Genetic Structure and Analysis of Host and Nonhost Interactions of *Striga gesnerioides* (Witchweed) from Central Florida

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ABSTRACT

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Striga gesnerioides is a root hemiparasite of wild and cultivated legumes, among which cowpea (*Vigna unguiculata*) and *Indigofera hirsuta* are suitable hosts. In this study, we examined the genetic structure and host-parasite interaction of a strain of *S. gesnerioides* parasitizing *I. hirsuta* (SGFL) from central Florida (United States). Amplified fragment length polymorphism analysis was used to compare genetic profiles from 71 individual *S. gesnerioides* plants (SGFL) representing four different populations in central Florida. Our results showed that these individuals are genetically similar, with pairwise genetic distances ranging from 0.00 to 0.037. A cluster analysis grouped all four *S. gesnerioides* populations from Florida, separating them from *S. gesnerioides* isolates parasitic on *I. hirsuta* and cowpea collected from West Africa.

One *EcoRI* and *MseI* selective primer combination generated a 510-bp fragment present in individuals from the SGFL and the West African isolate parasitic on *I. hirsuta*, but absent in isolates parasitic on cowpea. Germination of seed from individuals of all four populations of *S. gesnerioides* parasitic on *I. hirsuta* from Florida was stimulated by root exudates from cowpea genotypes Blackeye and TVX-3236, known to be highly susceptible to all races of *S. gesnerioides* parasitic on cowpea in West Africa. SGFL seedlings failed to parasitize cowpea, with the development of attached SGFL seedlings arrested at the tubercle stage. The very high level of genetic uniformity observed within and among the central Florida populations suggests that there was likely a single introduction of the parasite or strong host-driven selection to genetic uniformity. These findings are important in assessing the potential of the parasite as an agronomically significant pest in the United States.

Additional keywords: germination stimulation, incompatibility.

The genus *Striga* (family *Scrophulariaceae*) is composed of ≈ 50 species, most of which are obligate parasites of tropical cereals and legumes (7). The most agronomically important species of the genus are *S. hermonthica* (Del.) Benth, *S. asiatica* (L.) Kuntz, and *S. gesnerioides* (5,33). All three species associate intricately with their hosts and constitute a serious threat to food crop production in Africa (24) by parasitizing the roots of crop plants and serving as water and nutrient sinks (35). *S. hermonthica* and *S. asiatica* are most devastating on cereal crops. In West Africa, they cause serious yield losses to staple food crops such as corn (*Zea mays*), sorghum (*Sorghum bicolor*), upland rice (*Oryza sativa*), and pearl millet (*Pennisetum glaucum*) (1). *S. gesnerioides*, on the other hand, attacks cowpea (*Vigna unguiculata*), and results in 100% yield loss in some instances (16). It also is reported to attack tobacco (*Nicotiana tabacum*) in South Africa (28,41).

Through an elegant system of chemical signaling, *Striga* spp. recognize a potential host plant and regulates its development in order to optimize its chances for survival. Germination, haustorial induction, attachment to, and penetration of the host vascular system are all critical events in the *Striga* life cycle. *Striga* seed are nearly microscopic, averaging ≈ 0.3 by 0.2 mm and weighing 6 μg (31), with each plant capable of producing between 50,000 and 500,000 seed (1) per generation. Prior to germination, the seed must undergo an after-ripening period of ≈ 2 to 3 months. This after-ripening period must be followed by a preconditioning period during which the seed imbibe water at a suitable temperature (44) of ≈ 27 to 30°C for at least 7 days. Seed germination in *S. hermonthica* and *S. asiatica* has been reported to increase with

the length of preconditioning period for up to 3 weeks (2,6,44). The final step for germination to proceed is the exposure of the preconditioned seed to a specific chemical signal produced by the host roots (36,44). An array of molecules which differ in chemical structure and specific activity stimulate the germination of *Striga* seed (4,10,18,44). Following germination, a second host-derived chemical signal from the root, known as the haustoria initiation factor (HIF), is required for the differentiation of the radicle into the haustorium, by which the *Striga* seedling attaches to and penetrates the host root (7,13,34). When the vascular connection is established between the host and the parasite following penetration of the stele by the haustorium, the development of the haustorium ceases (13,37). As the vascular connection is established, the *Striga* seedling enlarges, forming a thick mass of tissue called the tubercle. This structure, which may be irregular in shape, enlarges as it differentiates.

Approximately half of the *Striga* life cycle is underground, during which time much of the damage to the crop host occurs, in part, through the diversion of host resources to the parasite. It has been estimated that the loss of carbon from the host by export to the parasite is more important than reduced photosynthetic capacity of the host imposed by the parasite in accounting for observed reduction in host growth in the cowpea-*S. gesnerioides* interaction (19). The damage done to the crop host is greater than can be explained by nutrient diversion alone. It is reported that the exchange of chemicals is bi-directional and that the parasite possibly releases some toxins that inhibit the growth and development of the host (15,19,20). This argument is supported by the fact that a small portion of ^{14}C -labeled CO_2 taken up by photosynthetically active *Striga* plants eventually could be traced in the host plant (35). Also, in vitro culture studies revealed that, after establishment on the host root, the host plant provides the parasite not only moisture, minerals, and photosynthate, but also addi-

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tional chemical signals required for further growth and development (8). Once above ground on a compatible host with adequate moisture, the parasite grows rapidly to produce numerous seed that can remain viable in the soil for well over a decade.

