Primary Haustorial Development of *Striga asiatica* on Host and Nonhost Species

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ABSTRACT

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Initial interactions of *Striga asiatica* with a susceptible host and non-host plants were examined by histological methods. Haustorial development was initiated when radicles of *S. asiatica* were placed in contact with host or nonhost roots. Reorganization of the *S. asiatica* root apical meristem was rapid and involved the formation of a distal group of cells that penetrated the host or nonhost root. Penetration of the epidermis of

the host (sorghum) roots and advance into the cortex occurred within 24 to 48 h of inoculation. Penetration of the endodermis by the developing endophyte was delayed for 72 to 96 h after initial contact. However, upon penetration vascular continuity was established between parasite and host. In contrast, interactions with nonhosts provided evidence of active resistance mechanisms. Penetration of lettuce, marigold, and cowpea roots by *S. asiatica* was most frequently arrested in the cortex, and endophytic cells were necrotic 72 h after inoculation. Some species-specific differences were observed in the reactions of nonhosts to penetration, although in their general nature the interactions with *S. asiatica* were similar.

Striga asiatica (L.) Kuntze (Scrophulariaceae), witchweed, is an obligate root parasite of most of the world's agronomically important cereal grasses. *Striga* parasitism causes severe chlorosis, wilting, and stunting of susceptible hosts, resulting in yield losses that range from slight to 100% (5,7). Primarily found in Africa and Asia, *S. asiatica* was first reported to be present in the southeastern United States in 1956 (8). Despite efforts to eradicate this pest through the use of improved cultural practices, chemical controls, and resistant or tolerant cultivars, *S. asiatica* persists in the United States and continues to be a major limitation on agricultural production in regions of heavy infestation (7).

Since the subsistence farmers who populate the most threatened regions are unable to afford expensive chemical treatments for control of the pathogen and often find it difficult to adopt new cultural practices, the development of high-yielding host cultivars with durable resistance is of utmost importance for reducing the agricultural and social impact of *S. asiatica* (7). Despite decades of effort by breeders, cultivars with complete resistance have yet to be obtained for the major grain crops, including maize, sorghum, millet, and rice, although some promising cultivars have been identified (7). The possible sources and mechanisms of host resistance to parasitism by *Striga* spp. were recently reviewed by Ejeta and Butler (7). Among the various resistance mechanisms operating in hosts of *S. asiatica*, low production of germination stimulants affecting *S. asiatica* and direct inhibition of infection processes by the development of chemical or physical barriers appear to be most important. While penetration of host cultivars by *S. asiatica* has been described (6,16), the nature of interactions between *Striga* and nonhosts and the possible basis for nonhost resistance are not well understood. Heath (11) suggested that non-host resistance is important by virtue of its prevalence and durability. Characterization of defenses against the parasite and identification of developmental stages at which the parasite is vulnerable

to such defenses are of particular relevance. Information of this kind may be gained from histological comparison of incompatible interactions between *Striga* and nonhosts and successful penetration of hosts by the parasite.

Like other parasitic angiosperms, *S. asiatica* infects host plants by first forming a haustorium (27). This infection structure attaches to host roots and penetrates and establishes vascular connections with them. We use the term *haustorium* to refer to this infection structure at all developmental stages, from initiation through the establishment of vascular connections. Haustoria may form at the radicle or root apex and at lateral positions on the mature root upon induction by exogenous signals (13). In any case, haustorial development begins with the enlargement of cells in the protoderm or epidermis and underlying ground tissue and the initiation of haustorial hairs (23). Cells in the apex of the developing haustorium become specialized for penetration (2,3,20,21), and growth through the host epidermis and cortex is rapid. Haustorial maturation is completed when the host stele is penetrated and vascular connections are constructed between the haustorium and the host (24). The *Striga* seedling becomes largely autotrophic upon emergence from the soil but continues to procure water and nutrients from the host root system. In this study we used sorghum as the susceptible host, since it has been suggested that sorghum is likely to be the species upon which the cereal-parasitizing *Striga* spp. evolved (25).

