Two cultivars of *Sorghum bicolor* in relation to *Striga hermonthica*

The effect of moment of attachment of *Striga hermonthica* on the development and biomass production of a sensitive and a tolerant cultivar of *Sorghum bicolor*

And

Root morphology of a sensitive and a tolerant cultivar of *Sorghum bicolor*

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Acknowledgements

The work presented here is just a small part of a bigger whole. For me it is the report of my experiments. For Aad van Ast, my supervisor, it is part of his PhD-project. For the people in Africa, it is, I hope, just a very small step towards a solution for the Striga-problem.

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During my practical period in Burkina Faso, I saw Striga and the problems it causes. In this way I know the significance of the problem. I liked to work on these experiments, although it was not always easy. The experience of working on a significant problem was a big motivation: if the results are interesting, it can be used to solve, or at least understand more of the Striga problem.
Abstract

Some *Striga* spp. behave like parasitic weeds, which can be very damaging for the host plant. The most common hosts for *Striga* are sorghum, millet, maize, rice and cowpea. *Striga* is found in tropical environments, mostly semi-arid areas. Due to the growing demand for food and the shortage of good arable land, the problem of *Striga* increases.

*Striga* is a hemi-parasite, which attaches itself to the roots of the host plant and withdraws water, assimilates and minerals from it. However, the problem is not only this extraction, the reduction in growth is bigger than can be explained by this. *Striga* has a “toxic effect” on the host plant; it interferes with the water-balance and with the photosynthesis of the host.

To find a solution for the problem of *Striga*, much research is still to be done. This research has two sides; on the one hand research is done on *Striga* itself, to find a way to combat it. On the other side, research is done on the host plants, to find a way of decreasing the reduction in growth of the host plant. The research described in this report is focussed on the latter.

A very important event in the life cycle of *Striga* is, of course, the attachment of *Striga* on the host. One of the hosts *Striga* attacks is sorghum. It is known that there are cultivars of sorghum that are more ‘tolerant’ to the attack of *Striga* than other varieties. The effects of *Striga* on these cultivars are rather small. To understand and reduce the problem of *Striga*, we want to know what mechanism(s) cause(s) that tolerance. In this report, two experiments are described.

In the first experiment the development of the sensitive and the tolerant host plant, both attacked by *Striga*, was compared. The aim of the research was to find out whether the moment of infection can (partly) explain *Striga* tolerance of the sorghum cultivar Tiemarifing.

In order to do that, the moment of infection was manipulated artificially: sorghum plants were ‘infected’ with preconditioned *Striga* seeds at the day of sowing of sorghum and one, two and three weeks later. The *Striga* sensitive sorghum plants that were ‘infected’ earlier, appeared to develop very slowly and biomass production was low. For all 4 different moments of infection, biomass production was much lower than that of the uninfected plants. Plants with *Striga* application at a later moment showed a better growth. The grain production of plants infected in the first two weeks after sowing was very low, plants infected after three weeks showed a better grain yield. *Striga* tolerant plants did not show this effect; there was no significant difference in growth between plants not infected and those infected at the different moments.

*Striga* plants that grew on the sensitive host produced more biomass than *Striga* on the tolerant host plant. The later the moment of application of *Striga* seeds, the faster the development of *Striga* plants. *Striga* that came into contact with a host plant seemed to ‘compensate for’ the delay; a lot of *Striga* plants developed and emerged. However, biomass production of *Striga* was lower, with a later infection moment.

In the second experiment morphology of the root systems of the two cultivars of sorghum, tolerant and sensitive, was studied. The assumption was that there would be a difference in morphology, which was (partly) responsible for the observed difference in time of attachment of both cultivars. A significant difference in root morphology was observed between the root system of a *Striga* tolerant and that of a sensitive sorghum plant. The sensitive plant started to produce a main root first, which grew in deep layers of the soil, while the root system of the tolerant cultivar immediately started to branch in the upper layers of the soil. The sensitive cultivar showed a strong reaction on infection with *Striga*: plants started to produce a lot of roots in the upper layers of the soil.
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In this first section a brief introduction on the problems around *Striga* is given. This introduction consists of three parts; one on the general agricultural problems faced in areas where *Striga* is prominent, the second on the physiology of *Striga* species and their life cycle and the last part on the possibilities to control *Striga*.

**Striga, an increasing problem**

Due to a growing demand for food, resulting from a growing population and a higher living standard for some people, agriculture in third world countries is faced with major problems. The increasing demand for food must be met by enlarging the total area (hectares) for agriculture or by intensification. Since in practice almost all of the area suitable for agriculture is already incorporated in the agricultural system, the only option left is the intensification process.

One of the main problems is that resulting from intensity, fallow periods get shorter and shorter all the time until they finally disappear completely. This intensification causes a reduction in soil fertility and is not sustainable in the long term. Also other problems will start to play a more important role. Pests, diseases and weeds are examples of this. One of the most important reasons for the declining production in the intensive production systems is the increasing pressure of parasitic weeds of which *Striga* spp. form the most important group. *Striga* spp. mainly attack cereals in the semi-arid parts of Africa and are also found in the western part of Asia, India incorporated. Most species attack monocotylic species but some are found on dicotyledeons as well (Press, 1995).

*Figure 1.1: Striga hermonthica causing the complete failure of early sown sorghum in Eritrea, while later sown crops in the background are less affected. (From: Parker and Riches: Parasitic weeds of the world; Biology and control, 1993)*
Striga infestation of cereals is a major problem in Africa, where approximately 21 million hectares are infested on a total area of 73 million hectares of arable land. The estimated loss of grain yield is up to 4.1 million tons (Press, 1995). There are about thirty species of Striga of which about ten species are important pests. Three species are very important in that they cause high economical losses. These species are S. hermonthica, S. asiatica and S. gesneroides. S. hermonthica and S. asiatica mainly attack cereals such as sorghum (figure 1.1), millet, maize and rice, while S. gesneroides parasitizes dicotyledons such as cowpea (Parker and Riches, 1993). Striga is a devastating species in many places in Africa but especially along a fringe below the Sahara. In this region the soils are generally poor and drought is more frequent. Particularly, it is difficult to eradicate a Striga-infestation from the soil once it is established, human activity has been identified as the biggest cause of spreading of seeds from field to field.

The life cycle of Striga

Striga belongs to the family of Scrophulariaceae. The genus Striga is characterized by opposite leaves, irregular flowers with a corolla divided into a tube and spreading lobes, herbaceous habit, small seeds and parasitism. The flowers are pink, red, white, purple or yellow (Ejeta and Butler, 1993). The life cycle of Striga is shown in figure 1.2.

A Striga plant can produce thousands (40,000-90,000) of very small seeds (about 0.4 mm). They may have a longevity of many years, depending on environmental conditions. Seeds stay dormant for several months. They are mostly situated in the surface-layer of the soil (Press, 1995).

The seeds are not capable of germinating the moment they are produced; it takes some time before the primary dormancy is broken. With this process we can distinguish the first step in the life cycle of the Striga: the after-ripening (Press, 1995).

After breaking of primary dormancy, the seeds are not yet able to germinate. The second step we distinguish, is the conditioning of the seed. The seeds are conditioned to increase the permeability of the seed coat to water and gases. Conditioning increases the sensitivity of the seeds to signals of the host (the root-exudates of the host-plant). These signals indicate the presence of the host and the distance and direction to that host (Press, 1995). Conditioning requires suitable temperatures (25-35°C) and adequate soil moisture conditions (van Delft, 1997); in fact it is an indication of the start of the rainy season.

After conditioning, the seeds are ready for germination. The roots of a potential host plant produce 'germination stimulants', which induce the germination of the Striga-seeds. The germination stimulants are exuded in a region of 3 to 6 mm from the root apex and induce germination of seeds when they are not more than about 4 mm away from the root surface. It has been found that host plants produce a mixture of stimulatory compounds, with one that is predominant over the others. These compounds are very active and are present in root exudates at extremely low levels. The main natural germination stimulants are 'strigol', 'sorgoleone' and 'sorgolactone'. The first seems to be an exudate of maize, the other two of sorghum. Strigol can be synthesized since 1974 (Parker and Riches, 1993). Because of the small seed reserves of Striga, germination is a very critical step in the life cycle; it is necessary for a seedling to attach itself to a host within a few days after germination.
Figure 1.2: The life cycle of Striga (from: the home page of Plant Ecology, VU Amsterdam).
Striga uses a chemical, produced by the host root, for **haustorial induction**. The radicle of the Striga seedling grows towards the host root and at the point of contact, the tip of the radicle swells (Ayensu et al., 1984). The radicle starts to produce hairs (resembling root hairs) which ‘glue’ the root to the host.