Morphological variation exists within *S. gesnerioides*, with isolates from various locations distinguishable by stem succulence, internode length, flower color, and size (26–28,31). Field observations also suggest that certain morphotypes may differ in their host specificity (25,28,31).

S. gesnerioides was first reported in the United States in 1980, from Polk County, Florida (22). This strain of *S. gesnerioides* (designated as SGFL) is distributed in five counties in central Florida where it mainly parasitizes *Indigofera hirsuta* and, less frequently, small-flowered morning glory (*Jacquemontia tamnifolia*) and one-leaf clover (*Alysicarpus vaginalis*) (28). Sunflower (*Helianthus annuus*) and sweet potato (*Ipomea batatas*) also have been reported to support the growth and flowering of SGFL in pot tests, although there are no reports of such attacks under field conditions (28).

The current distribution and host range of *S. gesnerioides* in the United States is not known, and there has not been any attempt to analyze the genetic variation within this strain, or the possibility of this parasite becoming an agronomically significant pest in the United States. As a result of its autogamous mode of reproduction, minimal disturbance, and the seclusion from its counterparts in Africa and Asia, the *S. gesnerioides* from central Florida offers an interesting system to study species evolution. In this study we report the first attempt to determine the genetic structure and variability of the Florida strain of *S. gesnerioides* at the molecular level. We also examine and report on the germination and postattachment interaction of this strain with host and nonhost species.

MATERIALS AND METHODS

Terminology. In this study, we use the term “strain” to refer to isolates of *S. gesnerioides* that are adapted to parasitize a specific host species. The strain of *S. gesnerioides* that parasitizes *Indigofera hirsuta* in Florida is designated as SGFL. A race is defined as a host species-specific variant, distinguishable by its differential ability to parasitize cultivars of the same host species. There are six defined races of the West African strain of *S. gesnerioides* that parasitize cowpea and are designated as SG1 to SG6.

Field collection of plant materials and seed. Four independent collections were made of *S. gesnerioides* plants parasitizing *I. hirsuta* in and around Ft. Meade, FL (81°56.62' and 27°47.03') in fall 2001. The collection sites were separated by at least 10 km. The different populations sampled are designated SGFL1, SGFL2, SGFL3, and SGFL4. At each site, *S. gesnerioides* seed were collected in bulk and grown on *I. hirsuta* in the laboratory to obtain fresh plant tissue for DNA extraction. In addition, 56 individual *S. gesnerioides* plants and mature seed were collected from the SGFL1 population and maintained separately on an individual plant basis for later use. The vegetative tissues were placed in polyethylene bags and immediately kept on dry ice overnight and sent to the University of Virginia, Charlottesville. Upon arrival, samples were removed and kept at –80°C for long-term storage until DNA extraction. All seed and plant materials were handled according to APHIS requirements.

Growth and preparation of plant and seed materials from *S. gesnerioides* in the laboratory. Following an after-ripening period of 3 months, the bulk-collected seed for SGFL2, SGFL3, and SGFL4 were threshed. Approximately 500 *S. gesnerioides* seed were infested in 6-in. plastic pots containing 30:70% (vol/vol) Metro-Mix250 (Scotts-Sierra Horticultural Product Company, Marysville, OH) and sand. Each collection was used to infest 12 pots (replications) and planted with six *I. hirsuta* seed (purchased from Wise Seed Co. Inc., Frostproof, FL). The number

of *I. hirsuta* seedlings per pot was reduced to 4 at 2 weeks postplanting. At 15 weeks postplanting, emerged individual *S. gesnerioides* plant tissues were collected, flash frozen in liquid nitrogen, and stored at –80°C until used for DNA extraction.

DNA extraction and amplified fragment length polymorphism profile analysis. Total genomic DNA was extracted from individual *S. gesnerioides* plants using the cetyltrimethylammonium bromide method as described previously (40), with the exception that a double phenol/chloroform precipitation was employed in order to remove phenolic compounds not removed during the first precipitation. Amplified fragment length polymorphism (AFLP) analysis was performed essentially as described previously (42), with some modifications. A selective amplification using 1 µl of a 10-fold dilution of the pre-amplification product was carried out using pairwise combinations of [γ -³²P]-ATP (Perkin-Elmer Inc., Boston) 3' end-labeled *Eco*RI and *Mse*I primers with three selective nucleotides (designated E for *Eco*RI and M for *Mse*I) using a “touchdown” procedure. The primer combinations and their selective nucleotides used in this analysis were E-ACC + M-CAT, E-AGA + M-CAC, E-AGG + M-CAC, E-AGG + M-CAT, and E-ACG + M-CAT. Profiles were generated from DNA extracted from 71 SGFL individuals and 10 cowpea-*S. gesnerioides* individuals. In addition, DNA isolated from 20 individual *S. gesnerioides* plants parasitic on *I. hirsuta* collected from Pahou, Benin Republic (6°23.14'N and 2°13.17'E) were included in the analysis. The *S. gesnerioides* plants parasitic on *I. hirsuta* from West Africa are collectively designated as SGIA.

