per plant species. In case more than one plant species was present, the dry weights were summed to obtain the total dry weight per pot. When per plant weight was used, total pot weight of rice or *M. villosus* monocultures with two plants (units) was divided by two. GenStat Edition 16.2 (VSN International Ltd.) was used for statistical analyses of data. Data were subjected to analysis of variance (ANOVA). If significant differences were present, comparison of means was done using Least Significant Difference (LSD).
3A. RESULTS –Striga–

First emergence of *M. villosus* was observed at four days after sowing (1 DAS of rice). It took three days before all pots contained emerged *M. villosus* seedlings. First rice emergence took place at 4 DAS, it was observed in 25% of the pots. At 6 DAS, all pots contained geminated rice seedlings. At 41 DAS of rice, first emergence of *S. asiatica* was observed.

**Dry weight of rice, *M. villosus* and *S. asiatica***

For per pot rice dry weight, an interaction was found between plant combination and parasite density (*P=0.009*). For all parasitic weed densities, rice biomass per pot was decreasing in the following order; two rice plants per pot (HH), one rice plant per pot (H), and one rice plant in the rice-weed mixture (HW) (Figure 4). The exception was found at the low parasite density (d1), where rice biomass in the treatments with two (HH) and one (H) rice plant per pot did not differ significantly.

For the plant combinations with two rice plants per pot (HH), the increasing parasitic plant density caused a significant decrease in rice biomass. In case of one rice plant per pot (H) only the high parasite density (d2) caused a significant decrease in rice biomass. In the rice-weed mixture, rice biomass was not significantly affected by parasite plant density.

Under an increasing parasitic plant density, the ratio in rice biomass between the treatment with two rice plants per pot (HH) and the rice-weed mixture (HW), gradually decreased from 1:0.49, to 1:0.52 and 1:0.57 for, control (d0), low (d1) and high (d2) density of *S. asiatica*, respectively. The decrease in ratio suggests that at higher parasite plant densities the ordinary weed became gradually more competitive.

The *M. villosus* biomass showed no interaction between plant combination and parasite density. There was a significant main effect for plant combination. In the absence of rice, a higher weed biomass was obtained (Figure 5). No difference was observed between pots with two (WW) and one (W) weed unit per pot. The ratio in weed biomass between the monoculture with two units of weed plants (WW) and the rice-weed mixture (HW) was 1:0.52, indicating that one rice plant was about as competitive as one unit of weed plants.

For biomass of the *S. asiatica* plants, an almost significant main effect was observed for parasite weed density (*P=0.059*). Mean biomass was 0.177 and 0.371 g·pot$^{-1}$ for the low and high density, respectively (Figure 6). Biomass of the parasitic weed was not significantly affected by plant combination (*P=0.137*).

For all plant combinations with rice (HH, H and HW) a significant interaction (*P=0.024*) for total per pot biomass (rice + *M. villosus* + *S. asiatica*) was found between plant combination and parasite density.

In the presence of the weed (HW) the total per pot biomass did not change with parasite density (Figure 7). For the other two treatments (HH and H), the total per plant dry weight reduced with parasite plant density. The only exception was found with treatment HH, where no significant difference was observed between the two parasite densities (d1 and d2). At each parasite density the total per pot dry weight was always highest for the rice-weed mixture (HW),
followed by the highest density of rice (H). Exceptions were found in the absence of the parasite (d0), when there was no significant difference between HW and HH and at parasite density d1, where no difference was found between pots with one (H) and two (HH) rice plants.

Focussing on all plant combinations with *M. villosus* (WW, W and HW), no significant differences were found for total per pot biomass (*M. villosus* + rice + *S. asiatica*) (Figure 8). Mean pot biomass was 8.53 g·pot⁻¹.

Therewithal, it appeared that 9 gram biomass per pot was about the maximum total above ground biomass per pot for rice without parasite (d0) (Figure 7) and *M. villosus* (Figure 8). One rice plant per pot (H) without parasite (d0) reached a biomass of 7.8 g. Pots with an additional rice plant (HH), extended the total pot biomass to 9.4 g, 21% more than one plant per pot (H).

Similar data were obtained from the *M. villosus* pots, where one plant unit (W) reached a total pot weight of 8.3 g. Two plant units of *M. villosus* per pot reached 8.7 g·pot⁻¹, what was 5% more biomass than one plant unit. Mean biomass for the rice-weed mixture over all parasitic densities (d0, d1, d2) was 8.6 g·pot⁻¹, close to maximal biomass of 9.4 g·pot⁻¹ in this experiment.