Species normally resistant to parasitism by *S. asiatica* are considered to be nonhosts. In general, they include most nongramineous plants. Some broadleaf dicots (i.e., leguminous species, tobacco, sweet potato), however, are susceptible to parasitism by other *Striga* species, notably *S. gesnerioides* (Willd.) Vatke (7,25). Plants expressing nonhost resistance might lack the chemical signals or nutritional components necessary for initiating or sustaining the development of *S. asiatica*, or they may possess constitutive or induced general resistance that prevents parasitism. Reports describing the interaction of *Striga* with nonhosts are limited, and they differ in their descriptions of the extent to which penetration progressed. While penetration of soybean halted in the nonhost cortex and only rarely reached the endodermis (24),

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haustoria on several leguminous nonhosts and cotton established vascular connections (19). Also, penetration of leguminous nonhosts by *Alectra vogelii* Benth. (also a member of the Scrophulariaceae) halted in the cortex, and the endophytic cells became encapsulated and necrotic (28).

The goal of the present study was to determine, using histological methods, how the initial interactions of *S. asiatica* with a susceptible host differ from interactions with nonhosts. Differences were observed in the development of *S. asiatica* and in the responses of various plants to penetration.

MATERIALS AND METHODS

Specimen preparation and inoculation. Seeds of *S. asiatica*, obtained from the USDA Methods Development Center (Whiteville, N.C.), were surface-sterilized in 70% (v/v) ethyl alcohol for 30 s, rinsed in sterile distilled water, subjected to mild sonication in 0.5% (w/v) sodium hypochlorite for 3 min, and then rinsed three times in sterile distilled water. Disinfested seeds were then pretreated in sterile distilled water in the dark for 7 to 10 days at 27°C (22). Prior to inoculation of host or nonhost roots, pretreated seeds were germinated by incubation in 10^{-9} M strigol (USDA Southern Research Center, New Orleans, La.) in the dark for 24 h at 27°C (4).

Sorghum bicolor (L.) Moench cv. Golden Acres Y-45-G was the host species in our studies of haustorial development. This cultivar has been shown to be susceptible to parasitism by *S. asiatica* (15). Nonhost species included *Lactuca sativa* L. cv. Bibb (lettuce), *Tagetes erecta* L. cv. Crackerjack (marigold), and *Vigna unguiculata* (L.) Walp. subsp. *unguiculata* (cowpea). Additional nonhost species were used only in preliminary studies of haustorial initiation on nonhost roots. These species included *Abelmoschus esculentus* L. (okra), *Vigna radiata* (L.) R. Wilczek (mung bean), *Ricinus communis* L. (castor bean), *Linum usitatissimum* L. (flax), and *Nicotiana tabacum* L. cv. Xanthi (tobacco).

Host and nonhost seeds were surface-sterilized in 0.5% (w/v) sodium hypochlorite containing 0.01% (v/v) Tween 80 for 10 min and rinsed three times in sterile distilled water. Surface-sterilized seeds were germinated and grown under aseptic conditions on moistened filter paper in petri dishes (100 × 15 mm). After germination, seedlings were placed on filter paper in separate dishes and grown hydroponically for 2 wk in 5 ml of sterile distilled water and an additional 2 wk in sterile augmented Hackett's nutrient solution (with Na_2MoO_4 substituted for $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$) at pH 5.2 (10). Throughout the course of the experiment the solution was changed at weekly intervals.