Preparation of host and nonhost root exudates and germination stimulation assays. Preconditioning of *Striga* seed from central Florida (SGFL) and West Africa (SG1 from Burkina Faso, SG3 from Nigeria, SG4 from Benin, and SG5 from Cameroon) was carried out essentially as previously described (1,3). The seed were surface sterilized in a solution of 1% sodium hypochlorite for 5 min, rinsed with distilled water, and then placed in a benomyl solution at 100 µg/ml for 30 min. This was rinsed five times with distilled sterile water. *Striga* seed then were soaked in distilled water in petri dishes sealed with parafilm. Petri dishes were wrapped in aluminum foil and placed in the incubator at 28°C. Seed were sonicated using a W-220 sonicator (Heat Systems-Ultrasonics, Inc., Farmingdale, NY) for 60 s and the water replaced with fresh distilled water every other day for over a period of 14 days.

Host root exudates were obtained from 12-day-old cowpea plants and 21-day-old *Indigofera* plants growing in vermiculite by washing the roots of 10 to 15 plants (per 6-in. plastic pot) with 250 ml of distilled water. In each case, the percolated water was collected in a beaker and then filter-sterilized using Nalgene disposable filter units (Nalge Nunc International, Rochester, NY) with a pore size of 0.45 µm. Root exudates were stored at 4°C until used. Prior to the exposure of the *Striga* seed to the root exudate, the seed were further surface sterilized with 25% (vol/vol) commercial bleach for 15 s and rinsed five times with distilled sterile water. In all, ~150 preconditioned *Striga* seed were placed in culture plates in three replications, and allowed to air dry in a laminar hood for 25 min. This was followed by the addition of 150 µl of root exudates, sealed with parafilm and then wrapped with aluminum foil. These set-ups then were placed in the incubator at 28°C for 3 days, after which germinated and ungerminated seed were counted using a light microscope. Sterile distilled water was used in place of host roots exudates as control.

Analysis of host- and nonhost-parasite interactions. In order to evaluate for the pathogenicity of the SGFL strain with cowpea, a second series of pot trials was set up in which seed of the four populations (SGFL1 through SGFL4) were used to infest pots planted with two cowpea genotypes, TVX-3236 and Black-eye, known to be susceptible to all races of *S. gesnerioides* parasitic on cowpea in West Africa (5). Pot trials involving the cowpea-specific strains SG1, SG3, SG4, and SG5 with TVX-3236

and Blackeye, and SGFL with *I. hirsuta* interaction, were included as controls. The experiments were set up in a completely randomized design with three replications and repeated three times.

Number of parasite attachments and *Striga* emergence data were recorded at 10 weeks postinfestation or planting for *S. gesnerioides*–cowpea set-ups (compatible interaction), and at 16 weeks for *I. hirsuta* plants infected with cowpea-adapted *S. gesnerioides* strains (incompatible interaction), and for the *I. hirsuta* plants infected with *Indigofera*-adapted *S. gesnerioides* (SGFL) (compatible interaction). Set-ups were sampled destructively to evaluate for postattachment interaction. The plant-per-soil mass from each pot was removed and immersed in a bucket of water, then agitated gently to loosen the soil mass off the host roots. Roots of the infested cowpea and *I. hirsuta* plants were washed free of soil and then examined under a dissecting microscope for attached *Striga* spp., necrotic hypersensitive lesions, and tubercles.

Histological analysis. Thin sections (25 μ M) of *Striga* tubercles were prepared from samples taken from each of the three types of *Striga*–host interactions described above. Specimens were sectioned using a freezing microtome (cryostat) at -26°C after fixing the specimen in tissue freezing medium (Triangle Biomedical Sciences, Durham, NC). Sections were stained with phloroglucinol (10 mg/ml) and visualized with the aid of a light microscope mounted with a Leica DC 200 digital camera. Images were captured at a resolution of 1,900 by 1,200 pixels.

Statistical analysis. Data from the germination assay and *Striga* spp. emergence counts each were subjected to analyses of variance (ANOVA) by the GLM procedure of the Statistical Analysis System (SAS, rel. 8.2; SAS Institute Inc., Cary, NC). ANOVA was done with square root-transformed values for the germination assay. For the *Striga* spp. emergence data, a constant was added in order to normalize data sets with values of zero prior to ANOVA. Separation of treatment means was done by the least significance difference.

Bands in the AFLP profiles of each individual plant were scored as present or absent (1 or 0, respectively) and subjected to sequential clustering algorithm using the unweighted pairgroup method with arithmetic means (UPGMA) (38). The relationships within populations and within and among different collections and strains of *S. gesnerioides* were generated using the phylogenetic analysis using parsimony (PAUP) software (version 4.0b10) (39) and presented in a dendrogram. The reliability of each cluster in the dendrogram showing plant-to-plant relatedness was confirmed by bootstrap analysis (14) with 1,000 replications using PAUP. Genetic distances were estimated using PAUP, applying pairwise comparison as described previously (29). The formula used was $Gd_{xy} = 1 - [2N_{xy}/(N_x + N_y)]$, where N_{xy} is the number of fragments shared between individuals x and y and N_x and N_y are the total number of fragments scored in x and y , respectively. Genetic distances are defined on a scale ranging from 0.00 (when all the bands for any two individuals are identical) to 1.00 (when the bands for any two individuals are all different).