![Figure 4](image4.png)

**Figure 4.** Rice biomass per pot in the *Striga* experiment. There was an interaction between plant combination and parasitic density was significant (*P*=0.009). Differences are marked with different letters (l.s.d.= 0.944). Error bars depict standard error (SE).

![Figure 5](image5.png)

**Figure 5.** *M. villosus* biomass per pot in the *Striga* experiment. There was a significant main effect for plant combination (*P*=<0.001), a regarding the letters used in the legend (l.s.d.= 0.536). Error bars depict SE.
Figure 6. *S. asiatica* biomass per pot, for low (d1) and high (d2) infestation level of *S. asiatica*, per plant combination. An almost significant effect occurred for infestation level ($P=0.059$). Error bars depict SE.

Figure 7. Total pot biomass (*Rice + M. villosus + S. asiatica*) for plant combinations with rice (HH, H and HW), per infestation level, per plant combination, in the *Striga* experiment. Interaction between density and plant combination was significant ($P=0.024$). Differences are marked with different letters (l.s.d. = 0.767). Error bars depict SE.

Figure 8. Total pot biomass (*M. villosus + Rice + S. asiatica*) for plant combinations with *M. villosus* (WW, W and HW), per infestation level, per plant combination, in the *Striga* experiment. No significant difference occurred. Error bars depict SE.
Rice plant height

The development of rice plant height is presented in Figure 9. Between 44 and 51 DAS the first height differences related to parasitic density became visual. All plant combinations at the control (0) density of \textit{S. asiatica} were taller as the same combinations at low (d1) or high (d2) infestation level. These differences became larger until 65 DAS. An exception were the pots with one rice plant at low (d1) infestation level, rice in these pots had a plant height in the same order as the non-infected (d0) pots. From 65 DAS, rice plants showed no to little growth in length. Two groups of heights became visible, the rice plants at control density (d0) with one rice plant per pot (H) at low density (d1) and the remaining rice plants with parasitic infection.

![Figure 9. Means of weekly observed rice plant height (in cm) as function of time (in DAS) in the \textit{Striga} experiment. Treatments with one rice plant (H) are depicted with solid lines, two rice plants (HH) are with long dashes and rice-weed mixture (HW) are in dotted lines. Control densities (d0) are in green with round markers, low densities (d1) are in black with triangle shaped markers and high densities (d2) are in red with squared markers.](image-url)
3B. RESULTS – *Rhamphicarpa* –

All *M. villosus* emerged at four days after sowing (1 DAS of rice). Rice, IR64, first emergence was at 4 DAS, with a 25% germination rate at that day. Two days later all rice seeds were emerged. First emergence of *R. fistulosa* occurred seven days after introduction of *R. fistulosa* seeds (4 DAS of rice).

**Dry weights of rice, *M. villosus* and *R. fistulosa***

In the *Rhamphicarpa* experiment, rice biomass per pot showed an interaction between plant combination and parasitic weed density (*P*=<0.001). At control density of *R. fistulosa* (d0), all plant combinations with rice were significantly different (Figure 10). Two rice plants per pot (HH) showed the highest biomass per pot, followed by one rice plant (H) and rice in the rice-weed mixture (HW).

At the low density of *R. fistulosa*, two rice plants per pot (HH) showed a significant higher biomass as one rice plant per pot (H) and the mixture (HW). At high parasite density (d2) there was a significant difference between two rice plants (HH) and a rice plant in the mixture (HW).

In the presence of *R. fistulosa*, two rice plants per pot (HH) demonstrated a significantly lower biomass. The same pattern appeared for rice biomass in the rice-weed mixture (HW).

*M. villosus* biomass showed no interaction between plant combination and density. The factor plant combination demonstrated a significant main effect (*P*=<0.001). Two *M. villosus* plant units (WW) showed the highest biomass (mean 4.038 g·pot⁻¹) (Figure 11), followed by one weed plant unit per pot (W) with a mean of 2.564 g·pot⁻¹. Lowest *M. villosus* biomass was found in the rice-weed mixture (mean 1.886 g·pot⁻¹).