Root tissue was produced by two additional methods. Seeds of host and nonhost species were sown in vermiculite and grown for 2 to 4 wk. The seedlings were watered daily and fertilized with augmented Hackett's nutrient solution at pH 5.2. Prior to inoculation, roots were rinsed free of vermiculite by washing them with sterile distilled water. The roots were then placed on filter paper in separate petri dishes and allowed to acclimate to hydroponic conditions for 24 h in sterile distilled water. Other seeds of host and nonhost species were sown in soil, allowed to mature for 2 wk, and transferred to an aeroponics system. The aeroponics system consisted of mist chambers continuously supplied with atomized half-strength Murashige and Skoog basal salt nutrient solution at pH 5.8 (18). The plants were grown aeroponically for 7 days and then transferred to petri dishes under hydroponic conditions. All host and nonhost plants were grown under discontinuous fluorescent lighting at approximately $65 \mu\text{mol m}^{-2}\text{s}^{-1}$ for 12 h per day at 28°C.

Roots were prepared for inoculation by positioning them over filter disks (3.5 cm in diameter) and aspirating the excess hydroponics solution, thus allowing the roots to settle on the disks. Inoculations were performed by placing radicles of *S. asiatica* in contact with roots. Sterile foam cylinders (2 × 1.5 cm) were placed

on the application sites in order to stabilize the radicle-root orientation. Following inoculation, 5 to 10 ml of sterile distilled water was added to each petri dish; the water was replaced daily throughout the remainder of the experiment. Roots were excised for histological observation at intervals from 1 to 4 days after inoculation.

Histopathology. A variety of histological methods were used to examine inoculated host and nonhost roots as well as preinoculation radicles of *S. asiatica*. To determine the progress of penetration, excised specimens were fixed (with 2.5% [v/v] glutaraldehyde in 0.2 M sodium cacodylate buffer, pH 7.2, at 20°C), rinsed for 1 h (in the same buffer), and cleared by autoclaving in 75% (v/v) lactic acid (20 min at 121°C). Cleared roots were stained for 4 s in 0.1% (w/v) acid fuchsin in 50% (v/v) ethyl alcohol and rinsed in distilled water. The tissue was then stained for 1 min in 0.05% (w/v) toluidine blue O (in 0.02 M sodium benzoate buffer, pH 4.4), destained for 30 to 120 s in 50% (v/v) ethyl alcohol, and rinsed in distilled water. Stained roots in 100% lactic acid were mounted on glass slides and stored at 4°C until observation.

Specimens were sectioned in order to observe the cellular morphology at the parasite-root interface. For light microscopy, specimen fixation and rinsing were followed by dehydration through a graded series of ethyl alcohol washes (30, 50, 70, 95, and 100%, v/v) at 12-h intervals at 4°C. Dehydrated specimens were infiltrated and embedded at room temperature in JB-4 Plus (Polysciences, Warrington, Pa.) and sectioned at a thickness of 2 to 3 μm with a glass knife. Sections in JB-4 were stained with toluidine blue O.

For transmission electron microscopy, specimen fixation and rinsing were followed by postfixation in 2% (w/v) OsO_4 at 20°C for 1 h. Postfixed specimens were rinsed three times, for 10 min each wash, in 0.1 M sodium cacodylate buffer, pH 7.2, at 20°C, dehydrated to 100% ethyl alcohol over 2 h in a graded series of alcohol, infiltrated with propylene oxide-resin mixture, and embedded in epoxy resin. Ultrathin sections were cut with a diamond knife, stained in 10% (w/v) uranyl acetate, and counter-stained in lead citrate (26). Sections were examined using a JEOL 100 CX Temscan microscope. For scanning electron microscopy, dehydrated specimens were dried with Tousimis Critical Point Drier, sputter-coated with gold palladium on a Technics Hummer Coater, and observed under an ETEC Autoscan microscope.

RESULTS

Vegetative radicle apex. The radicle apex of *S. asiatica* was diminutive prior to inoculation, approximately 60 to 80 μm in length and width. Well-defined protoderm, ground meristem, and procambial tissues were recognized, and two or three vacuolated root cap cells characterized the meristem (Fig. 1A). An extracellular pectin matrix was conspicuous on the root cap cells. The apical initials, numbering four in most roots, were subtended by densely protoplasmic cells that gave rise to the primary-tissue meristems. A quiescent center was not observed. The procambial zone was 20 to 25 μm wide and consisted of narrow elongated cells.