The haustorium acts as a morphological and physiological bridge between the host and the parasite. Simple compounds, produced by the host root are the primary signal that induces haustorial development and **attachment** to the host (van Delft, 1997).

If the host is suitable, the **penetration** stage starts: the haustorium forms a link with the host vascular system (xylem). Penetration partially takes place by cell wall degrading enzymes, produced by the parasite, and partially by enlargement of intrusive cells that push away host root cells. *Striga* forms secondary roots, which, by means of secondary haustoria attach the same or other nearby roots. At this (underground) stage, the parasite is completely dependent on host resources for growth.

The **seedling development** starts underground. During this time it is completely dependent on its host and causes the most extensive damage to the host plant. It exerts a powerful influence on the growth-regulating systems of the host by altering hormonal balances, resulting in stimulation of root production, decrease of photosynthesis and decrease of dry matter production. (Press, 1995).

**Emergence**: after about 4-6 weeks, the parasite emerges above ground. It produces stems and leaves with chlorophyll and becomes a hemi-parasite, which produces some of the required assimilates itself (about 30%). The parasite remains dependent upon water and minerals from the host (Press, 1995).

The last stage in the life cycle of *Striga* is the **flowering**, which occurs within one month after emergence. The *Striga* produces seeds, which mature 2-4 weeks after pollination. With this last stage, the life cycle of *Striga* is completed (Press, 1995).

**Control of Striga**

There are three factors that cause the *Striga* problem to be persistent and difficult to solve:
1. The *Striga* life cycle is difficult to disrupt by control methods
2. The *Striga* problem is usually embedded in a range of problems that farmers have. A big problem is often the lack of financial means, which results in a lack of agricultural means.
3. Damage to the crop is already caused when the parasite is still underground, so many control methods aimed at killing the emerged *Striga* plant will not prevent loss in crop production (van Delft, 1997, Pieterse, 1994).

However, there are several existing and proposed *Striga* control methods:
- One of the traditional methods of weed control is hand weeding (figure 1.3). This method requires much labour and, as indicated above, can not reduce much of the damage. However it is useful in the long term *Striga* control, because it prevents the build up of a *Striga* seed bank in the soil, if the *Striga* is weeded before seeds are produced (Parker and Riches, 1993).
Figure 1.3: *Striga hermonthica* being hand-pulled by a farmer in Ethiopia (From: Parker and Riches: Parasitic weeds of the world; Biology and control, 1993).

- Application of herbicides has proven not to be a very great success. Many herbicides affect the crop as well as the parasite. Apart from that, resistance may evolve in parasitic weeds within a few years (van Delft, 1997).
- High levels of nitrogen fertilizer can help to reduce the harmful effects of *Striga*, but do not kill the parasite (van Delft, 1997).
- In theory, synthetically produced root exudates can be used to induce ‘suicidal germination’ of the *Striga* seeds. The main obstructions appear to be high costs for production of these synthetic exudates (van Delft, 1997).
- ‘Trap crops’ are plants that release root exudates that can cause germination of *Striga* seeds, but are not parasitised themselves. These plants can be used in rotation or mixed cropping systems with the sensitive crop. However, in dryer area’s it is not very accepted by farmers because of the small benefit of the trap crops (Kim, 1991).
- Soil fumigation is a very effective but expensive method to kill *Striga* seeds (van Delft, 1997).
- Fallow periods, crop rotation and mixed cropping are traditional agronomic practices that can control *Striga*, but they may not be adopted anymore by farmers for economic reasons; in a fallow period there is no production (Kim, 1991; Parker and Riches, 1993).
- Considerable efforts have been made by researchers and crop breeders to develop partially resistant or tolerant varieties (van Delft, 1997). The first chapter of this report deals with this subject.
Chapter 1

The effect of moment of attachment of *Striga hermonthica* on the development and biomass production of a sensitive and a tolerant cultivar of *Sorghum bicolor*.

**Introduction**

Once the *Striga* is attached to the roots of its host, it might cause much damage to the host plant. The first mechanism through which *Striga* affects the host plant is the extraction of nitrogen and assimilates from it. However, this seems to be not the most important effect. About 20% of the growth reduction of the host can be explained by the simple removal of substances. Reduction in the production of assimilates is a far more important factor, estimated to be responsible for about 80% of the growth production of the host (Press, 1995). The mechanisms underlying this reduction are not completely clear. Figure 1.4 shows a diagram of the relations that are assumed to be important. *Striga* is known to influence the hormonal balance (ABA, cytokinins) of the host, which in turn causes a decrease in chlorophyll-concentration.

The symptoms, which are shown by the infected host plants, include stunting, wilting and leaf chlorosis. Growth of the root system is being stimulated by the parasite, while shoot growth is reduced, which results in a strong decrease in shoot/root ratio. Although, the underlying processes taking place within the host plant are still not completely clear.

However, not all sorghum plants show such a strong reaction on *Striga*. There are cultivars that show defense mechanisms against *Striga*.

This experiment focussed on the effects of *Striga* on the host plant investigation. The mechanism(s) of host plant defense is an interesting subject. This is still not completely found out by researchers. For a good understanding of this subject, some terms, used in the previous part, will be explained. There are four different types of mechanisms:

- **Low stimulators** have a low production of germination stimulants. This is also called immunity. There are no emerged *Striga* plants and even no attachments. Immunity is not found in practice, it would not be durable because only a few genes are responsible for it.
- **Plants** that are (partly) resistant reduce the growth and vigour of *Striga* after attachment. The number of attachments, although, is lower than on the susceptible sorghum plant.
- **Tolerance**: the number of *Striga* attachments on the sensitive and on the tolerant plant is more or less the same. The tolerant plant is not suffering much from the intoxination effect (Ejeta, 1993; Parker and Riches, 1993).
- **Avoidance** of contact between the host roots and the *Striga* seeds. This can be affected by e.g. development of a root system that grows very deep, under the layer of *Striga* infected soil.
Photosynthesis parameters

Photosynthesis

Assimilates

Respiration

Photosynthetically active radiation

Intercepted radiation

Green leaf area

Dead leaf area

Leaves

Stems

Roots

Grain

Striga

1. Reduction in photosynthetic rate

2. Increase in maintenance respiration

3. Altering assimilate distribution

4. Reduction in light interception

Figure 1.4: Relational diagram of the model, showing the various ways through which Striga can affect the growth rate of a sorghum crop (van Ast, unpublished, 1998).
At the Laboratory of TPE, an experiment was conducted in which the development of a sensitive and a tolerant cultivar of sorghum (CK-60 and Tiemarifing, respectively), attacked by *Striga*, were compared. The aim of this experiment was to proof a difference in moment of attachment between a tolerant and a sensitive cultivar of sorghum. The development of the host and the parasite was investigated during the first 5 weeks after sowing the sorghum. A clear difference between the two cultivars in moment of attachment of *Striga* on the host roots was found. *Striga* attached to the tolerant cultivar ten days later than to the sensitive one. This difference of ten days in moment of attachment resulted in a delay of emergence of 18 days of the *Striga* on the tolerant cultivar. At the same time *Striga* was found to have a smaller effect on weight, length and development of the tolerant sorghum cultivar.