RESULTS

Genetic distances and phenetic relationships. In order to evaluate the intra- and interpopulation genetic structure of *S. gesnerioides* parasitic on *I. hirsuta* (SGFL) in central Florida, and the relationship of this strain to other *S. gesnerioides* strains, AFLP profile analysis was carried out using five different combinations of *Eco*RI- and *Mse*I-selective primers. In all, 71 plants representing four different populations, SGFL1 to SGFL4, were evaluated along with individuals from *S. gesnerioides* populations parasitic on *I. hirsuta* from Benin, West Africa (SGIA) and individuals representative of different races of *S. gesnerioides* parasitic on cowpea from West Africa (e.g., SG1, SG3, and so on). The

number of scorable AFLP markers ranged from 87 (E-AGA + M-CAC) to 148 (E-ACC + M-CAT) and, in all, 680 fragments were scored from analysis using five different *Eco*RI- and *Mse*I-selective primer combinations.

Over 98% of all the bands scored were identical among the 71 individual SGFL plants evaluated. Pairwise genetic distances between individuals within the SGFL1, SGFL2, SGFL3, and SGFL4 populations ranged from 0.000 to 0.067. Genetic distances between the individuals from the four SGFL populations and SGIA ranged from 0.240 to 0.330. Slightly lower genetic distances (ranging from 0.214 to 0.274) were found between *Striga* seedlings parasitic on cowpea and *I. hirsuta* from West Africa (SGIA). The largest pairwise genetic distances were obtained between individuals in the SGFL populations and those parasitic on cowpea from West Africa, with values ranging from 0.320 to 0.390.

Each of the five primer combinations used in this study readily generated at least one marker fragment that discriminated between *S. gesnerioides* strains parasitic on *Indigofera* spp. (SGFL) and those parasitic on cowpea. The *Eco*RI- and *Mse*I-selective primer combination E-AGG + M-CAC generated a fragment of ≈ 510 bp that was present in SGFL individuals and the West African isolate parasitic on *I. hirsuta* (SGIA), but absent in *S. gesnerioides* representative of the races of the parasite that parasitize cowpea in West Africa. The primer combination E-AGA + M-CAC generated fragments of ≈ 415 , 435, and 465 bp that discriminated the cowpea–*S. gesnerioides* interaction from all four SGFL populations (i.e., SGFL1 through SGFL4).

The phenetic relationships among SGFL, SGIA, and cowpea–*S. gesnerioides* are shown in Figure 1. Two major clusters are formed, with all individuals in the SGFL1, SGFL2, SGFL3, and SGFL4 populations forming one cluster, and SGIA and cowpea–*S. gesnerioides* the other. The cowpea–*S. gesnerioides* from West Africa is the most distantly related of the forms analyzed. Genetically, cowpea–*S. gesnerioides* is more similar to SGIA (parasitic on *I. hirsuta* from West Africa) than any of the populations (SGFLs) parasitic on *I. hirsuta* in the United States.

Germination stimulation and postattachment development on host and nonhost species. To evaluate the host–parasite interactions, germination stimulation assays and pot tests of parasite growth and emergence were carried out on host and nonhost plants (i.e., *Indigofera* spp. and cowpea). The mean percent germination following 72 h of exposure is presented in Table 1. Germination was observed in all combinations of host root exudates and parasite seed tested, with root exudates from the cowpea genotypes (Blackeye and TVX-3236) and *I. hirsuta* stimulating seed germination of both SGFL and SG1-5. The differences in the level of germination were highly significant ($P = 0.01$), with cowpea–*S. gesnerioides* race SG5 from Cameroon and root exudates from cowpea cv. Blackeye having the highest germination (97%). Of the *S. gesnerioides* races parasitic on cowpea, SG3 from Nigeria showed the lowest germination response to root exudates from *I. hirsuta* (66%). All the control germination assays using sterile distilled water in place of the root exudates produced no germination.

The number of emerged *Striga* plants following parasitism on cowpea and *I. hirsuta* was significantly different ($P = 0.01$), with absolute values ranging from 9.0 (cowpea–*S. gesnerioides* race SG1 from Burkina Faso and *I. hirsuta* combination) to 96.0 (cowpea–*S. gesnerioides* race SG5 from Cameroon on TVX-3236 combination) (Table 2). The *Striga* spp. emergence count for all combinations with cowpea cv. Blackeye was low relative to the level of germination stimulated by root exudates from this genotype, when compared with a corresponding data set for TVX-3236 (Tables 1 and 2). Interestingly, although cowpea genotypes Blackeye and TVX-3236 are known to be susceptible to all races of *S. gesnerioides* from West Africa, and could stimulate germination of *Indigofera*–*S. gesnerioides* (SGFL) seed from central

Florida (Table 1), no *Striga* spp. emergence was recorded for any of the combinations, Blackeye and SGFL or TVX-3236 and SGFL (Table 2). Postattachment evaluations of all interactions in the combinations Blackeye + SGFL and TVX-3236 + SGFL showed that the development of attached SGFL *Striga* plants on these cowpea genotypes is arrested after tubercle formation (Fig. 2B). In the compatible interactions involving cowpea-*S. gesnerioides* from West Africa with cowpea genotypes Blackeye and TVX-3236 (Fig. 2A), and *S. gesnerioides* strain SGFL from central Florida with *I. hirsuta* (Fig. 2C), the development of attached *S. gesnerioides* plants progressed to the completion of the life cycle.