*M. villosus* biomass was significantly affected by parasitic density (*P*=<0.001). Highest *M. villosus* biomass occurred in pots under control (d0) and low (d1) density of *R. fistulosa*, with means of 3.152 and 2.987 g·pot⁻¹, respectively. Lowest *M. villosus* biomass was found at high parasitic density (d2) (mean 2.349 g·pot⁻¹).

*R. fistulosa*, in pots with rice (+ *R. fistulosa* control), showed a significant main effect for plant combination (*P*=<0.001) and density (*P*=<0.001). Most *R. fistulosa* biomass was found in pots with one (H) and two (HH) rice plants, where means were 2.327 and 2.304 g·pot⁻¹, respectively (Figure 12). *R. fistulosa* biomass in the rice-weed mixture (HW) had significantly less biomass, 1.789 g·pot⁻¹. Lowest *R. fistulosa* biomass was found in the *R. fistulosa* control pots, without other plants (mean 0.727 g·pot⁻¹).

*R. fistulosa* showed significantly more biomass at high (d2) density, compared to low (d1) density, with amounts of 2.051 and 1.522 g·pot⁻¹, respectively.

*R. fistulosa* biomass in combination with a *M. villosus* plant unit (+ *R. fistulosa* control) showed a significant main effect for plant combination (*P*=<0.001) and parasite density (*P*=<0.001). The rice-weed mixture (HW) showed significantly the highest parasite biomass per pot, 1.789 g (Figure 13). Second highest parasite biomass occurred in control pots (*R. fistulosa* only), and pots with one weed unit (W). Lowest biomass of *R. fistulosa* was found in pots with two weed units (WW), with a mean weight of 0.352 g·pot⁻¹.
Again, more *R. fistulosa* biomass was found in pots with a high infestation level (1.049 g·pot\(^{-1}\)) than in pots with a low (d1) parasite level (0.672 g·pot\(^{-1}\)).

For the plant combination with rice (HH, H and HW), significant main effects for total pot biomass (*rice + M. villosus + R. fistulosa*) were found for plant combination (*P*=<0.001) and density (*P*=<0.001). The rice-weed mixture (HW) and the monoculture of two rice plants (HH) showed significantly the highest total pot biomass with means of 4.728 and 4.487 g·pot\(^{-1}\), respectively (Figure 14). Pots with one rice plant (H) had the lowest weight, with 3.652 g·pot\(^{-1}\). Pots without *R. fistulosa* (d0) had the highest total pot biomass (mean; 5.141 g·pot\(^{-1}\)). Total per pot biomass was not significantly affected by parasite density.

Plant combinations with *M. villosus* (HH, H and HW) showed a significant main effect for plant combination (*P*=<0.001) and parasite density (*P*=<0.011), regarding total pot biomass (*M. villosus + rice + R. fistulosa*). The total pot biomass mean of 4.728 g·pot\(^{-1}\) in the rice-weed (HW) mixture was significantly the highest (Figure 15). Two weed plant units (WW) demonstrated the second highest biomass (4.272 g·pot\(^{-1}\)). One weed unit (W) had the lowest total plant biomass (2.947 g·pot\(^{-1}\)).

Total pot biomass at high (d2) parasitic density was significantly lower as total biomass in pots with low density (d1) and control pots in the absence of a parasite (d0). Means of total pot biomass for high, low and control densities were 3.663, 4.052 and 4.232 g·pot\(^{-1}\), respectively.

Even though, the interaction between plant combination and parasitic density was not significant (*P*=0.068), it was close to an interaction. The mixture of rice-weed (HW) can be attributed to this almost interaction. The difference between rice-weed (HW) and two weed units per pot (WW) was decreasing over an increasing parasite density. At control density (d0) this difference was 0.998 g·pot\(^{-1}\) while at high parasite density (d2) it was 0.177 g·pot\(^{-1}\).

![Figure 10](image-url). Rice biomass per pot in the *Rhamphicarpa* experiment. There was an interaction between plant combination and parasitic density was significant (*P*=<0.001). Differences are marked with different letters (l.s.d. = 0.482). Error bars depict SE.
Figure 11. *M. villosus* biomass per pot in the *Rhamphicarpa* experiment. There was a significant main effect for as well plant combination, as parasite density ($P<0.001$). Differences are regarding the letters used in the legend (l.s.d. = 0.351). Error bars depict SE.

