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A time-course study of early establishment stages of parasitic angiosperm *Striga asiatica* on susceptible sorghum roots†

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Summary

A time-course study of the early establishment stages of *Striga asiatica* was carried out on a susceptible sorghum hybrid, CSH 1, using polyethylene bags and whole-root clearing and staining techniques. Preconditioned *Striga* seeds were applied to different aged segments of primary root but the results did not differ for these different aged segments. Most of the *Striga* seeds (63%) germinated within 24 h of inoculation on the host roots. The attachment of *Striga* radicles to host root was rapid and it occurred between 36 and 48 h after inoculation. Only 9% of the germinated *Striga* seeds attached to the host root but 65% of these attachments successfully penetrated through the epidermis and entered the host cortex within 72 h. Penetration through the cortical cells was difficult; only 17% of attachments were able to reach the endodermis. Penetration took from 12 to 43 h after the first appearance of haustorial cells in the cortex; a total of 84 to 120 h after inoculation on the host root. Penetration through the endodermis and establishment on the host stele was relatively easier, as most of the haustoria reaching the endodermis were able to establish on the host stele. But this is a slow process taking a minimum of 24 h, and a maximum of 60 h after first contact of haustorial cells with the endodermis. The minimum time taken from inoculation of ungerminated *Striga* seed on the host root to establishment is about 108 h. The results are discussed in relation to published reports on other parasitic species such as *Agalinis purpurea*.

Key words: *Striga asiatica*, sorghum, *Striga* seed germination, haustoria, attachment, penetration, establishment

Introduction

Striga asiatica (L.) Kuntze is a root parasite belonging to the family Scrophulariaceae. It attacks almost all rainfed cereals, particularly, sorghum (*Sorghum bicolor* (L.) Moench), millet (*Pennisetum glaucum* (L.) R.Br), and maize (*Zea mays*) causing serious yield losses. Although *Striga* plants were first described as early as 1753 by Linnaeus, their biology, particularly in the early stages of development, is still not fully understood. These development stages include: germination, haustorial initiation, and host penetration through various host root tissues such as the epidermis, cortex, endodermis, and finally the host stele. An understanding of these early development stages may reveal some weaker links between the parasite and its host, which could be exploited in *Striga* control. In this paper the results of a time-course study of the early establishment stages of *Striga asiatica* are reported.

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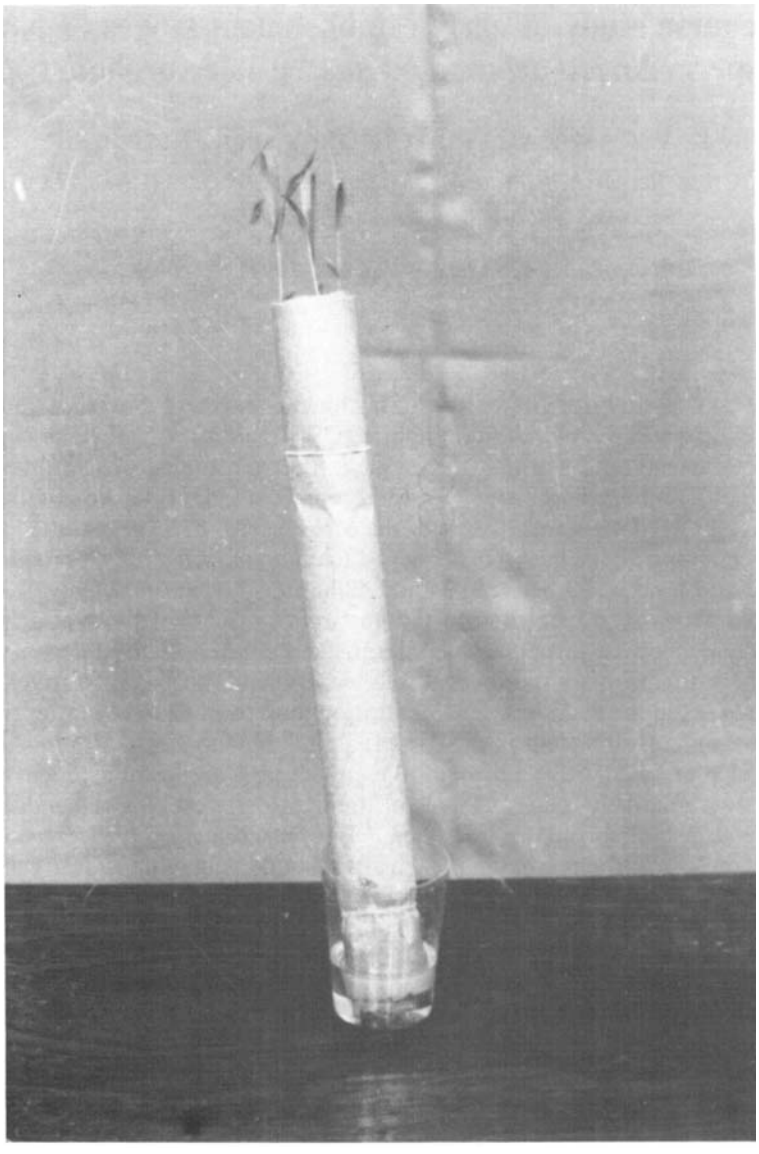