Histological analysis of tubercles formed in compatible and incompatible interactions. To further evaluate the nature of the

TABLE 1. Germination of *Striga gesnerioides* seed after 72 h of exposure to root exudates from two cowpea genotypes (Blackeye and TVX-3236) and *Indigofera hirsuta*

Source country	Race, strain	Host root exudate, mean germination(%) ^z		
		Blackeye	TVX-3236	<i>I. hirsuta</i>
Burkina Faso	SG1	92 ab	94 ab	72 d
Nigeria	SG3	96 a	NA	66 d
Benin Republic	SG4	72 d	86 bc	87 bc
Cameroon	SG5	97 a	NA	90 abc
United States	SGFL	83 c	92 ab	96 a

^z Means followed by the same letter in the same column are not significantly different (*P* = 0.01, least significant difference). NA = data not available.

Fig. 1. Dendrogram showing the phenetic relationship among different strains of *Striga gesnerioides* parasitic on *Vigna unguiculata* (cowpea-*S. gesnerioides* from West Africa), and *Indigofera hirsuta* (SGIA from West Africa and the United States [SGFL1 through SGFL4]). Dendrogram was generated as described in Materials and Methods, and based on amplified fragment length polymorphism profile analysis data of individual *S. gesnerioides* plants obtained using five *Eco*RI- and *Mse*I-selective primer combinations. Bootstrap values are indicated at each branch node.

host–parasite interactions, histological studies were carried out on thin sections prepared from tubercles obtained from a compatible interaction involving the *Striga* spp.-susceptible cowpea cv. Blackeye and *S. gesnerioides* race 3 (SG3 from Nigeria), and an incompatible interaction involving cowpea cv. Blackeye and *S. gesnerioides* strain SGFL (from Florida). In contact with a susceptible host plant such as Blackeye, SG3 penetrates the host root cortex, develops a tubercle, and forms xylem–xylem linkage with the host vascular system. Tubercles from such interactions (Fig. 3A and C) show organized vascular tissues that extend to the developing cotyledons. In contrast, in incompatible interactions, such as that occurring when the *Indigofera* spp.-adapted strain SGFL attempts to parasitize the highly SG3-susceptible cowpea cv. Blackeye, development of the parasite is arrested at tubercle formation. Although a tubercle develops, it appears to lack internal organization and there is no evidence of either vascular development or connection between parasite and host (Fig. 3B and D).

TABLE 2. Number of emerged *Striga gesnerioides* plants following parasitism on two cowpea genotypes (Blackeye and TVX-3236) and *Indigofera hirsuta*

Source country	Race, strain	Mean <i>Striga</i> spp. emergence ^z		
		Blackeye	TVX-3236	<i>I. hirsuta</i>
Burkina Faso	SG1	18 ef	73 b	9 g
Nigeria	SG3	23 d	54 c	13 fg
Benin Republic	SG4	29 d	80 ab	22 de
Cameroon	SG5	27 d	96 a	NA
United States	SGFL	0 h	0 h	24 d

^z Means followed by the same letter in the same column are not significantly different ($P = 0.01$, least significant difference). NA = data not available.

DISCUSSION

The level of genetic uniformity reported here within and among different collections of *Indigofera*–*S. gesnerioides* (SGFL) from central Florida is very high. The extremely low genetic variation, with pairwise genetic distances between individuals ranging from 0.000 to 0.037, may be attributed to a number of factors. First, the species is autogamous, with a floral biology that makes any possibility for outcrossing very low. Unlike most other *Striga* spp. where powdery pollen is formed, *S. gesnerioides* produces pollen plugs and, thus, genetic variation that may result from outcrossing is practically impossible. With autogamy, it would be expected that even a single *Striga* sp. seed that successfully parasitizes a host could colonize a given locality readily, whereas minor variations rapidly become genetically fixed, and allele frequencies would be either 1 or 0. Apart from the very strong autogamous mode of reproduction, *Indigofera* witchweed from central Florida is isolated from its counterparts in Africa and Asia, so that changes of population structure or composition through mechanisms such as gene flow is highly restricted. It is likely that SGFL became established in the five counties in central Florida following a single introduction in Polk County and then spread to other counties with time, possibly with *Indigofera* seed. It might well be that there were multiple introductions that experienced a host-driven selection leading to genetic uniformity in this strain of the parasite. SGFL has been reported to parasitize mainly *I. hirsuta* in five counties in central Florida (28). We have shown that race formation in cowpea–*S. gesnerioides* was largely a result of host-driven selection (5). Given that SGFL shares a common marker fragment (510 bp) with the *S. gesnerioides* isolate from West Africa that parasitized *I. hirsuta*, it is likely that SGFL experi-

Fig. 2. Representative post-attachment interactions between two strains of *Striga gesnerioides* host and nonhost species. A, *S. gesnerioides* race SG1 from Burkina Faso developing on the susceptible cowpea cv. Blackeye. Arrows indicate various individual *Striga* seedlings. B, *S. gesnerioides* strain SGFL, adapted for growth on *Indigofera hirsuta* growing on the susceptible cowpea cv. Blackeye. Arrows indicate various attached *Striga* seedlings arrested at the tubercle stage of development. C, *S. gesnerioides* strain SGFL developing on its host, *I. hirsuta*.