Figure 12. *R. fistulosa* biomass per pot for low (d1) and high (d2) parasite density, per rice plant combination. Plant combinations and density showed both a significant main effect ($P<0.001$) regarding the letters used in the legend (Pl.Comb. l.s.d. = 0.220; Density l.s.d. = 0.155). Error bars depict SE.

Figure 13. *R. fistulosa* biomass per pot for low (d1) and high (d2) parasite density, per *M. villosus* plant combination. Plant combinations and density showed both a significant main effect ($P<0.001$) regarding the letters used in the legend (Pl.Comb. l.s.d. = 0.195; Density l.s.d. = 0.138). Error bars depict SE.
Figure 14. Total pot biomass (Rice + *M. villosus* + *R. fistulosa*) for plant combinations with rice (HH, H and HW). Plant combinations and density showed both a significant main effect (*P*=<0.001) regarding the letters used in the legend (l.s.d. = 0.352). Error bars depict SE.

Figure 15. Total pot biomass (*M. villosus* + Rice + *R. fistulosa*) for plant combinations with *M. villosus* (WW, W and HW). Plant combinations and density showed both a significant main effect (*P*=<0.001 and 0.011, respectively), regarding the letters used in the legend (l.s.d. = 0.368). Error bars depict SE.

Figure 16. Means of weekly observed rice plant height (in cm) as function of time (in DAS) in the *Rhamphicarpa* experiment. Treatments with one rice plant (H) are depicted with solid lines, two rice plants (HH) are with long dashes and rice-weed mixture (HW) are in dotted lines. Control densities (d0) are in green with round markers, low densities (d1) are in black with triangle shaped markers and high densities (d2) are in red with squared markers.
R. fistulosa, rice plant height

Observations for rice plant height development in the Rhamphicarpa experiment are depicted in Figure 16. First differentiation in height became visible at 23 DAS. Rice plants without R. fistulosa were found to be higher than rice plants in combination with a parasite. From 37 DAS, this difference became even larger, since rice plants in pots with parasites showed hardly any growth.
4A. DISCUSSION – *Striga* –

*M. villosus* and rice biomass

Based on field observations in Kyela, Tanzania it was hypothesized that *S. asiatica*’s reducing effect on hosts biomass production gave ordinary weeds the possibility to enhance its competitive strength to rice for available resources, and increase its biomass. The observation, in the current pot experiment, that total biomass in the rice-weed mixture was not affected, could be in line with this hypothesis. The reduction in rice biomass due to infection with *S. asiatica* was expected to be compensated by additional growth of the ordinary weed. However, in the pots with rice-weed (HW) mixture there was no decrease of rice’s biomass observed and consequently the biomass of the ordinary weed was not increased. Clearly the overall effect was as expected, but the underlying contributions of rice and the ordinary weed were different than expected. Possible explanations for this observation are described in the following sections.

*S. asiatica* data and the parasitizing effect

Biomass, as well as number, and time of emergence of *S. asiatica* showed high variation between, and in, different plant combinations and infestation levels. First emergence of *S. asiatica* was at 41 DAS of rice and occurred over a long period of time (41-78 DAS). First emergence was rather late compared to previous studies like that Kabiri (2013, unpublished) and Van Dijk (2011, unpublished), in which first emergence of *S. asiatica* appeared about one to two weeks earlier.

The unpredictable emergence of *S. asiatica* in pot and laboratory experiments is known from former studies. Precondition period, temperature and moisture level do influence the time to emergence (Osman et al., 1991). These findings are in line with the variation in emerged *S. asiatica* plant numbers and, following from that, variation in plant height, in this study (data not shown). Furthermore, tests showed that *S. asiatica* seeds stored under laboratory conditions become less viable over years (Bebawi et al., 1984; Kanampiu et al., 2003). Hence, it might well be that seeds, collected in 2012, had become less viable.

A late emergence of *S. asiatica* might be related to a late germination, followed by a late root attachment to the host plant. A xylem-to-xylem connection in a later stage of the rice plant development might cause less damage to the rice plant biomass accumulation, as was reported in a previous rice- *R. fistulosa* study (Pflug, 2013, unpublished).