The question that remained from this experiment was whether the smaller effects of *Striga* on the tolerant cultivar was completely caused by real tolerance mechanism(s), or that this reduction was partly or completely caused by the delay in attachment. The present experiment was conducted to separate the effect of real tolerance mechanisms and the effect of a delaying attachment in the tolerant cultivar. Or, to put it differently, the question was asked whether there is a functional relation between the delayed attachment of *Striga* on the tolerant cultivar of the sorghum and the tolerance of that cultivar. In order to do that, the time of attachment was artificially delayed.
**Materials and Methods**

**Materials**

The experiment was executed in a greenhouse, using the following materials:

- The plants were grown in square pots of 12 liter, with a surface of 25x25 cm and a depth of 20 cm.
- The pots were filled with a mixture of soil: sandy arable soil and white sand (1:3).
- The nutrient requirement is answered by fertilization: 0.41 g Urea, 1.25 g Patentkali and 0.93 g Triple Superfosfaat per pot comparable to 60:30:50 (N:P:K kg/ha), applied as surface-dressing at 20 days after emergence.
- The temperature in the greenhouse was about 30°C during daytime and 20°C during night.
- RH: 70-80%. Water was given every two days or every day, considering the needs of the plants.
- In the greenhouse a day/night regime of 11/13 was maintained; the greenhouse was kept dark with black screens between 19.00 h PM and 8.00 h AM.
- For plant protection biological control of ‘red-spider mite’ and ‘trips’ was applied.
- 2 cultivars of *Sorghum bicolor* were used for the experiment: the highly Striga-sensitive inbred cultivar CK60 B (origin: 96006 Nov ’96 ICRISAT) and the Striga-tolerant local Malian landrace Tiemarifing (origin: 96005 Nov ’96 ICRISAT). 3 Pre-germinated seeds were sown at a depth of 1.5 cm in each pot. After emergence the sorghum was reduced to 1 plant per pot.
- *Striga hermonthica* seeds: 20 mg or 4900 seeds mixed through the upper 9 cm of the soil (to obtain a density of 5.7 seeds per cm³). Origin *Striga* seeds: 95016; harvest 1995 Samanko, Mali. Percentage of germination February ’99: 83%.
- At the destructive harvests, plants were harvested and dried in an oven at a temperature of 70°C for at least 24 hours to determine the dry weight.

**Experimental design**

**Treatments:**

* 2 cultivars of sorghum: Tiemarifing and CK-60
* With or without *Striga* infestation: application of *Striga hermonthica* seeds at 4 different moments, to obtain 4 different moments of *Striga* infection:
  1. Immediately at sowing the sorghum (natural situation)
  2. 7 days after sowing the sorghum
  3. 14 days after sowing the sorghum
  4. 21 days after sowing the sorghum
* 4 replicates (=blocks). Within each block each treatment was represented with 1 pot.

A split-plot block design with four blocks was used, the main plot (block) was divided in two, by the different cultivars. Within these two blocks, the position of pots with different treatments was chosen randomly. To assure regularity (in the greenhouse there is always the problem of local differences in incoming radiation and temperature) and statistical justification, four blocks were used.
The delay of the moment of attachment was achieved by growing the plant in a pot, filled with *Striga*-free soil, surrounded by 4 buried plastic cups (see Appendix I). These cups were initially filled with *Striga*-free soil. 14 Days before the desired moment of seed application, the cups were removed and the soil in the cups was mixed with *Striga* seeds. After that, the cups were returned at their position in the pots. During 14 days, the *Striga* seeds were allowed to condition in the moist soil. After which the cups were removed from the pot and the *Striga* infected soil was thrown back in the holes, left after removal of the cups. From then on, the *Striga* seeds were potentially able to infect the roots of the host plant.

The first 'infection' of the host plant with *Striga* was simultaneously with the sowing of the sorghum, the *Striga* infected soil was released from the plastic cups in the first group of plants. For the other treatments, the moment of *Striga* application was delayed with one, two or three weeks. The time plan of the experiment is explained in figure 1.8.

To exclude a possible effect of the cups in the soil, the uninfected plants had cups also, however without *Striga* seeds. The soil in these cups was thrown back in the holes at the same time as in the infected pots. However, no significant effect of the cups was found after the experiment.

**Harvests:**

* 4 moments of major harvest:
  1. 4 weeks after sowing the sorghum
  2. 6 weeks after sowing the sorghum
  3. 8 weeks after sowing the sorghum (at 50% flowering of the sorghum/ end of the vegetative stage)
  4. After ripening of the sorghum seeds (end of the life cycle)

These major harvests were conducted mainly to investigate the growth and biomass production of sorghum. They were executed at 4, 6, 8 and 12 (Tiemarifing) or 13 (CK-60) weeks after sowing of sorghum (figure 1.8). Final harvest was conducted at 90 days after sowing of sorghum, that is the time the sorghum needs to mature.
* 3 small harvests for following the *Striga* development
  1. 3 weeks after sowing the sorghum...m1
  2. 5 weeks after sowing the sorghum...m3+m1
  3. 7 weeks after sowing the sorghum...m3

These small harvests were conducted, mainly to give extra information about the growth of *Striga*. However, only plants infested with *Striga* seeds were harvested at that time. The small 'intermediate' harvests were executed 3 and 5 weeks after application of *Striga* seeds, for the moments 1 and 3 (figure 1.8).

**Size of the experiment:**
With these treatments, \((2 \times 2 \times 4 \times 4 \times 4) + (2 \times 1 \times 2 \times 2 \times 4) = 288\) pots were needed for the experiment. To create conditions as equal as possible for each plant, border rows are used. In total 240 plants in pots form the border rows, these plants were not infected with *Striga*.

![Figure 1.6: Experimental set-up of the experiment in the greenhouse in Wageningen.](image)

The blocks were situated about 2 m from the side of the greenhouse and were surrounded by border rows. Also between the different treatments there were border row plants to minimize the influence of unwanted external factors (temperature and radiation) within the blocks and between treatments.

![Figure 1.7: Experimental set-up of one block of the experiment.](image)
Figure 1.8: Time plan of the experiment.
Observations and measurements

Observations made during the whole life cycle:
- Photosynthesis was measured every week, for 5 weeks, starting in week 4. Photosynthesis measurements were carried out with an ADC leaf-photosynthesis meter, which measures gas exchange. The measurements were done at a CO₂ level of 350 ppm and an incoming radiation of 393 W m⁻² (1958 umol/m²/s), which was provided by a special lamp fixed onto the ADC meter. For every plant, the photosynthetic rate of the youngest, fully developed leaf was measured. A square leaf chamber was used with an area of 6.25 cm². The temperature was 25°C, at each measurement. Plants were given at least 10 to 15 minutes to adapt to the conditions of the measuring room.

Figure 1.9: The set-up of the equipment to measure photosynthesis.

- The appearance of leaves of sorghum was registered by counting the number of leaves 2 times per week.
- Every two days, the number of emerged Striga plants was counted.
- The time of flowering of the Striga plants was registered.

Observations made at (destructive) harvests:
- The dry weight of the aerial plant parts of sorghum was determined: leaf, stem, inflorescence and grain (only at the last harvest).
- The length of the stem was measured.
- The total leaf area was measured.
- The dry weight of the roots was determined.
- Striga dry weight was determined.
- The number of Striga attachments on the sorghum roots was counted.
The destructive harvests were divided in major (1-4) and intermediate (x-z) harvests (figure 1.8). The moments of major harvests were chosen at 4, 6 and 8 weeks after sowing the sorghum and the final harvest after ripening of the grains. The major harvests were meant to give in the first place information about sorghum.

For the intermediate harvests, only plants, infested with *Striga* seeds were taken. The moments of the intermediate harvests were done 2 and 4 weeks after infection of sorghum with *Striga* (only for moments 1 and 3). These intermediate harvests gave especially information about *Striga*.

Data processing

Data were collected in a database in Excel 97 and statistically analyzed.
Results

Striga growth

Figure 1.10: Number of attachments of Striga found on the roots of sorghum cultivar, a. CK-60 and b. Tiemarifing, plotted against time after sowing the sorghum, for four different moments of application of Striga seeds to the soil: 0(■), 1(▲), 2(●), and 3(★) weeks after sowing of sorghum.
Figure 1.10a shows that *Striga*, applied to the soil at moments 3 and 4 (2 and 3 weeks after sowing of sorghum) reached a higher number of attachments on CK-60, than *Striga* applied at moment 1 and 2 (0 and 1 week after sowing of sorghum). The number of *Striga* attachments on sorghum decreased after some time.

In figure 1.10b can be seen a similar effect as in figure 1.10a; *Striga* applied at moments 3 and 4 reached a higher number of attachments on Tiemarifing than *Striga* applied at moments 1 and 2. However, there was no reduction in number of attachments at the end of the life cycle of sorghum.