Fig. 1. Sorghum seedlings in filter paper roll placed in polyethylene bag with opening at both ends. A brown paper was used outside the roll to prevent light interference.

Materials and Methods

The experimental materials were CSH 1, a susceptible sorghum hybrid, and *Striga asiatica* seeds collected in March 1975 from a sorghum field at the ICRISAT Center, Patancheru, India. *Striga* seeds were preconditioned as described by Parker, Hitchcock & Ramaiah (1977). Some of the other methods used in this study were briefly described earlier (Anon., 1978). Parker & Dixon (1983) adopted this method, after modification, to suit their purpose of study of *Striga* spp. on crop roots. The techniques used for the present study are described below in detail.

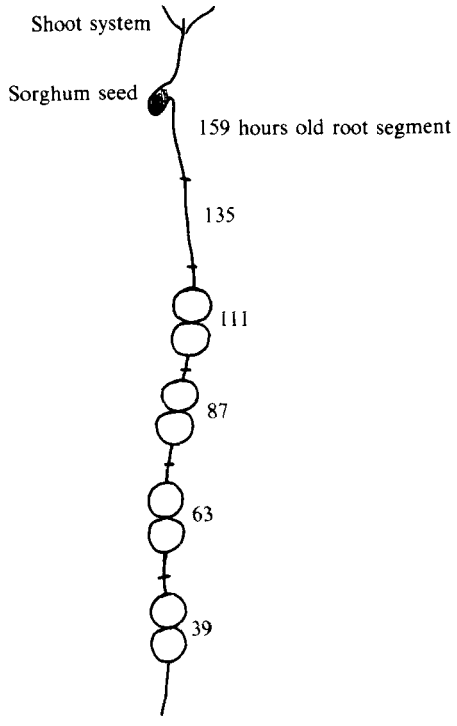

Fig. 2. Young sorghum seedling grown in Whatman No. 1 filter paper showing root segments aged 39 to 159 hours and glass fibre filter paper discs with *Striga* seed facing the root (○).

Raising sorghum seedlings

Seeds of sorghum hybrid CSH 1 were surface sterilised with sodium hypochlorite solution (1% available chlorine) for 25 min, followed by rinsing several times with distilled water until the chlorine smell disappeared. The seeds were germinated for 24 h in a Petri dish at room temperature (25 ± 5 °C). The germinated seeds were placed between two layers of Whatman filter paper (20 × 50 cm), approximately 2 cm below one edge of the paper. The filter papers were introduced into a polyethylene bag, open on both sides and watered to moisten it. The sheets were rolled with a brown paper on the outer surface and tied with a rubber band to prevent unrolling. The rolls were placed vertically in a glass container containing water (Fig. 1). Each roll had three to four seedlings. Each day the rolls were opened and the tips of the primary root marked to record daily growth (Fig. 2). The seedlings were grown at room temperature (25 ± 5 °C).