As parasitic weeds are related to the decrease in yield, biomass and height of their host plants (Musselman, 1980; Parker, 2009), the rice plant height of rice might be an indicator of parasitic infestation and attachment. Hence, in the *Rhamphicarpa* experiment the height of the rice plants showed the first differentiation, whether they were infested or un-infested, around 23 DAS. Contrary, it took about 44 DAS before this differentiation in rice plant height occurred in the *Striga* experiment (Figure 9 and Figure 16). These observations suggest that the infection with *S. asiatica* was rather late.

Total emerged *S. asiatica* numbers per pot at low infestation were 4.3 and 6.7 plants for one (H) and two (HH) rice plants per pot, respectively. At high infestation levels those number were 22.7 and 17.8 plants for one (H) and two (HH) rice plants per pot, respectively. For both infestation levels with these plant combinations, the emerged numbers were corresponding to
the data of a rice pot experiment with changing *S. asiatica* infestation levels of Kabiri (2013, unpublished). However, for rice-weed (HW) mixture, plant numbers of emerged *S. asiatica* were only a share of two-fifth of the expected numbers, based on previous pot experiments (2.2 and 8.2 emerged *S. asiatica* for low (d1) and high (d2) infestation, respectively). It might be that the roots of *M. villosus*, as a non-host plant, were accountable for the low number of emerged *S. asiatica*.

**Possible influence of non-host root system on *S. asiatica* emergence**

The introduction of a non-host plant (*M. villosus*) might inhibit the optimal development of the expected increasing rice root system, since the non-host plant might be a strong root competitor for space and nutrients, and both are in a bordered system of a 6 L pot. Consequently, this will result in less well developed root system of the host and thus a lower emergence of the parasitic plants.

The emergence of *S. asiatica* might be interfered by the root system of *M. villosus*. *M. villosus* might occupy the space around the rice roots and hinder *S. asiatica*’s radicle to find the host roots, or form attachments. Alternatively, it might be possible that the expanding root system of *M. villosus* is able to disrupt the newly formed xylem-to-xylem connection, since they are known to be fragile.

In this study, due to a lack of time, root systems were not cleaned, analysed and determined. It is recommended to do further research on the influence of non-host root systems on the emergence of *S. asiatica*, and analyse the root systems. As a non-host root system seems to inhibit the negative effect of the parasite on the host, a rice intercrop with a non-host crop might be a practical solution to reduce the host yield decrease due to *S. asiatica*. Studies with finger millet and maize, in *S. hermonthica* infested fields, showed that an intercrop with the fodder legumes of the genus *Desmodium* and the food legumes *Crotalaria, groundnut* and mung bean had a reducing effect on *Striga* infestation, and positively influenced crop yield (Midega et al., 2010; Midega et al., 2014).

**Rice-weed competition**

The relative, above soil yields of rice and *M. villosus*, can be used as an indication for the competition between both plant species. Relative yield of a particular crop ‘a’ (RY<sub>a</sub>) is calculated as; the yield of crop a in mixture with crop b (Y<sub>a,mix</sub>) divided by the yield of crop a as monocrop (Y<sub>a,mono</sub>), so RY<sub>a</sub> = Y<sub>a,mix</sub>/Y<sub>a,mono</sub>. The calculation of the RY of crop (b) is similar. Regarding the data for rice and *M. villosus* without *Striga* infestation, RY of rice was 0.49 and RY of *M. villosus* was 0.51. It can be concluded, that the original assumption, that three plants of *M. villosus* would have the same competitive ability as one rice plant was correct. At low density of *S. asiatica*, rice had a RY of 0.52 and *M. villosus* 0.53. At high *S. asiatica* density RY’s of rice and *M. villosus* were 0.57 and 0.50, respectively. Although, an increasing parasite density did not change the rice biomass when it was grown in the rice-weed mixture, there was a decrease in rice biomass, when it was grown as monocrop. This explains the slight increase in the RY of rice.
4B. DISCUSSION –Rhamphicarpa–

The effect of R. fistulosa on M. villosus and rice biomass

In the Rhamphicarpa study a decrease in biomass of rice and the biomass of the ordinary weed, was expected when grown in mixture in the presence of the parasitic weed. The expected decrease occurred for rice already at low density (d1) of R. fistulosa, with 67% less biomass. And was even more severe at high density, with 81% less biomass. These findings are in line with previous field and pot studies (e.g. Raynal-Roques, 1994; Ouédraogo et al., 1999; Rodenburg et al., 2011a; Pflug, 2013, unpublished). For M. villosus the decrease in biomass became visible at high parasitic density (d2), resulting in 20% less biomass for the ordinary weed. This biomass decrease at high parasitic density was in line with observations done in the Tanzanian field trial.