If we compare figures 1.10a and b of the number of *Striga* attachments found on the two sorghum cultivars, two remarking things can be seen. On Tiemarifing, *Striga* attachments were found about 3 weeks later than on CK-60: week 6, versus week 3 on CK-60. After this late attachment, the number of attachments increased very fast on Tiemarifing. At final harvest, there was no significant difference between the number of *Striga* attachments on the two cultivars.
Figure 1.11: Number of Striga plants emerged on sorghum cultivar, a. CK-60 and b. Tiemarifing, plotted against the time after application of pre-conditioned Striga seeds in the soil, for four different moments of application: 0(■), 1(▲), 2(●), and 3(*) weeks after sowing of sorghum.
Figure 1.11 shows the number of emerged Striga plants, plotted against time after application of Striga seeds. This scale is different from that of figure 1.10, in 'days after infection with Striga' in stead of in 'days after sowing of sorghum' in order to show clearly the difference in development of number of attachments between the different moments of Striga seed application.

In figure 1.11a can be seen that the Striga plants had a fixed moment of emergence (about after 25-30 days after application) on CK-60. The later the moment of application of Striga seeds, the more Striga plants emerged; Striga applied 3 weeks after sowing of sorghum showed a higher number of emerged plants than Striga applied at sowing of sorghum. However, the pattern is not very clear, Striga applied after 3 weeks showed a lower number of emerged plants than Striga applied after 2 weeks.

Another observation is that with a later moment of application, the number of emerged Striga plants increased and the time-interval between two plants emerging got shorter. In figure 1.11b a similar effect was found at Tiemarifing, however even clearer; Striga applied 3 weeks after sowing of sorghum caused a higher number of emerged plants than Striga applied after 2 weeks, followed by 1 and 0 weeks after sowing of sorghum. Striga on Tiemarifing showed also a more or less fixed moment of emergence (about 40-45 days after application).

Comparing figures 1.11a and b it can be seen that Striga on CK-60 emerged much earlier than that on Tiemarifing: 25–30 days after application of the Striga seeds at CK-60 versus 40-45 days at Tiemarifing. In general, the number of emerged Striga plants on Tiemarifing was lower than that on CK-60.

Figure 1.12: Biomass production of Striga on sorghum plants (Striga biomass per sorghum plant) cultivar CK-60 and Tiemarifing, for four different moments of application of Striga, at final harvest.

Figure 1.12 shows the mean biomass production of Striga on a sorghum plant. On CK-60, it becomes clear that the later the moment of infection, the less biomass Striga produced. There is a major difference between the different moments of application. On Tiemarifing, it is remarkable that the difference in Striga biomass between the moments of infection was small. The biomass production of Striga, did not differ significantly between the different moments of Striga seed application.
Comparing the two cultivars, it can be seen that *Striga* biomass production on Tiemarifing was far less than that on CK-60 (figure 1.12). With this result, the question arises, whether the difference of *Striga* biomass production on CK-60 between the different moments of application was caused by the difference in growing period or by a difference in growth rate. Figure 1.13 answers that question.

**Figure 1.13: Biomass production of Striga on sorghum cultivars, a. CK-60 and b. Tiemarifing, plotted against time after application of pre-conditioned Striga seeds to the soil, for four different moments of infection of Striga: 0(■), 1(▲), 2(●), and 3(★) weeks after sowing the sorghum. The dotted line shows the presumed development in time.**
In figure 1.13, *Striga* biomass production is plotted against time after application of *Striga*, in order to make clear the development of the *Striga* plants. On CK-60, it can be seen that *Striga* plants, with seed application at different moments, had still the same development; the different moments seem to form one line in figure 1.13, what would mean that they had more or less the same growth rate. However, the growth of *Striga* plants on Tiemarifing was low and the different moments did not seem to have the same growth rate (they do not seem to form one line in the figure).
Figure 1.14: Biomass production of sorghum cultivars, a. CK-60 and b. Tiemarifing, plotted against time after sowing the sorghum, for four different moments of application: 0 (■), 1 (▲), 2 (●), 3 ( *) weeks after sowing of sorghum and without ( • ) application of Striga seeds.
CK-60 (figure 1.14a) reacted very strongly on *Striga* infection and apart from that the moment of application was found to strongly influence the level of biomass production; sorghum plants with early application of *Striga* seeds produced very little biomass, plants infected later had a higher production. No significant difference was found between the first three moments of *Striga* application in terms of biomass production. Mean biomass production of plants of moment 2 was very high, according to the assumed regularity. This can possibly be explained by one replicate plant with a very high biomass, compared to the other 3 replicates; the effect of this can be seen in all results on sorghum. The difference between plants with *Striga* application 3 weeks after sowing of sorghum and plants without *Striga* was still significant.

For Tiemarifing (figure 1.14b) only a small difference in biomass production was observed between the plants without *Striga* and the infected plants. Also no significant differences were found between the plants of the different moments of infection. Comparing the two cultivars (figures 1.14a and b), it can be seen that the total sorghum biomass production of plants without *Striga* of CK-60 did not differ significantly from the biomass of Tiemarifing at final harvest. Although, the effect of different moments of infection with *Striga* is different for the two cultivars of sorghum.

![Image of sorghum plants with and without Striga application](image)

*Figure 1.15: The effect of different moments of application of Striga on sorghum cultivar CK-60 at final harvest. From left to right: “(natural) infection”, application at the day of sowing the sorghum and 1, 2 and 3 weeks later.*

In figure 1.15 the results for CK-60 are illustrated in a picture. Note that apart from the reduction in biomass, the development was affected. Only plants without *Striga* (right in the photo) and those in which *Striga* was applied 4 weeks after sowing of sorghum, an
Inflorescence was formed. Plants that were infested with pre-conditioned *Striga* seeds at the day of sowing were very small and had no inflorescence (left in the photo); the *Striga* plants in this pot were large and flowering.

**Figure 1.16:** Biomass production of different plant organs: kernels (■), shoot (■) and root (□) of sorghum cultivars CK-60 and Tiemarifing, for the four different moments of application and without application of *Striga*, at final harvest.

**Figure 1.17:** Biomass partitioning over the different organs: kernels (□), shoot (■) and root (□) of sorghum cultivars CK-60 and Tiemarifing, for the four different moments of application and without application of *Striga*, at final harvest.

For CK-60 the differences in grain yield were even stronger than the differences in total biomass; in absolute (figure 1.16), but also in relative figures (figure 1.17), the grain
production of CK-60 decreased dramatically due to application of *Striga* seeds. Plants with *Striga* application 3 weeks after sowing of sorghum produced significantly more grain than plants with application at 0, 1 or 2 weeks after sowing of sorghum. This is still a significantly lower amount compared to the plants without *Striga*. Differences between application moments 1, 2 and 3 were not significant. Biomass production of infection moment 2 was relatively high, due to one replicate plant with a very high biomass production. In Tiemarifing, no significant decrease in production of grain was observed. Biomass of shoot and root of both cultivars was also reduced due to *Striga* infestation, although differences were observed between shoot and root. Shoot rates were much more reduced compared to roots, resulting in relatively low shoot/root ratios in infected plants.
Figure 1.18: Leaf appearance of sorghum cultivars, a. CK-60 and b. Tiemarifing, plotted against time after emergence of sorghum, for four different moments of application: 0(■), 1(▲), 2(●), 3(☆) weeks after sowing of sorghum and without(♦) application of Striga. The flag-leaf is the last leaf before flowering, this is the last leaf that is observed.

The effect of the different moments of Striga application on leaf appearance of CK-60 can be seen in figure 1.18a; CK-60 plants appeared to produce more leaves when Striga seeds were applied at a later moment. CK-60 showed a significant reaction to the different moments of infection. Tiemarifing did not show a significant difference in leaf appearance between the different moments of Striga application. Comparing figures 1.18a and b it can be seen that CK-60 produced significantly more leaves. It also becomes clear that for CK-60 it took 70 days to reach maturity (flowering) and for Tiemarifing it took about 55 days (figure 1.18: the last leaf before flowering appeared after 70 or 55 days respectively).
In CK-60, the difference in leaf area of uninfected and infected plants and between the different moments of application of *Striga* seeds was significant (figure 1.19). Plants that were infected earlier had a significantly smaller leaf area than plants that were infected at a later moment. There was no significant difference between the leaf area of the uninfected plants and the plants with *Striga* application 3 weeks after sowing of sorghum. The differences between the plants with different moments of application decreased at the end of the life cycle of sorghum. However, only the difference between moment 1 and 4 was significant. The difference between application moments 1, 2 and 3 was not significant. The value of plants of moment 2 was higher as a cause of one plant with a very big leaf area.