Haustorial initiation and attachment. Haustorial development began when radicles of *S. asiatica* were placed in contact with host or nonhost roots. Reorganization of the *S. asiatica* meristem occurred within 12 h of contact. The apical initials became indiscernible and primary-tissue meristems less clearly defined within 12 h of application. Densely protoplasmic cells of the protodermal and ground meristem regions ceased rapid division and began hypertrophy within 24 h of inoculation (Figs. 1 and 2). Haustorial hair development was acropetal during the early stages of haustorial differentiation but occurred more randomly in the later stages. Vacuolation of the densely protoplasmic protodermal and ground meristem regions also occurred in progression toward the apex, excluding a wedge-shaped cluster of protoplasmic cells located in the distalmost two or three cell layers and extending basipetally

into the haustorium. Numerous small vacuoles, densely staining regions, and distinct nuclei characterized the cytoplasm of cells in this cluster. The distalmost protoplasmic cells divided, flattened, and became abutted to the epidermis of the root during haustorial attachment. Haustorial initiation and attachment were observed on all nonhost species investigated in our preliminary studies. To provide a more quantitative assessment, observations of 1,000 seeds applied to roots of each of three species were made. Attachment frequencies were similar for sorghum (0.264), marigold (0.256), and lettuce (0.253).

Host penetration and establishment of vascular continuity.

On sorghum, further development of the haustorium principally involved differentiation of the distalmost protoplasmic cells. Elongation of these cells resulted in penetration of the host epidermis by mechanical force. Penetration of the host cortex was characterized by anticlinal and periclinal divisions in the distalmost cells and by further acropetal vacuolation of the haustorium. Vacuolation of the haustorium lagged behind penetration into the host cortex, so that the wedge of protoplasmic cells enlarged. The ultrastructure of the cytoplasm of these cells was characterized by numerous mitochondria; abundant endoplasmic reticulum; a large, distinct nucleus; and nonhomogeneous intercellular depositions (Fig. 3E). There was little detectable evidence of disruption by mechanical force in the host cells in the area where the parasite advanced into the cortex. Host cortical cells in close proximity to the penetration site did not exhibit hypertrophic or hyperplastic reactions. However, dark discoloration of the host tissue often occurred around the endophytic cells and was particularly notable at sites of multiple penetration.

Fig. 1. Vegetative radicle apex and haustorium of *Striga asiatica*. **A**, Vegetative apex prior to inoculation, with apical initials (arrowhead) and procambial zone (arrow) (bar = 10 μ m). **B**, Haustorium (right) on a sorghum root 72 h after inoculation (bar = 50 μ m).

Penetration of the host cortex was completed 48 to 72 h after inoculation, at which point the parasite was in contact with the endodermis. Each of the distalmost cells lengthened (by about 50%) and underwent anticlinal division upon reaching the endodermis. The result was the formation of a palisade arrangement of 20 to 30 densely protoplasmic cells (Fig. 3A). Vacuolated cells flanking the protoplasmic cells also elongated during cortical penetration. The cells in the palisade region were further subdivided by an oblique anticlinal division and were characterized by enlarged vacuoles, accumulated granular materials, and elongated, spindle-shaped nuclei.

Penetration of the host endodermis was typically delayed for 72 to 96 h after parasite-endodermis contact (Fig. 4A). Sixty percent of penetrations had reached the endodermis 48 h after inoculation; however, most did not begin to establish vascular connections until 144 h after inoculation. Acropetal and basipetal differentiation of vascular elements within the haustorium occurred concurrently with endodermal penetration. Penetration of host vessels followed endodermal penetration, and the vessel-penetrating cells differentiated into xylem elements and established vascular continuity between *S. asiatica* and sorghum (Fig. 3B). Typically, cotyledons of *S. asiatica* enlarged and broke free from the seed coats 24 h after xylem-to-xylem connections were constructed.