Inoculation of Striga *seeds on the host roots*

Striga asiatica seeds were surface sterilised and preconditioned at 25 ± 1 °C for 10 to 14 days as described by Parker *et al.* (1977). Preconditioned seeds were used to inoculate root segments that were 39, 63, 87 and 111 h old. Two glass microfibre filter paper (Whatman GF/A) discs, 8 mm diameter, each containing 25 to 30 *Striga asiatica* seeds, were kept on each root segment (Fig. 2). To initiate the *Striga* seed germination, 50 µl of 0.01 ppm GR-7 (Johnson, Rosebery & Parker, 1976) were added to each disc. Every 12 h, starting from 24 h after inoculation to 168 h, two sorghum seedlings (each seedling = one replication) were sampled and observations on *Striga* seed germination, attachment, and stage of penetration of haustorial cells through the host root were taken. A stereozoom microscope was used to

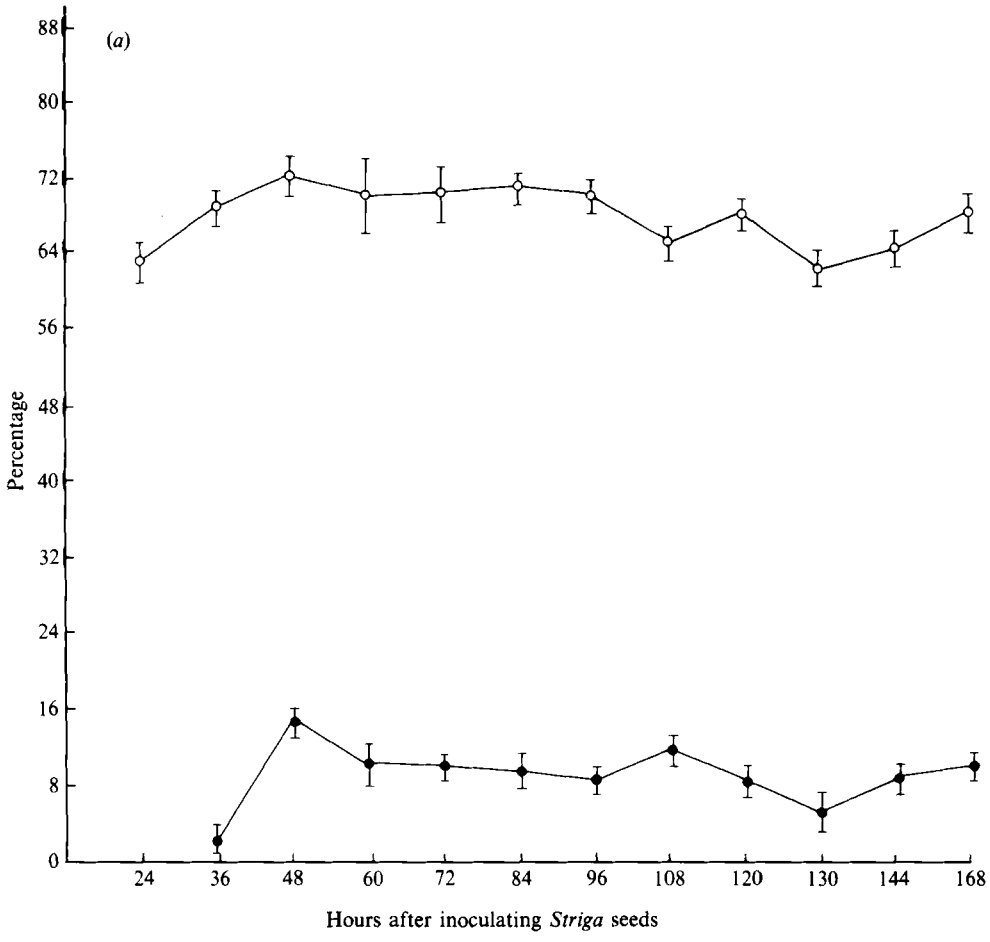

Fig. 3 (*a*). The relationship of percentage *Striga* seed germination (-○-○-) and percentage attachments (-●-●-) are shown against time (hours) after inoculation of seeds of *Striga asiatica* on root of susceptible sorghum hybrid, CSH-1. Number of attachments are expressed as percentage of number of germinated *Striga* seeds. Bar: S.E. of mean.