In Tiemarifing, no significant difference between the moments of infection and between infected and uninfected plants was shown.

Comparing the two cultivars, it can be concluded that the leaf area of Tiemarifing (without *Striga*) was lower than the leaf area of CK-60.
Figure 1.20: Time of 50% flowering of sorghum cultivars, CK-60 (■) and Tiemarifing (●), for four different moments of application and without application of Striga. CK-60 Plants with Striga application at the moment of sowing of sorghum (moment 1) did not reach flowering.

In figure 1.20, it can be seen that the effects on plant development of the different moments of infection with Striga were different for the two cultivars. With CK-60, time of flowering was delayed when Striga seeds were applied to the sorghum; application at sowing of sorghum even caused a lack of flowering plants in this experiment. Striga application had no significant effect on the time of flowering of Tiemarifing.
Photosynthesis

Figure 1.21: Photosynthesis of the last fully developed leaf of sorghum plants, a. CK-60 and b. Tiemarifing, plotted against time after application of pre-conditioned Striga seeds to the sorghum plants, for four different moments of application: 0 (■), 1 (▲), 2(○), and 3 (★) weeks after sowing of sorghum and without (●) Striga.

In figure 1.21 the overall standard error is showed, it includes all treatments. CK-60 (figure 1.21a) showed a significant reduction in photosynthesis due to Striga infection. Apart from that, plants with Striga applied at an early moment showed a lower photosynthesis than plants that were infested later. At the end of the sorghum
life cycle, photosynthesis decreased. However, no significant differences in decrease were found between plants with early application of *Striga* seeds (0 and 1 week after sowing of sorghum) and later application (2 and 3 weeks after sowing of sorghum). The decrease in photosynthesis at the end of the life cycle was found not to be significantly stronger with later application of *Striga* seeds.

Tiemarifing also showed a decrease of photosynthesis at the end of the life cycle of the plant. No significant reduction in photosynthesis was found due to *Striga* infection and apart from that, no significant difference was found between the different moments of *Striga* application.

Comparing the two cultivars (figure 1.21a and b), photosynthesis of Tiemarifing was found significantly higher than of CK-60, over the whole period of life of the plants. CK-60 showed a stronger decrease of photosynthesis if it is infected with *Striga* than Tiemarifing.
Discussion

In this discussion, the results on *Striga* will be discussed first, followed by the results on sorghum biomass production and its photosynthesis. The last part of the discussion is a general part, in which some implications of the experimental results for practical situations are discussed.

In the experiment a difference in moment of attachment of *Striga* on the two cultivars was shown: Tiemarifing was attacked almost two weeks later than CK-60, while *Striga* seeds were present in the soil at the same time. This confirms the results of the research of van Ast (1998). However, time between application and first attachment found was a fixed number of days, for CK-60 this was about 3 weeks after application of the *Striga* seeds, on Tiemarifing, first attachments were found after 5 or 6 weeks. On Tiemarifing, the number of attachments increased quicker than on CK-60, resulting in a number of attachments almost as high as on CK-60. On both sorghum cultivars it was found, that at a later moment of application, the number of attachments was higher than at earlier application. This could possibly be explained by the size of the sorghum root system; if *Striga* seeds are applied to the soil at a later moment, it could be assumed that the sorghum plants and also the root systems would be bigger. On this bigger (and presumably more branched) root system, *Striga* could find many places to attach to the roots, which would result in a high number of attachments. Another factor that played a role here is that the big, branched root system can produce easily new roots to enter the fresh (*Striga* infested) soil that came out of the cups.

Emergence of *Striga* plants occurred a fixed number of days after application of the seeds to the soil, as well on CK-60 as on Tiemarifing. However, conform to the findings of van Ast (1998), on Tiemarifing, *Striga* emerged at a later moment than on CK-60. The development of *Striga* was apparently not influenced by the different moments of seed application. With a later moment of infection, the number of emerged *Striga* plants appeared to increase faster and finally, more *Striga* plants emerged than with earlier application. Late applied *Striga* seemed to "compensate" for the time sorghum was uninfected. This could possibly be explained by what is explained above about the big, branched root system. The host plant would be strong enough for the *Striga* attachments to fully develop.

Between the two sorghum cultivars, a difference in number of emerged *Striga* plants was found; on Tiemarifing, the number of emerged *Striga* plants stayed lower than on CK-60. On CK-60 the difference between the moments of application was not very clear, however on Tiemarifing a clear pattern was found: at application of *Striga* seeds at a later moment after sowing of sorghum, more *Striga* plants emerged than on earlier moments. This could mean that *Striga* emergence was influenced more by Tiemarifing than by CK-60.

*Striga* biomass production on CK-60 was higher than on Tiemarifing. *Striga* on CK-60 produced more biomass when seeds were applied at an earlier moment. When *Striga* biomass production is plotted against time after infection (figure 1.13a) it was found that points of the different application moments lie on a straight line. From this we could draw the conclusion that growth rate of *Striga* was the same for the different moments of seed application. CK-60 did not influence the biomass production of the *Striga* plants, however, plants were smaller with a later infection moment. On Tiemarifing, no significant differences in biomass were found between the different moments of seed application (figure 1.12). When *Striga* biomass is plotted against
time after seed application (figure 1.13b) no line was found; this would mean that *Striga* growth rate was influenced by Tiemarifing. This could possibly be caused by the tolerance mechanism of Tiemarifing.

Biomass production of sorghum CK-60 was considerably reduced as a result of *Striga* infection. Apart from that, the time of seed application had a significant effect; biomass production of plants that were infected three weeks after sowing of sorghum was significantly higher than plants infected during the first 3 moments. This indicates that a delay in the moment of application (and indirectly delay of infection) causing the reduction of sorghum growth due to *Striga* was less severe. *Striga* not only reduced the total growth of CK-60, but also formation of an inflorescence and grain production. Tiemarifing did not show this 'reaction' on different moments of application and did not even show a significant reaction on *Striga* infection. This brings us back to our main question of this experiment: is the difference in time of attachment of *Striga* between CK-60 and Tiemarifing, THE cause of a difference in plant biomass production? At this point, this question can be answered: this difference in time of attachment is certainly not the most important cause of difference in biomass production between the tolerant and the sensitive cultivar.

Grain production of CK-60 decreased even stronger than overall biomass production due to *Striga*. Plants that were infected 3 weeks after sowing appeared to have a significantly higher grain biomass than plants infected earlier. There was no significant difference between the first three moments of infection. It could be imagined that somewhere between moment 3 and 4 (between 2 and 3 weeks after sowing) the process of grain forming or filling is induced and can not be disturbed by *Striga* anymore at that time.

There was no significant decrease found on grain production of Tiemarifing. Van Ast (1998) found a CK-60 harvest index that was slightly lower than the harvest index of Tiemarifing. Conform these results, the harvest index of uninfected CK-60 was found lower than that of Tiemarifing in our experiment. It is known that the harvest index of an inbred cultivar like CK-60 should be higher than that of a landrace like Tiemarifing (van Ast, 1998 unpublished). A higher grain yield was the reason to develop the cultivar CK-60 (van Ast, unpublished). However this was only reached under very controlled circumstances, in pot experiments and in the field harvests were always low.

As found in former research, the shoot-root ratio decreased due to infection of *Striga* (Parker and Riches 1993). Press et al. (1987) already found that *Striga* causes a reduction in photosynthesis of the host plant; the capacity to fix CO2 is reduced. A difference in this reduction was found between a tolerant and a sensitive cultivar of sorghum. This is confirmed in this experiment; photosynthetic activity of Tiemarifing was significantly higher and was reduced less, affected by *Striga* infestation than that of CK-60. The experiment was executed to find out whether this difference was caused by a difference in time of attachment of *Striga* on the two cultivars. CK-60 showed a difference in reduction between the different moments of infection. However, this was not big enough to explain the difference between the two cultivars.