From the results of total pot biomass in the rice-weed mixture it was learned that R. fistulosa took a large share of the total biomass for its account. However, compared to un-infested pots, total pot biomass in the rice-weed mixture was less when infested with R. fistulosa. As suggested in the study of Pflug (2013, unpublished), the disturbance in the host photosynthesis after parasite infection might be responsible for the difference in total pot biomass between pots with and without infestation of R. fistulosa.

R. fistulosa biomass

In line with prior research on R. fistulosa, the parasites’ biomass was significantly less when it did not rely on a host (e.g. Ouédraogo et al., 1999). Nevertheless, at high density, R. fistulosa was able to negatively affect the biomass of M. villosus, even without being attached to a host plant. Even though in the current study the final sampling occurred before flowering, it may be assumed that R. fistulosa is able to complete its life cycle without a host and disperse seeds (Ouédraogo et al., 1999). Consequently, it might be assumed that even without host presence, R. fistulosa is able to sustain, or even may increase, over the seasons. This is confirmed by the results of a repeated survey of inland valleys in Benin in 2007 by Rodenburg et al. (2011) where an increase of infestation level of R. fistulosa over 9 years was found.

On the other hand, R. fistulosa biomass was negatively affected by an increased plant number of M. villosus (Figure 13). These data suggest that crop rotation with a highly competitive non-host, sown at high density, might be able to decrease R. fistulosa infestation over years.

Biomass of R. fistulosa was not affected by host plant density. This was in line with the study of Pflug (2013, unpublished), where rice of different ages (and so biomass) was infested by R. fistulosa. This suggests that, rather than source strength, the sink-strength is leading for a R. fistulosa infection.

Rice-weed competition

In the rice-weed mixture relative yields of both rice and M. villosus decreased over an increasing density of R. fistulosa. RY’s decreased from 0.6 and 0.5, respectively for rice and M. villosus in the absence of the parasite (d0) to 0.5 and 0.4 at high density (d2) of R. fistulosa. It might be suggest that the increasing competitive ability of the parasite, due to the additional
dry matter gain of the attached parasite on its host, was responsible for the decrease in relative yield, and thus suppressed the rice-weed competition.

*M. villosus*, the right ordinary weed species?

*M. villosus* behaved different in the *Striga* experiment as in the *Rhamphicarpa* experiment. In the *Striga* experiment there was no biomass difference measured between one (W) or two (WW) plant units per pot. Whereas in the *Rhamphicarpa* there was a significant lower biomass of *M. villosus* in pots with one (W) weed unit, implying that the intraspecific competition of the weed was less in the *Rhamphicarpa* experiment. Possible explanations might be a shorter period of growth and the suboptimal (more moist) water conditions in the *Rhamphicarpa* experiment (compared to the *Striga* experiment). Under natural circumstance *M. villosus* thrived in dryer upland environments (Verdcourt 1976; Neumann et al., 1989). An ordinary weed, more adapted to the more moist lowland conditions, may be an option for a new pot experiment.

However, the total host range of *R. fistulosa* is not yet known (Ouédraogo et al., 1999; J. Rodenburg personal communication). It is of great importance to test whether an ordinary weed (or other plant species) is a possible host of *R. fistulosa*. For example *Ammannia auriculata*, an ordinary weed in lowland rice cultivation (Graham, 1979), and very common throughout Tanzania, tends to be a host for *R. fistulosa*. Since *A. auriculata* showed difficulties with emergence and development at pot level, an experiment was set-up with a range of different hydrological conditions; upland, lowland, flooded, upland + flooding after emergence, lowland + flooding after emergence. Seeds of *R. fistulosa* were added in the same pots to observe its development under different hydrological conditions. In pots where both *A. auriculata* and *R. fistulosa* emerged, the growing patterns differ from those pots where only one of the species develop as a sole crop. *R. fistulosa* developed much taller when it grew together with *A. auriculata*. Meanwhile, the latter grew smaller, and even died in a pot at upland moist conditions. The pictures that shows the differences in plant height, and the xylem-to-xylem connections after cleaning the root systems of *A. auriculata* and *R. fistulosa* can be found in Appendix 2.
5. CONCLUSIONS

An observed change in biomass accumulation of the ordinary weed community in rice fields infested with *S. asiatica* and *R. fistulosa* in Tanzania was the driving factor to set-up pot experiments, to understand to what extent the ordinary weed biomass would change in the presence of two different parasitic weeds. As observed in the field, *S. asiatica* infestation was beneficial for ordinary weeds, as a response to the decrease in rice biomass, due to the parasitizing effect, ordinary weeds could increase their biomass. Fields infested with *R. fistulosa* showed for both, rice and the ordinary weed, a suppressed biomass production, suggesting that *R. fistulosa* develops in a strong competitor when it is parasitizing its host.