observe *Striga* seed germination. Attachment was defined as physical gluing between host root and the *Striga* radicle cells following contact between them. It does not necessarily indicate penetration by the cells of *Striga* radicle through the host tissue as many such unions were superficial. Therefore, a whole-root clearing technique described below was used to observe penetration through the host root.

Whole-root clearing and staining technique

a) Preparation of stain: 0.1% aniline blue (water soluble) was prepared in lactophenol (Johansen, 1940).

b) To soften the root, root regions of sorghum with *Striga* seedling attachments were placed in a small test tube containing the stain, which was then boiled over a flame for 2 min and allowed to cool.

c) The softened roots were placed in a microscope slide in a drop of stain. To flatten the specimen, a gentle pressure on the cover slip was applied.

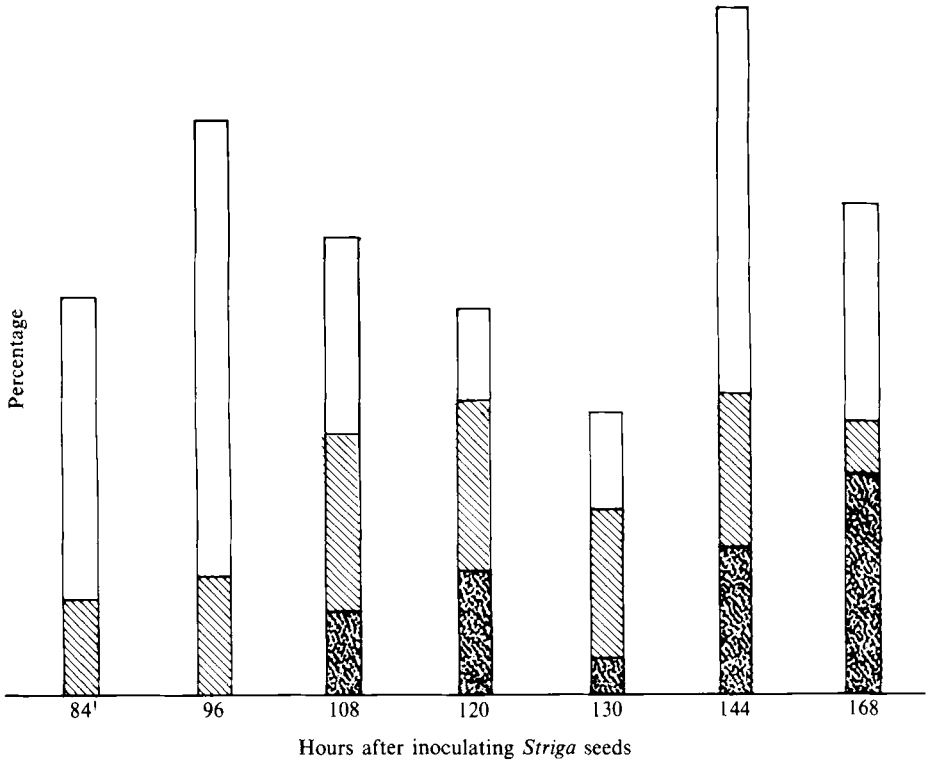

Fig. 3 (*b*). The relationship of percentage attachments penetrated through the epidermis (includes those in cortex and those reached endodermis/established on host stele) □, those reaching endodermis (includes those established on host stele) ▨ and those established on host stele ▩ shown against time (hours).