Van Ast (1998, unpublished) found a very big reduction of photosynthesis of CK-60 as a cause of *Striga* infestation. Although, the reduction found in the experiment was not very big. A possible explanation for this could be found in the method of measurement. Photosynthesis was, at all measurements, measured at the youngest fully developed leaf of the plant. This gives a result that is not representative for the
whole plant. A better method of photosynthesis measurement would be to measure the photosynthesis of more (developed) leaves of the plant, on different heights.

From the results of this experiment we can draw some conclusions, these are summarized in the table in figure 1.25 and explained below.

Figure 1.25: The effect of Striga infestation on the development and production of the sensitive (CK) and the tolerant (Tie) cultivar of sorghum. The number of symbols show the amount of effect and the kind of symbol shows whether the effect is negative (-), positive (+) or whether there is no effect (0) for the plant or the parasite.

<table>
<thead>
<tr>
<th>Striga biomass production</th>
<th>CK Inf 1</th>
<th>CK Inf 2</th>
<th>CK Inf 3</th>
<th>CK Inf 4</th>
<th>Tie Inf 1</th>
<th>Tie Inf 2</th>
<th>Tie Inf 3</th>
<th>Tie Inf 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of emerged Striga plants</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Number of Striga attachments</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
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<tr>
<td>Sorghum biomass production</td>
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<tr>
<td>Sorghum harvest index</td>
<td>- - - -</td>
<td>- - - -</td>
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<td>- - - -</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Sorghum leaf appearance</td>
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<tr>
<td>Sorghum leaf area</td>
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<td>- - - -</td>
<td>- - - -</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sorghum flowering</td>
<td>- - - -</td>
<td>- - - -</td>
<td>- - - -</td>
<td>- - - -</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Sorghum photosynthesis</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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</tbody>
</table>

In general, the results of the experiment showed that there was a significant difference between CK-60 plants with Striga application immediately after sowing and plants that were infected a few weeks later. However, the differences found in growth of CK-60 between the different moments were bigger than could be explained by the difference in moment of infection. On this point, the main question can be answered: difference in time of infection cannot explain the difference in growth between the tolerant and the sensitive cultivar. However, for the sensitive cultivar, a delay in moment of infection can cause a big difference in yield and growth of the plant. Therefore, it could be interesting to try to find a method to delay that moment in the field. In the experiment plastic cups were used to delay the moment of attachment. In the field, a method like that is not possible: Striga seeds are always present in the soil. If there can be found a method to delay the moment of attachment in the field, this may help to avoid growth reduction due to Striga. Striga would still be there, however a reasonable harvest of sorghum can be gained.

One idea to use this method of avoidance in the field is to make a conical shaped plant-hole. This is made in a way that the lowest point is about 15 cm below the
surface of the soil and, more important, below the layer with the most *Striga* seeds (van Ast, 1998). In this way the plant forms its (first) roots below the *Striga* infested layer and the moment of attachment is delayed. This method is likely to be used in the field.

Another idea would be to make a nursery with *Striga*-free soil. The first weeks the sorghum plants are grown in the nursery, and later they are transplanted to the field. Even if *Striga* attacks the plants, the moment of infection is delayed and accordingly the effect would not be very harmful: a harvest can be gained. This method will cost a lot of time and labour, therefore it is not likely to be used in the field.

**Future research**

This experiment generated a lot of answers, but also a lot of questions are raised. This experiment focused on the development and growth of sorghum, however also some observations are made on *Striga*. Because some information on the development of *Striga* was missing, it would be interesting to do some more research that, also after harvest or dying of the sorghum. From this experiment information about the biomass production of *Striga* was gained, however, no conclusions can be drawn for the future from this. Information about the quantity of seed production is lacking. To get an estimation of the growth of the seed bank in the soil the number of flowers, for example, could be counted. *Striga* still lives for some time after the host plant dies; this period seems to be important for seed production and release.

In this experiment, different organs of sorghum were examined. A big effect of *Striga* on the grain production was found; what happened in the plant, that grain production was affected? Does the initiation of inflorescence or grain filling play a role? To understand the life-strategy and mechanisms *Striga* uses to survive, it would be interesting to look at the development stages of sorghum. What stages in plant development can be distinguished with sorghum and at what time is which process induced? Combined with the results of this experiment, it could give a rather complete vision of the whole process. E.g. the reduction of grain biomass production could be explained with the moment of grain setting of sorghum.
References


Chapter 2

Root morphology of a sensitive and a tolerant cultivar of *Sorghum bicolor*

**Introduction**

To find a solution for the *Striga* problem, it is necessary to understand the problem, therefore much research must be done. This research has two sides; on the one hand research is done on *Striga* itself, to find a way to combat it. On the other side, research is done on the host plants, to find a way of decreasing the reduction in growth of the host plant.

One way to decrease the growth reduction, could be to avoid or diminish the contact between roots of the host plant and *Striga* seeds. In the General Introduction it was already mentioned that the roots of the host plant produce certain exudates which stimulate the germination of the *Striga* seeds. The most important are Sorgholeone and Sorgholactone (Parker and Riches, 1993). The host roots must produce these exudates within a certain distance, about 4 mm (Press, 1995) from the seeds to attain successful germination. From field observations it is known that the seeds of *Striga* are mostly found in the upper 10 cm of the soil (van Delft, 1997). To induce germination, the production of germination stimulants and thus the development of the root system must take place in that upper soil layers. In deeper layers of the soil, fewer seeds are present and less germination and attachment of *Striga* occurs.

With this aspect kept in mind, there must be a way to avoid germination and attachment of *Striga* on the sorghum roots. We can think of different methods:

- The spatial separation of the host roots and the *Striga* seeds.
- The sorghum can develop a root growth pattern in which it forms a physical obstruction to the attachment of the *Striga* seeds; this is called resistance.
- There is also the possibility of ‘chemical avoidance’: to prevent the host plant to produce sufficient or different germination stimulants to cause germination of the *Striga* seeds.


This experiment focused on the first method, the spatial separation of host roots and *Striga* seeds. One of the most important aspects of spatial separation as a key factor in *Striga* management is that it should lead to an easily applicable method in problem areas (mostly developing countries). First however, more fundamental information is required for instance on root formation and root architecture of the host plants.

To develop a management strategy based on spatial separation, more knowledge should be gained about the morphology of the root system and the distribution of *Striga* seeds in the soil. For the latter, some information is already available in literature: most of the seeds are present in the upper 10 cm of soil, if the soil is not tilled (van Delft, 1997). The morphology of the root system of sorghum might differ between cultivars as was reported by Cherif-Ari et al. (1990). A cultivar classified as resistant showed a reduced root mass in the upper layers of the soil. In a field experiment, Dixon and Parker (1984) found less root branching and root length in a resistant sorghum line, than in a susceptible variety.

In continuation of former research, a comparison of the root morphology of a tolerant and a sensitive sorghum cultivar was made. It might be possible that root morphology is one of the underlying mechanisms of tolerance, given the fact that *Striga* seeds are positioned mostly in the upper 10 cm of the soil. (Cherif-Ari O. et al. 1990).

In Chapter 1 it was already mentioned that there is a difference in time of attachment of *Striga* on sorghum between the sensitive and the tolerant cultivar (van Ast, 1998). In this experiment, the role of root morphology in the observed delay of attachment of *Striga* on roots of sorghum cultivars that are classified as being tolerant was examined. There might be a physical difference between the root systems, for instance in the depth of roots, the branching pattern, total root length or number of root tips. The excretion of germination...
stimulants from the roots is assumed to be situated just behind the root tips. This would mean that a root system, with a relatively high number of root tips, produces more germination stimulants and has a bigger chance to be attacked by *Striga* than a root system with less root tips.

In this experiment, root development was observed and the two cultivars were compared. Total root length, the number of root tips, branching pattern and root dry weight was determined for different layers in the soil.

**Materials and Methods**

**Materials**

- Sorghum plants were grown in root-boxes.
  The principle of the root-box system is based upon the fact that under natural conditions roots are able to grow through perforations in the soil, such as wormholes and mole tracks. There is no noticeable change in branching of roots and formation of root hairs as long as perforations are smaller than 15 mm and light is excluded. At the same time the relative air humidity in the perforations should be kept at a high level. With aid of an ‘intrascope’, the root environment can be studied and root length and the number of root-ends can be estimated. These root-boxes are stainless steel boxes, being tapered. The front and the back have a perforated plate and the sides and the bottom are of transparent material. Sliding panels cover all 4 sides. By aid of the perforated plates at the front and the back, perforations in the soil are being made with a boring tube. Through the mixture of different soil types, perforations maintained open for the whole length of the period. The hold volume of the root-boxes: 21,9 dm³

![Figure 2.1: Schematic representation of a root box](image1)

![Figure 2.2: Schematic representation of a root box with a growing plant](image2)
• Root-map-paper was used to register the observations on root development; this is a drawing of the front panel of the root box (an example is added to this report as appendix II). On this paper, the newly produced roots of the plants in the root-boxes were drawn every day.