Nonhost penetration and characterization of incompatible interactions. Although haustorial initiation and development through attachment occurred on nonhost roots in a manner similar to that observed in associations between *Striga* and host roots, further haustorial development typically did not progress beyond the cortex of nonhost roots (Fig. 4B). The average extent of penetration of the nonhost cortex was not more than 25% of the distance from the root surface to the endodermis ($n = 25$) in cowpea and marigold. Penetration occurred to a lesser extent (10%) in lettuce. By 72 to 96 h after inoculation, the cytoplasm of the endophytic cells typically appeared degenerated (Fig. 3C and D). No further cell differentiation was observed in *S. asiatica* following the degeneration of the distal cells. Haustorial maturation, including vascular connection, was observed very infrequently (in less than 1% of cases) on cowpea, marigold, and lettuce. In these cases, no resistance reaction was evident in the nonhost cortex, and no change was found in the extent to which other endophyte penetrations progressed in the cortex of the same plant.

Some differences were observed among nonhosts in the response of cortical tissues to attempted penetration by *S. asiatica*. On lettuce, cortical cells in proximity to the penetration site exhibited little evidence of cytological activity or morphological changes 72 h after inoculation. However, these cells were necrotic in appearance, and their cell walls appeared degraded at later stages (Fig. 3C). On marigold, cytological activity was apparent in

Fig. 2. Differentiation of haustorial tissues of *Striga asiatica* at the time of inoculation of sorghum roots and 12 and 24 h after inoculation.

the cortex 72 h after inoculation. A notable increase occurred in the density of the cytoplasmic components of cortical cells in front of the endophyte, particularly numerous small vacuoles, densely staining regions, and enlarged nuclei (Fig. 3D). Intracellular wall appositions were observed in cortical cells of marigold 144 h after inoculation (Fig. 3F). These appositions appeared as electron-dense globules and were most prominent on the cell walls directly adjacent to the nonhost-parasite interface. Cortical cells containing appositions appeared necrotic at 144 h after inoculation. In marigold, cortical cells near infection sites also exhibited an unusual and distinctive green staining when treated with toluidine blue O (data not shown). In cowpea, no notable differences from other nonhosts were observed at the interface of cortex and endophyte. Cell necrosis along the flanks of the endophyte was often accompanied by dark-staining wall accumulations typical of all nonhost invasions.

Thickening of the cell wall at the *S. asiatica*-nonhost interface was observed in all of the nonhost plants examined. The thickening was predominantly associated with the cell walls of the endophyte. In addition, we also saw evidence of intercellular ac-

cumulation of deposits of unknown composition at the *Striga*-nonhost interface. This was most evident in the *Striga*-marigold associations.

DISCUSSION

Histological comparisons of sorghum, a typical host of *S. asiatica*, and several nonhost species during penetration by the parasite have provided initial information on the progression of haustorial development and the elicitation of resistance mechanisms in compatible and incompatible *Striga*-plant interactions. Our studies indicate that haustorial initiation and development through attachment occur on nonhost roots in a manner similar to that observed on roots of host species. However, the extent of endophyte development following attachment differed in host and nonhost species, suggesting that the later stages of parasite establishment may have greater importance in determining host specificity. It has been suggested that the host range of *Striga* spp. is mediated by a variety of factors that affect the ability of the parasite to recognize a specific host (25). Host recognition is thought to occur