Results

The primary root segments of CSH 1 seedlings that were 39-, 63-, 87- and 111-h old did not exhibit significant differences between themselves for percentage *Striga* seed germination, percentage attachments, and percentage establishment. Therefore, for all the observations reported here, the overall mean of 4 root ages was taken as an experimental unit for analysis. The results are shown in Fig. 3.

Striga *seed germination*

The percentage of *Striga* seeds that germinated at different periods of sampling, ranging from 24 to 168 h, were not significantly different. Most of the *Striga* seeds (63%) germinated within 24 h after inoculation on host roots and addition of GR-7, a germination stimulant. The germination reached maximum after 48 h, but it was not significantly different from that at 24 h. Subsequent sampling periods did not show a significant increase in germination. The mean *Striga* seed germination was 67.5%.

Attachment to host roots

Striga radicles started attaching to the host roots from 36 h after inoculation, reaching a maximum after 48 h. After 36 h the tip of the *Striga* radicule became slightly swollen and produced hairs and a sticky substance(s) that helped the attachment of the radicle to the host root. There were no significant differences in the frequency of attachment to host roots among the sampling periods from 36 to 168 h. The mean percentage of attachments was only 9% of the germinated *Striga* seeds.

Striga *haustorial cells in host cortex*

The haustorium in *Striga* spp. appears as a bulbous structure on the *Striga* radicle or root. It develops on contact with the host roots. A few cells, greater in length than in diameter, develop from within the haustorium. These cells are normally perpendicular to the host vessels and are known as penetrating cells or intrusive cells. These penetrating cells were first seen in the host cortex 72 h after inoculation. This was also the time when the maximum number of haustoria (65% of attachments) penetrated through the epidermis and entered the cortex. There was a gradual decline in penetrating cells in the cortex from then until 120 h. This may be a result of differences in cell-type of those that had not only penetrated the cortex but also reached and/or penetrated the endodermis.

Striga *haustoria to host root endodermis*

The number of haustoria that successfully advanced their penetrating cells as far as the host root endodermis was counted. The first appearance of the penetrating cells at the outside of the endodermis was at 84 h. By this time penetrating cells of 12.5% of the attachments had reached the endodermis. A maximum of 25.6% of the attachments appeared at the endodermis 120 h after the inoculation of *Striga* seeds. By this time an additional 14.6% of the attachments had already established on host stele. However, the sampling periods from 84 – 168 h for penetration up to the endodermis were not significant.

Striga *seedling establishment on host stele.*

The first contact between *Striga* haustorial cells, that is, penetrating cells and the host stele, was observed at 108 h, with a maximum number of haustoria (26.5% of attachments) making contacts with host stele by 168 h. There were no significant differences among the sampling periods from 108 – 168 h. The mean percentage of attachments that successfully established on the host stele was 14.8%.

Discussion

Striga seeds are extremely small, measuring about 0.15 × 0.31 mm; in bulk, they form a fine dust. They germinate when they are within 3 to 4 mm of the host root (Brown & Edwards, 1944). *Striga* radicles can grow 2 – 4 mm (Kasasian, 1971; Williams, 1961) and should attach to the host root, penetrate, and establish on the host stele within a short period of time, since their food reserve is very small. In *Agalinis purpurea*, developing radicles that have not contacted a host by 60 – 72 h of germination fail to attach when contact is established (Baird & Riopel, 1983). It has been reported that it is only after successful establishment on a host root that the plumular end of *Striga* starts growing (Yoshikawa, Worsham, Moreland & Eplee, 1978). Therefore, the timings of these early stages are very critical in the life cycle of *Striga*.