• 2 cultivars of *Sorghum bicolor* were used in the experiment: the highly Striga-sensitive inbred cultivar CK-60 B (origin: 96006 Nov ’96 ICRISAT) and the Striga-tolerant local Malian land-race Tiemarifing (origin: 96005 Nov ‘96 ICRISAT). Pre-germinated seeds were sown at a depth of 1.5 cm, 3 seeds per pot. After emergence, the sorghum was reduced to 1 plant per pot.

• *Striga hermonthica* seeds: 20 mg (4900 seeds) per pot, resulting in 1.3 seeds per cm³ (in the upper 6 cm of the soil). Origin *Striga* seeds: 95016; harvest 1995 Samanko, Mali. Percentage of germination February ’99: 83%.

• The root boxes were filled with a mixture of soil: sandy arable soil and white sand (1:2). The proportion of the quantities of arable soil and sand was selected to obtain a soil that was sufficiently solid to maintain the perforations made for observation.

• Nutrient application rate: N: 60 kg/ha, resulting in 0.13 g N per box = 0.29 g urea per box.
P: 30 kg/ha, resulting in 0.067 g P per box = 0.33 g triple superfosfaat per box.
K: 50 kg/ha, resulting in 0.12 g K per box = 0.49 g patentkali per box. Fertilizer was applied 11 days after sowing of sorghum.

• The temperature in the greenhouse was about 30°C during daytime and 20°C during the night

• RAH: 70/80%. Water was given regularly to the plant at the top of the soil.

• In the greenhouse a day length of 11 hours (nighttime = 13 hours) was maintained; the greenhouse was artificially kept dark with black screens between 19.00 h PM and 8.00 h AM. Lamps were used to give additional light: HPIT-lamps 400 Watt.

• The location of the set-up of the experiment: greenhouse Haarweg, Wageningen

• At the destructive harvests, plants were harvested and dried in an oven at a temperature of 70°C for at least 24 hours to determine plant dry weight, for the different organs: roots, stems and leaves.

*Figure 2.3: Making the perforations in the soil in the root-boxes.*
**Research method**

Growth and development of the root system of infected and non-infected sorghum plants were observed during the first 4 weeks. The first 4 weeks seem to be the most important period in the development of the root system of sorghum (van Delft, 1997) in relation to *Striga* infestation.

The following treatments were applied in three replicates in the experiment:
- 2 varieties of sorghum: Tiemarifing (tolerant) and CK-60 (sensitive)
- with or without *Striga*

With these treatments $3 \times 2 \times 2 = 12$ boxes were needed.

The experiment was conducted from half September to the end of October 1999. The development of the plants was observed for four weeks. To resemble a natural situation, the upper 6 cm of the soil was infected with *Striga* seeds. This is also the most upper layer, distinguished in the experiment.

The number of roots observed in the perforations was counted, roots that grow totally through the perforation are counted as 1; roots that do not totally grow through the perforation are counted as 0.5. This number was used to determine the total root length and the total number of root tips.

The total root length was estimated by using the following formula (Schouten, 1989):

$$RL = n.r. \times (w \times h \times \pi) / (2 \times m \times d)$$

- **RL** = root length
- **n.r.** = number of roots counted.
- **w** = width of the perforated front plate in m.
- **h** = height of the soil column in m.
- **m** = number of perforations
- **d** = average diameter of the perforations in m (0.007 m).

$$NRT = n.t. \times (w \times h) / (m \times \pi \times d^2 \times 0.25)$$

- **NRT** = number of root tips
- **n.t.** = number of root tips counted. All the roots are counted as 1
- **w, h, m** and **d**, as above.

**Observations and measurements**

- The root-box observations on the development of the root system were made daily. These observations were denoted “root-maps” of the different root systems. The drawing of the root-maps started when the first root became visible in one of the perforations.
- At the end of the experiment, four weeks after sowing, the plants were harvested. The dry weight of the aerial parts was determined and the length of the stem was measured. The soil column was divided in 7 layers of 6 cm high (Appendix III) and the dry weight of the roots was determined per soil layer. Thin metal bars were put into the perforations and a
rubber stop at the end of the bar closes the holes in the front panel. The back panel was removed and the box was turned on its front. The soil was washed out of the root-box in such a way that the root system stayed more or less in its natural position. The number of Striga attachments per soil layer was counted and after counting, the attachments were removed and dry weight of the Striga was determined.
Results

The development of the roots in the root boxes was recorded by making frequent observations (nearly every day) and depicting the development on a root map (Appendix IV). Total root length and number of root tips were calculated with formula per layer (appendix V).

![Figure 2.4: Root biomass plotted per layer of 6 cm, for CK-60 without (a) and with infestation of Striga (b) and Tiemarifing, without (c) and with (d) infestation of Striga, at 4 weeks after sowing of sorghum.](image)

Figure 2.4 shows the root profiles of root biomass of the sorghum cultivars as determined through destructive harvesting, 4 weeks after sowing. Total biomass production of uninfected plants of CK-60 and Tiemarifing differed significantly (figure 2.4a and c): root biomass production of Tiemarifing was higher than that of CK-60. However, CK-60 produced significantly more root biomass in deeper layers than Tiemarifing, resulting in a more evenly distributed vertical root pattern.

Comparing the root biomass of CK-60 with and without *Striga* (figure 2.4a and b), a significant increase can be observed; total biomass of infected plants was higher than that of uninfected plants. The major root biomass of infected plants was situated in the upper soil layers. Already after 4 weeks, the effect of *Striga* on root development of sorghum was visible.

The effect of *Striga* on Tiemarifing (figure 2.4c and d) appeared to be a significant reduction of root growth. The vertical root distribution pattern did not change significantly due to infection of *Striga*. 

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The root maps in figure 2.5 illustrate the difference in root morphology between CK-60 and Tiemarifing. CK-60 first produced a 'main root', which grew into deeper layers. Later the root system started to branch. Tiemarifing started to branch directly in the upper soil layer, resulting in a shallow root system; consequently it did not reach the lower soil layers during the experiment.
The total root length of uninfected plants of Tiemarifing was significantly higher than that of CK-60 (figure 2.6 a and c). Root length distribution over the different soil layers was similar to the root weight distribution: CK-60 produced more roots in deeper layers than Tiemarifing. However, the difference between the total root length of the two cultivars appeared to be much bigger than the difference in root biomass production. The relation between these parameters can be described by the term specific root length: root length (m)/ root biomass (g). SRL of Tiemarifing was significantly higher than SRL of CK-60 (figure 2.8).

The effect of Striga on the root length distribution of CK-60 (figure 2.6 a and b) was an increased production of root length, especially in the upper layer of the soil. SRL increased as a result of Striga infection. In Tiemarifing (figure 2.6c and d) no significant effect of Striga infection on the total root length was found. However, the decrease in root biomass resulted in a higher SRL due to Striga infection.

Figure 2.6: Total root length, plotted per layer of 6 cm, for CK-60 without (a) and with infestation of Striga (b) and Tiemarifing, without (c) and with (d) infestation of Striga, at 4 weeks after sowing of sorghum.
The number of root tips gives information about the level of branching of the root system. A significant difference was found in the number of root tips comparing uninfected plants of CK-60 and Tiemarifing (figure 2.7a and c); Tiemarifing produced more root-tips than CK-60 and the major part was found in the upper soil layers. However, CK-60 had also produced root tips in deeper layers of the soil. The number of root tips per meter root length of uninfected CK-60, was very high (figure 2.8) compared to Tiemarifing. Concerning the effect of Striga on the number of root tips, CK-60 produced significantly more root tips due to infection, the number of tips per meter root length decreased. The most root tips were produced in the upper soil layers. Tiemarifing did not show a significant effect to Striga infestation.
Figure 2.8: Specific root length number of root tips per meter root length and shoot/root ratios for CK-60 and Tiemarifing, with and without infestation of Striga at 4 weeks after sowing of sorghum.