enced a host-driven selection to genetic uniformity. Jerome and Ford (23) showed that host identity (host-driven selection), among other factors, contributed to race formation in dwarf mistletoe (*Arceuthobium americanum*). Allozyme analysis of *S. asiatica* collected from well-separated locations within North and South Carolina showed that the populations were monomorphic at 32 genetic loci encoded by 18 different isoenzymes (43). Pavlista (32) suggested that the establishment of *S. asiatica* in the United States likely involved one or a few seed which, in conjunction with low recombination rate, may have led to the rapid loss of variation during early stages of the infestation.

How *S. gesnerioides* was introduced into the United States remains unknown. One possibility is that seed of the parasite were present as contaminants in *I. hirsuta* seed (28). As a result of its ability to fix nitrogen, *I. hirsuta* currently is being used for the reclamation of phosphate mining sites in central Florida. Unlike *S. asiatica*, which was first reported in the United States in 1956, spread rapidly, and was considered a major threat to U.S. agriculture (17), *S. gesnerioides* thus far has not achieved agronomic significance in the United States. In fact, despite the lack of any

conscious effort at containment, there is little evidence for spread of the parasite outside of the initial infestation areas. This may be the result of the very narrow host range exhibited by the SGFL strain. We recently observed that *S. gesnerioides* seedlings growing on *I. hirsuta* in a farmer's field in West Africa were able to parasitize cowpea under laboratory conditions (5). With increased disturbance throughout central Florida as a result of natural disasters such as hurricanes, the spread of SGFL seed to areas under intensive agricultural use is enhanced. Thus, the potential for this species to shift host and become a threat to U.S. agriculture exists.

The ability of nonhost species to stimulate the germination of *S. gesnerioides* has been described previously by Berner and Williams (3). These authors showed that a cultivar of *Sorghum bicolor* (a nonhost species) stimulated significantly more germination of *Striga gesnerioides* than any other species, including various *Vigna* spp. This is the basis for suicidal or abortive germination of *Striga* seed, a measure that is being used for the control of *Striga* spp. in many parts of the world (3,6,9,11). It also has been reported that exudates from cowpea stimulated the germination of a strain of *S. gesnerioides* that parasitize small-flower

Fig. 3. Vascular organization at the host-parasite interface in *Striga gesnerioides* tubercles of compatible and incompatible interactions. Thin sections were prepared from tubercles from the roots of **A and C**, the susceptible cowpea cv. Blackeye parasitized by SG3 or **B and D**, Blackeye parasitized by SGFL. Sections were stained with phloroglucinol and visualized by light microscopy. **A**, Arrow indicates the organized central vascular tissue in compatible host-parasite interactions. **C**, A portion of this vascular core showing developed vessel elements is magnified. **B**, A corresponding lack of organized vascular tissue in the tubercle formed in an incompatible interaction. **D**, A close-up of the host-parasite interface. Note the intact host vascular bundle unconnected to the parasite. Arrows indicate where lignification of host cells has occurred at the interface.

morning glory but failed to parasitize cowpea (30). Our data clearly show that selection of a host by *S. gesnerioides* is not mediated solely by response to host-derived germination stimulants because root exudates from all the species tested elicited germination of *Striga* seed (Table 1). Our findings indicated that developmental processes occurring postgermination and attachment are likely more important in limiting successful host-parasite interaction. Musselman and Parker (28) previously have reported that SGFL failed to parasitize cowpea, but did not explore the causative effect of this failure. Here we demonstrate that the inability of *Indigofera*-adapted SGFL to parasitize cowpea results from incompatibility between parasite and host that commences postattachment.

The cowpea genotypes Blackeye and Txv3236 are susceptible to all known races of *S. gesnerioides* from West Africa, but resistant to parasitism of SGFL. In compatible interactions, as in the case of SG3 with Blackeye, following parasite attachment, rapid connection of the parasite and host vascular systems occurs through xylem-xylem linkage. Once the vascular connection is established, the parasite uses this connection to divert host resources (water and other nutrients) (34). In the SGFL-Blackeye interaction, incompatibility appears to be the result of the failure of the parasite to establish proper vascular connection with the host. Why this connection is not established, despite the fact that the parasite has attached and begun to develop its tubercle, remains unclear. One possibility is that there is an improper exchange of developmental cues from host to parasite directing its development. It also is possible that, in the absence of a specific gene-for-gene resistance response (found when a resistant cowpea cultivar is parasitized by a specific race of *S. gesnerioides*), a default nonhost resistance or basal resistance response is mounted, leading to cessation of further invasion by the parasite and arrest of parasite development.