Fig. 3. Histology of the *Striga*-root interface. **A**, Palisade cells formed upon contact of the endophyte with the endodermis (arrowhead) of a sorghum root 72 h after inoculation (bar = 20 μ m). **B**, Connection of vascular elements of the endophyte (arrowhead) with vascular elements of sorghum 144 h after inoculation (bar = 20 μ m). **C**, Arrested development of the endophyte in the cortex of a lettuce root 120 h after inoculation. Note the necrotic appearance of endophytic cells at the endophyte-root interface (arrowhead) (bar = 20 μ m). **D**, Arrested development of the endophyte in the cortex of a marigold root 72 h after inoculation. Note the presence of cortical cells (arrowhead) containing dense cytoplasm in front of the endophyte-root interface (bar = 20 μ m). **E**, Ultrastructure of central cells of a haustorium on a sorghum root 72 h after inoculation. Note the presence of numerous mitochondria (arrowhead), endoplasmic reticulum, and non-homogeneous intercellular depositions (arrow) (5,000 $\times$). **F**, Wall appositions in cortical cells of a marigold root adjacent to the interface with the endophyte (arrowhead) 144 h after inoculation (3,000 $\times$). The appositions are on the cell walls closest to the endophyte.

during several developmental stages early in the parasite life cycle. For example, seed germination occurs only in response to certain exogenous plant-derived chemical signals (e.g., strigol, strigol-like compounds, sorgoleones). Although some expression of partial host resistance has been attributed to low production of germination stimulant, roots of numerous nonhost species exude compounds that are capable of stimulating germination (25). Haustorial initiation is also known to be induced by specific classes of plant-derived compounds and has been considered a potential site for regulating host recognition (14,19).

In the studies described above, we observed that haustorial initiation and attachment occurred on the roots of all nonhost species tested, suggesting that later (i.e., postattachment) stages of parasite development may have greater importance in determining host specificity. Among the later developmental stages of *S. asiatica* are penetration of host tissue, physiological compatibility following the establishment of vascular connections, and maturation of the parasite to reproductive stages (19,25). Haustorial development on sorghum has proved to be a valuable model of successful penetration by *S. asiatica* and has allowed us to document developmental events that characterize the compatible interaction. In the current study, postattachment haustorial development on sorghum can be summarized as follows: (i) the host epidermis was penetrated by elongating distal cells; (ii) continued periclinal and anticlinal divisions of these cells led to rapid advancement into the host cortex, but with little mechanical disruption; (iii) upon reaching the endodermis, the distalmost cells elongated and divided anticlinally and oblique-anticlinally, so that a palisade arrangement of cells was formed; (iv) penetration of the endodermis was delayed, but upon its occurrence vascular continuity was established between parasite and host.

The endodermis is generally considered a substantial barrier to vascular penetration by root pathogens, and in fact it has been reported to be the site of resistance expression in sorghum cultivars resistant to *S. asiatica* (17). The delay in endodermal penetration we observed in the sorghum cultivar Golden Acres Y-45-G may represent the expression of a minimal level of partial resistance. However, since there is little or no information available in the literature, it is not possible for us to directly compare the results of our analysis of the temporal aspects of endodermal penetration with previous findings.

Our studies did reveal that nonhost resistance to *Striga* parasitism is expressed after haustorial attachment, typically as the endophyte begins cortical penetration. Although the nature and extent of the resistance response differed among nonhost species, penetration was most often arrested in the outer cortex of lettuce, marigold, and cowpea. In each of these species, degeneration of the distalmost cells of *S. asiatica* was observed 72 h after inoculation. The accumulation of cytotoxic compounds is more likely to have caused the observed degeneration of the endophytic cells than the expiration of the nutrient reserves of the developing *Striga* seedlings. This hypothesis is supported by the necrotic appearance of cortical cells near the nonhost-parasite interface and by the observation that haustorial maturation on sorghum requires significantly longer than 72 h. One cannot rule out the possibility that the endophyte is capable of acquiring nutrients or other components necessary for growth from host tissue prior to haustorial maturation and that these compounds are either absent or unavailable in nonhost tissues during the same period. Cortical necrosis was most pronounced in lettuce and involved substantial degradation of cell walls. In contrast, the initial reaction of cortical cells of marigold indicated increased cytological activity, with elevated quantities of cytoplasmic components in cortical cells several layers internal to the nonhost-parasite interface. The deposition in cortical cells of marigold is likely to have resulted from this increased metabolic activity.