<table>
<thead>
<tr>
<th></th>
<th>CK-60 -</th>
<th>CK-60 +</th>
<th>Tiemarifing -</th>
<th>Tiemarifing +</th>
</tr>
</thead>
<tbody>
<tr>
<td>SRL</td>
<td>16</td>
<td>81</td>
<td>46</td>
<td>48</td>
</tr>
<tr>
<td>Tips/m</td>
<td>172</td>
<td>74</td>
<td>72</td>
<td>71</td>
</tr>
<tr>
<td>Shoot/root</td>
<td>0.23</td>
<td>0.26</td>
<td>0.32</td>
<td>0.51</td>
</tr>
</tbody>
</table>

Figure 2.9: Dry biomass production per plant organ, leaves ( ), stem (■) and root (□) of sorghum CK-60 and Tiemarifing, with and without Striga at 4 weeks after sowing of sorghum.

Between the two (uninfected) cultivars, a small difference in total biomass production was observed, biomass production of Tiemarifing was higher. Between the infected and uninfected plants a significant difference in biomass production was observed. At CK-60, infection of Striga caused a reduction in biomass production. The shoot-root ratio increased a little. On Tiemarifing biomass production, Striga had a reducing effect, especially on the roots. Shoot-root ratio increased significantly.
Discussion

Housely et al. (1987) and Baltus et al. (1993) already assumed that susceptibility of host plants to *Striga* parasitism may depend on architecture and the amount of roots that are produced by the host plant, which can be different for different cultivars (van Delft, 1997). In a field trial, Cherif-Ari, Housely and Ejeta (1989) found that also the vertical positioning of the roots might be important; two resistant cultivars of sorghum, P-967083 and Framida, showed a reduced root mass in the upper portions of the soil core relative to the susceptible cultivar Dabar.

In this experiment the root morphology of the tolerant cultivar Tiemarifing and the sensitive CK-60 was compared. The results of the experiment show that the root systems of CK-60 and Tiemarifing were clearly different when not infected. CK-60 roots reached deeper layers than roots of Tiemarifing, most likely since the root system of Tiemarifing started to branch earlier in the upper layers of the soil. Taken the fact that *Striga* seeds are positioned in that upper layer, CK-60 seems to be best 'protected' against infestation of *Striga*. In their discussion, Cherif-Ari, Housely and Ejeta (1989) assume that root development might be one of the mechanisms of sorghum to avoid *Striga* parasitism. A resistant cultivar would produce less root biomass in the ‘*Striga*-infected soil layer’ and would reduce parasitism. In this experiment it is found that this is not what is happening in practice. Tiemarifing produced more root biomass and root tips than CK-60. From this it can be concluded that Tiemarifing produced more root exudates than CK-60 and should be attacked more by *Striga*. However, from this experiment no information can be gained about the quality, activity or moment of production of the root exudates. For these results a new experiment is required.

At this point, the main question of this research can be answered: whether root morphology plays a role in the mechanism of tolerance, the delay of attachment of *Striga* on tolerant cultivars. If plants were not infected, there was a difference in vertical root distribution pattern between the two cultivars. However, this difference cannot explain the difference in tolerance: CK-60 had the most fortunate root morphology of the two, a big part of the roots were situated in deeper layers of the soil, where no *Striga* was situated. Tiemarifing root system branched in the upper layers of the soil, which were infected with *Striga*.

However, other interesting observations were made when plants were infected with *Striga*. The strategy of CK-60 concerning root morphology appeared to change; CK-60 produced more root biomass, -length and number of roots in the upper layer of the soil, when it was infected. Because in this upper layer the *Striga* seeds were situated, the chance of infection increased. CK-60 plants seem to make the situation worse for itself: more roots in the *Striga* infested layer results in more attachments of *Striga* on the roots, which causes more reduction on growth and production of the whole plant later in development.

When Tiemarifing was infected with *Striga*, root biomass production was reduced and not only in the upper layer. Tiemarifing did not significantly change its root morphology when it was infected with *Striga*. This could explain its tolerance. However, CK-60 did change its root morphology when it was infected and affected a fast attack of *Striga* itself. This shows the sensitivity of CK-60.

Probably, root morphology plays a role in delay of attachment of *Striga* on tolerant cultivars of sorghum. However, a better way to pronounce it would be that root morphology plays a role in the quicker attachment of *Striga* on sensitive cultivars of sorghum. Only on one plant *Striga*-infestations were found: 2 attachments. This has been a reason to draw no conclusions from the figures on numbers of *Striga* plants. However, it is assumed that there should have been more attachments, but these were not visible or lost with the
cleaning of the root systems. An other explanation may be that the *Striga* seeds were not preconditioned very well: 5 days after mixing the *Striga* seeds with the moist soil, the sorghum is sown. From literature and former research we know that preconditioning period of one week to 10 days is optimal (IITA, 1997). This may have caused a delay in development of *Striga*.

### Future research

After execution and interpretation of this experiment, there are still questions to be answered and new questions are born. From this experiment information was gained about the number of root tips of the tolerant and the sensitive cultivar of sorghum. We assumed a certain relation between these root tips and the production of root exudates. It would be interesting to further research that relation. At the moment, an experiment starts at Wageningen University, in which this relation will be examined.
References

- Borg, S.J. ter and Ast, A. van, Soil Moisture, Root Architecture and Broomrape (Orobanche crenata) Infestation in Faba Bean (Vicia faba). Department of vegetation Science, plant ecology and weed science, Agricultural University, Wageningen, The Netherlands.


- Manual root-box system
Method of the set-up of the experiment

Figure 1: Per pot, 4 coffee cups were filled with soil and dug in the soil in the pot. The pot and the cups were filled with Striga-free soil.

Figure 2: At the different moments of infection (0, 1, 2 or 3 weeks after sowing) the coffee cups were taken out of the pots.

Figure 3: At later moments, the Sorghum roots were already well developed and grown around the coffee cup.

Figure 4: The soil of the 4 coffee cups of each pot was mixed with 20 mg Striga seeds.

Figure 5: The infected soil was thrown back in the holes. From this moment on, contact was possible between the soil in the pot and the Striga infected soil from the cups, and, more important, between the roots of sorghum and the Striga seeds.
Example of a root map
Different layers are distinguished in the root-boxes. All layers are 6 cm in height, also layers I and VII, the root-box is bigger than the pannel. The soil in layer I is mixed with pre-conditioned Striga seeds.
Root system CK-60

Root map of a sorghum cultivar CK-60. This plant was infected with Striga.
Root map of a sorghum cultivar Tiemarifing. This plant was infected with Striga.
Appendix V

<table>
<thead>
<tr>
<th>Layer</th>
<th>m</th>
<th>w (m)</th>
<th>h (m)</th>
<th>d (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>13</td>
<td>0.32</td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td>II</td>
<td>20</td>
<td>0.3</td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td>III</td>
<td>17</td>
<td>0.281</td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td>IV</td>
<td>16</td>
<td>0.261</td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td>V</td>
<td>15</td>
<td>0.243</td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td>VI</td>
<td>14</td>
<td>0.244</td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td>VII</td>
<td>4</td>
<td>0.211</td>
<td>0.06</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Constant values, per layer, for the calculation of root length and number of root tips.

\[
\text{RL} = n.r. \times \left( \frac{w \times h \times \pi}{2 \times m \times d} \right)
\]

\[
\text{NRT} = n.t. \times \left( \frac{w \times h}{m \times \pi \times d^2 \times 0.25} \right)
\]

Formula, per layer, for the calculation of root length and number of root tips.

\[\text{RL} = \text{root length}\]
\[n.r. = \text{number of roots counted. Roots which grow totally through the perforation are counted as 1, roots which do not totally grow through the perforation are counted as 0.5.}\]
\[w = \text{width of the perforated front plate in m.}\]
\[h = \text{height of the ground in m.}\]
\[m = \text{number of perforations}\]
\[d = \text{average diameter of the perforations in m (0.007 m).}\]
\[\text{NRT} = \text{number of root tips}\]
\[n.t. = \text{number of root tips counted. All the roots are counted as 1}\]

These constant values and formula are based at values from: Theo Schouten, 1989.