It is interesting that, in the incompatible interactions, tubercle development proceeds and that the tubercle is able to survive for several weeks without a connection with the host vascular system. It has been reported that, in *Orobanche* spp., a parasite related to *Striga* spp., high amounts of mannitol accumulate and thus lower the osmotic potential to a value much more negative than that of the host (21). Thus, it is believed that inhibitors of mannose 6-phosphate reductase (a key enzyme in mannitol biosynthetic pathway) would control *Orobanche* spp. during the early stages of development (12). It is plausible that, despite not establishing vascular connections, the tubercles develop for a short period of time in the incompatible interactions between cowpea and SGFL by maintaining a higher osmotic potential (more negative) than that found in the host. Once the lack of vascular connection becomes limiting, tubercle growth ceases. Metabolic profiling or the identification and characterization of genes that are differentially expressed following compatible and incompatible interaction of *S. gesnerioides* and its host or nonhost will illuminate these process further and may provide a key to identifying potential inhibitors of genes or metabolic pathways that are involved in compatible interactions.

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LITERATURE CITED

- Aggarwal, V. D. 1985. Cowpea *Striga* research. Pages 335-340 in: Cowpea Research, Production, and Utilization. S. R. Singh and K. O. Rachie, eds. John Wiley & Sons, London.
- Babiker, A. G. T., Ejeta, G., Butler, L. G., and Woodson, W. R. 1993. Ethylene biosynthesis and strigol-induced germination of *Striga asiatica*. Plant Physiol. 88:359-365.
- Berner, D. K., and Williams, O. A. 1998. Germination simulation of *Striga gesnerioides* seeds by hosts and nonhosts. Plant Dis. 82:1242-1247.
- Boone, L. S., Fate, G., Chang, M., and Lynn, D. G. 1995. Seed germination. Pages 14-38 in: Parasitic Plants. M. C. Press and J. D. Graves, eds. Chapman & Hall, London.
- Botanga, C. J. 2005. Genetic and molecular analyses of factors defining host-specificity and virulence in *Striga* species (*Scrophulariaceae*). Ph.D. dissertation, University of Virginia, Charlottesville.
- Botanga, C. J., Alabi, S. O., Echeckwu, C. A., and Lagoke, S. T. O. 2003. Genetics of suicidal germination of *Striga hermonthica* (Del.) Benth by cotton. Crop Sci. 43:438-488.
- Butler, L. G. 1995. Chemical communication between parasitic weed, *Striga* and its host crop: A new dimension in allelochemistry. Pages 156-168 in: Allelopathy: Organisms, Process, and Applications. K. M. Inderjit, M. Dakshini, and F. A. Einhelling, eds. ACS Symp. Ser. 582, American Chemical Society, Washington, DC.
- Cai, T., Babiker, A. G., Ejeta, G., and Butler, L. G. 1993. Morphological response of witchweed (*Striga asiatica*) to in-vitro culture. J. Exp. Bot. 44:1377-1384.
- Carson, A. G. 1988. Studies on *Striga* in Gambia. Pages 37-43 in: Proc. FAO/OAU All-African Government Consultation on *Striga* Control. T. O. Robson and H. R. Broad, eds. FAO, Rome.
- Chang, M., and Lynn, D. G. 1986. Haustoria and the chemistry of host recognition in parasitic angiosperms. J. Chem. Ecol. 12:561-579.
- Dejongh, K., Van Damme, P., and Berner, D. K. 1993. Suicidal germination of *Striga* seed using several trap crop varieties. Meded. Fac. Landbouwwet. Rijksuniv. Gent. 58(3a.):983-993.
- Devault, P., Simier, P., Séverine, T., Véronési, C., Fer, A., and Thalouarn, P. 2002. Isolation of manose-6-phosphate reductase cDNA, changes in enzyme activity and manitol content in broomrape (*Orobanche ramosa*) parasitic on tomato roots. Physiol. Plant. 115:48-55.
- Dörr, I. 1997. How *Striga* parasitizes its host: ATEM and SEM study. Ann. Bot. 79:463-472.
- Efron, B., and Gong, G. 1983. A leisure look at the bootstrap, the jackknife, and cross-validation. Am. Stat. 37:36-48.
- Efron, Y., Kim, S.K., Parkinson, V., and Boxque-Perez, N. A. 1988. IITA's strategies to develop *Striga* resistant maize germplasm. Pages 141-153 in: Proc. FAO/OAU All-African Government Consultation on *Striga* Control. T. O. Robson and H. R. Broad, eds. FAO, Rome.
- Emechebe, A. M., Singh, B. B., Leleji, O. I., Atokple, I. D. K., and Adu, J. K. 1991. Cowpea-*Striga* problems and research in Nigeria. Pages 18-28 in: Combating *Striga* in Africa. S. K. Kim, ed. Proc. Int. Workshop organized by IITA, ICRISAT and IDRC. IITA, Ibadan, Nigeria.
- Eplee, R. E. 1992. Witchweed (*Striga asiatica*): An overview of management strategies in the USA. Crop Prot. 11:3-7.
- Estabrook, E. M., and Yoder, J. I. 1998. Plant-plant communications: Rhizosphere signaling between parasitic angiosperms and their hosts. Plant Physiol. 116:1-7.
- Graves, J. D., Press, M. C., Smith, S., and Stewart, G. R. 1992. The carbon canopy economy of the association between cowpea and the parasitic angiosperm *Striga gesnerioides*. Plant Cell Environ. 15:283-288.
- Graves, J. D., Press, M. C., and Stewart, G. R. 1989. A carbon balance model of the sorghum-*Striga hermonthica* host-parasite association. Plant Cell Environ. 12:101-107.
- Harloff, H. J., and Wegmann, K. 1987. Manitol pathway in *Orobanche*. Pages 295-308 in: Proc. Fourth Int. Symp. Parasitic Flowering Plants. H. C. Weber and W. Forstreuter, eds. Phillips University, Marburg.
- Herbaugh, L., Upton, N. P., and Eplee, R. E. 1980. *Striga gesnerioides* in the United States of America. Proc. South. Weed Sci. Soc. 33:187-190.
- Jerome, C. A., and Ford, B. A. 2002. The discovery of three genetic races of the dwarf mistletoe (*Arceuthobium americanum* [Viscaceae]) provides insight into the evolution of parasitic angiosperms. Mol. Ecol. 11:387-405.
- Lagoke, S. T. O., Parkinson, V., and Agunbiade, R. M. 1991. Parasitic weeds and control methods in Africa. Pages 3-14 in: Combating *Striga* in Africa. S. K. Kim, ed. Proc. Int. Workshop organized by IITA, ICRISAT and IDRC. IITA, Ibadan, Nigeria.
- Lane, J. A., Moore, T. H. M., Child, D. V., and Cardwell, K. F. 1996. Characterization of virulence and geographic distribution of *Striga gesnerioides* on cowpea in West Africa. Plant Dis. 80:299-301.
- Mohamed, K. I., Musselman, L. J., and Riches, C. R. 2001. The genus *Striga* (*Scrophulariaceae*) in Africa. Ann. Mo. Bot. Gard. 88:60-103.
- Musselman, L. J. 1980. The biology of *Striga*, *Orobanche* and other root-parasitic weeds. Annu. Rev. Phytopathol. 18:463-489.
- Musselman, L. J., and Parker, C. 1981. Studies on *Indigo* witchweed, the American strain of *Striga gesnerioides* (*Scrophulariaceae*). Weed Sci. 29:594-596.