If one considers the nature of the resistance response expressed by each nonhost, it is possible to speculate about the possible factors that evoke these incompatible interactions. Nonhosts used in the current study fall into one of two broad categories based upon their biological interactions with *Striga* spp. Lettuce and marigold are of one type; cowpea is of the second. Neither lettuce nor marigold commonly serve as a host for any *Striga* species. *S. asiatica* would therefore be considered to elicit a general or basic resistance response in these plants (12). This type of response is thought to be not the direct result of selection imposed by the pathogen in question but rather the result of "diffuse coevolution" between the plants and their antagonists (9). Even though the mechanisms of resistance may vary, attempted penetration of lettuce and marigold demonstrates the effectiveness of general resistance against haustorial penetration. Even in the rare cases in which haustorial penetration of the vascular tissue occurs, matu-

Fig. 4. Penetration of host and nonhost roots by *Striga asiatica*. **A**, Penetration into sorghum root tissues by tissue type over time ($n = 50$). The distance across the cortex was divided into thirds to delineate the outer, mid-, and inner cortex regions. Note the delay between reaching the host endodermis (48 to 72 h after inoculation) and the establishment of vascular connections (144 h after inoculation). **B**, Advance of *S. asiatica* into sorghum and nonhost roots 144 h after inoculation (sorghum, $n = 29$; cowpea, $n = 27$; marigold, $n = 23$; lettuce, $n = 27$). Penetration was most often arrested in the cortex of nonhost roots.

ration of the parasite does not appear to proceed further in seedling development (19), suggesting the possibility of a fundamental physiological incompatibility between *Striga* and these nonhosts. Therefore, while nonhost determination of lettuce and marigold may not involve recognition at seed germination or haustorial initiation, multiple barriers to parasitism may exist at later developmental stages.

Although cowpea is not parasitized by *S. asiatica*, it is a suitable host of *S. gesnerioides*. This species differs from *S. asiatica* principally in host range, haustorium size, and minor aspects of foliar morphology. However, the general nature of parasite development is the same for the two species, and by inference we assume that the majority of the physiological processes during parasitic establishment are also similar. Cowpea is native to regions of Africa also thought to be the origin of *S. gesnerioides*. These two species have thus coexisted, and resistance found in certain cultivars of cowpea is thought to be an adaptation resulting from selection imposed by *S. gesnerioides* (1). Cowpea resistance to *S. gesnerioides* consists of varying levels of strain-specific resistance in different cultivars (7). It is possible that resistance mechanisms in cowpea, if expressed, are similar when the plant is challenged by *S. asiatica* or *S. gesnerioides*.

The in vitro inoculation method developed for this study is very similar to that presented and discussed by Lane et al. (14). These authors, investigating the penetration of cowpea by *S. gesnerioides*, found the method suitable for cytological studies of host resistance. Benefits include precise application of *Striga* radicles, easy monitoring and excision of inoculated roots, and control of the physical and chemical environments. Although many environmental factors differed from naturally infested soil, the consistency of our observations with previous reports of compatible host penetration suggests this is an appropriate method for the study of *Striga*.

Further investigations concerning resistance elicitation and expression in hosts and nonhosts of *Striga* spp. are needed. Comparisons of reciprocal interactions of *S. asiatica* and *S. gesnerioides* with leguminous and gramineous hosts of each may be especially important. Also, documentation of the expression of nonhost resistance, such as that observed in lettuce and marigold, may provide more details about the specificity of exogenous signals at stages of *S. asiatica* development and about the expression of durable, general resistance by nonhosts.

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