*S. asiatica*

In a rice-weed mixture, the ordinary weed *M. villosus* did not increase in biomass under an increasing density of the rice parasitic weed *S. asiatica*. Since rice biomass in the rice-weed mixture was not affected by the parasite, the distribution of rice and ordinary weed biomass did not change at an increasing parasite density. Late overall emergence, and a high variation in biomass of *S. asiatica* might be partly responsible for this. On the other hand, the interference of the root system of the ordinary weed with the root system of rice might have inhibited the possible *S. asiatica* emergence. Additional research focussing on the influence of non-host root systems on the emergence of *S. asiatica* is recommended. The possibility of intercropping rice intercrop with a non-host plant to supress *S. asiatica* infection should also be taken into consideration.

*R. fistulosa*

Similar to what was observed in the rice field, biomass of the ordinary weed *M. villosus* was reduced in the pot experiment. In a rice-weed mixture, at a high *R. fistulosa* infestation, there was a significant decrease of the *M. villosus* biomass. Since *R. fistulosa* could take advantage of the host plant to compete against the ordinary weed, the biomass of the ordinary weed decreased with 37%. A decrease in ordinary weed biomass also happened when *M. villosus* grew in monoculture. For pots with one weed unit, biomass decreased with 25%. Pots with the double plant number of ordinary weeds encountered a decrease of 20%, when grown at a high *R. fistulosa* density. Therefore, a high field density of *R. fistulosa*, even without a host plant, is a serious pest, since the parasitic weed competes with other plants and completes its life cycle even in the absence of a host.

The existence of a broader host range makes the *R. fistulosa* problem even more serious. *A. auriculata*, a common weed in Tanzanian lowland rice fields, can be considered as a newly discovered host plant for *R. fistulosa*, since the parasite showed enlarged development in growth and seed capsules, when grown together. These alarming observations stress again the need for more research on this devastating pest.
REFERENCES


APPENDIX 1

Schematic overview of the layout of the pot experiments carried out in the greenhouse of Wageningen UR. First scheme is for the *S. asiatica* experiment and the second belongs to the *R. fistulosa* experiment.

For both schemes, left: The full experiment with corresponding dimensions. Right: A close-up of one replicate with the corresponding dimensions on pot level.

Codes should be interpret as follows: Characters, **HH**= Host + Host (rice), **HW**= Host + Weed (*M. villosus*), **WW**= Weed + Weed, **H**=Host, **W**= Weed, **C**= Control (*R. fistulosa*, only in *Rhamphicarpa* experiment). Digits, **0**= No parasitic infestation, **1**= Low parasitic infestation, **2**= High parasitic infestation.
APPENDIX 2

Pictures of the possible parasitic interaction between *A. auriculata* and *R. fistulosa*.

Figure 17. Three photos of pots with different combinations of *A. auriculata* and *R. fistulosa*. *A. auriculata* is indicated with red arrows. Height of *R. fistulosa* per pot is indicated with a white bar. **Upper photo** (upland); *R. fistulosa* only (left), *R. fistulosa* with *A. auriculata* (right). **Middle photo** (flooded); *R. fistulosa* only (left), *R. fistulosa* with *A. auriculata* (middle), *A. auriculata* only (left). **Bottom photo** (flooded); *R. fistulosa* with *A. auriculata* (left), *R. fistulosa* only (middle), *R. fistulosa* with *A. auriculata* (right).
Figure 18. Cleaned root systems of *R. fistulosa* (*Rf*) and *A. auriculata* (*Aa*). Where the photos at the right side are a close-up of the photos left. Xylem-to-xylem connections are indicated with the red circles. Note the difference in root colour, white/purple for the *R. fistulosa* roots and brown/yellow for the *A. auriculata* roots.