Our present results demonstrate that most of the *Striga* seeds, after meeting their preconditioning requirements, germinate within 24 h of exposure to host root exudates, as has been reported earlier (Brown & Edwards, 1944; Parker *et al.*, 1977). Germination may, in fact, have been triggered within a few hours of exposure to the stimulant, but the present study and those of others do not indicate exactly the minimum time that is required to trigger the germination. Attachments to the host root were observed after 36 h and reached the highest frequency at 48 h. The sticky substance(s) that is found at the tip of the *Striga* radicle in our study was also reported to be produced by haustorial hairs of *Agalinis purpurea* (Baird & Riopel, 1983), which helped in attachment to the host root. Baird & Riopel (1983) also reported that attachments occur in significant numbers by 12 – 18 h of their initiation and reach a maximum level of attachment by 36 h. Our results with *Striga asiatica* closely agree, in

that *Striga* seeds needed 48 h of exposure to stimulant to reach a maximum attachment frequency and from then onwards the frequency of attachment did not increase.

A small percentage (9%) of the total *Striga* seeds that germinated successfully attached to the host root. They either fail to form haustoria or the radicles may fail to respond to chemotropism. The importance of chemotropism in host selection has been discussed by Dixon & Parker (1984). Failure of over 90% of germinated *Striga* seeds to attach to the host root is explained by some observations on *Agalinis purpurea*, which show that attachment is a non-discriminatory event, and induced haustoria will adhere to a variety of biological and non-biological substrates (Baird & Riopel, 1983). In *Agalinis*, laboratory and field observations of soil-grown plants reveal that haustorial attachment frequencies are variable (25 – 80%). In the soil, most haustoria are usually attached to the roots very close to them, and others are either not attached or attached to a variety of inanimate objects. This has also been reported in other root parasites (Kuijt, 1969; Werth & Riopel, 1979).

In this study, penetration of the host root epidermis took about 36 h from first attachment, and a total of 72 h after exposure to the stimulant substance. Although this stage of haustorial penetration of host root took relatively more time, the relatively high percentage of attachments passing through this stage indicated absence of interference by the host root tissue. The maximum number of haustoria (65%) attachments and cortical penetration was noticed after 72 h, and it declined gradually to 7% after 120 h. This decline corresponded to a progressive increase in the number of haustoria that had their cells reach the endodermis or established on host stele. The haustoria with their cells in the cortex after 120 h may represent those *Striga* seeds which may have delayed their germination and penetrated slowly through the host root epidermis.

Only a few attachments were able to reach the endodermis. After entry into the cortex, the haustorial cells took a minimum of 12 h to penetrate through the cortical cells and reach the endodermis. The minimum time required to reach the endodermis after attachment was 48 h, in contrast to the 8 – 24 h reported by Saunders (1933). Saunders reported that host cortical cells seriously obstructed the penetration of the haustorium in a resistant cereal cultivar. Our study shows that although many haustorial cells had penetrated through the epidermis there had been no penetration through the cortical cells. However, once they reached the endodermis, most cells successfully penetrated through the endodermis and established on the host stele. This may be due to the relative youth of the root segments that were inoculated in the present study. However, this is a slow process, taking a minimum of 24 h and attaining maximum establishment 60 h after contact with the endodermis. In this study minimum time taken from attachment was 72 h, and from exposure to host root to establishment was about 108 h.

Varietal differences exist in sorghum for various root morphological characters, such as cell wall thickenings, the presence of silica crystals in endodermis cells, and lignification of pericyclic cells (Saunders, 1942; Maiti, Ramaiah, Bisen & Chidley, 1984). The mechanical barriers in host root tissue may delay the penetration process of *Striga* haustorial cells to the extent that it consequently fails to establish. Therefore, screening host cultivars for their resistance to penetration through the various root tissues could be useful. The simplicity of the present technique could help identify cultivars that delay or inhibit the penetration process.

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