29. Nei, M., and Li, W.-H. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc. Natl. Acad. Sci. USA* 76:5269-5273.
30. Parker, C., and Reid, D. C. 1979. Host specificity in *Striga* species—some preliminary observations. Pages 79-90 in: *Proc. 2nd Symp. Parasitic Weeds*. L. J. Musselman, A. D. Worsham, and R. E. Eplee, eds. North Carolina State University, Raleigh.
31. Parker, C., and Riches, C. R. 1993. *Striga* and *Alectra* on cowpea and other broadleaved crops. Pages 75-109 in: *Parasitic Weeds of the World: Biology and Control*. C. Parker and C. R. Riches, eds. CAB International, Wallingford, UK.
32. Pavlista, A. D. 1981. Why hasn't witchweed spread in the United States? *Weeds Today* (special issue):16-19.
33. Pieterse, A. H. 1985. Control of *Striga* at the level of small-scale farmer. Pages 24-36 in: *Proc. FAO/OAU Workshop on Striga*. FAO, Rome.
34. Riopel, J. L., and Timko, M. P. 1995. Haustorial initiation and differentiation. Pages 29-79 in: *Parasitic Plants*. M. C. Press and J. D. Graves, eds. Chapman and Hall, London.
35. Rogers, W. E., and Nelson, R. R. 1962. Penetration and parasitism of *Striga asiatica*. *Phytopathology* 52:1064-1070.
36. Sahai, A., and Shivanna, K. R. 1982. Seed germination and seedling morphogenesis in parasitic angiosperms of the families *Scrophulariaceae* and *Orobanchaceae*. *Seed Sci. Technol.* 10:565-583.
37. Saunders, A. R. 1933. Studies in phanerogamic parasitism with particular reference to *Striga lutea* Lour. Ph.D. diss., University of Pretoria, South Africa.
38. Sneath, P. H., and Sokal, R. R. 1973. *Numerical Taxonomy: The Principles and Practice of Numerical Classification*. W. H. Freeman, San Francisco.
39. Swofford, D. L. 1999. PAUP: Phylogenetic Analysis Using Parsimony, version 4.0beta10. Sinauer Associates, Sunderland, MA.
40. Van Gysel, A., Cnops, G., Breyne, P., Van Montagu, M., and Cervera, M. 1998. Chromosome landing using AFLPTM-based strategy. Pages 305-314 in: *Arabidopsis* Protocols. *Methods in Molecular Biology*, Vol. 82. J. Martinez-Zapater and J. Salinas, eds. Humana Press, Totowa, NJ.
41. Visser, J. 1981. *South African Parasitic Flowering Plants*. Juta & Co. Ltd., Capetown, South Africa.
42. Vos, P., Hogers, R., Bleeker, M., Reijmans, M., Van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M., and Zebeau, M. 1995. AFLP: A new technique for DNA fingerprinting. *Nucleic Acids Res.* 23:4407-4414.
43. Werth, C. R., Riopel, J. L., and Gillespie, N. W. 1984. Genetic uniformity in an introduced population of witchweed (*Striga asiatica*) in the United States. *Weed Sci.* 32:645-648.
44. Worsham, A. D. 1987. Germination of "witchweed" seeds. Pages 45-61 in: *Parasitic Weeds in Agriculture*, Vol. 1, *Striga*. L. J. Musselman, ed. CRC Press, Boca Raton, FL.