Plant Physiol. (1990) 93, 201–207
0032-0889/90/93/0201/07/$01.00/0

Received for publication June 12, 1989
and in revised form December 12, 1989

Control of Germination in *Striga asiatica*: Chemistry of Spatial Definition[1]

Gwendolyn Fate, Mayland Chang[2], and David G. Lynn*

Searle Chemistry Laboratory, The University of Chicago, Chicago, Illinois 60637

ABSTRACT

***Striga asiatica* (Scrophulariaceae), a member of a heterogeneous group known as the parasitic plants, is totally dependent on host root attachment for survival. In agar, *Striga* seeds germinated in high percentages within 5 millimeters of a sorghum (*Sorghum bicolor* (L.) Moench) host root surface, and no germination was observed at distances greater than 1 centimeter. This spatially restricted germination may be explained by the chemistry of a single compound, 2-hydroxy-5-methoxy-3-[(8′Z, 11′Z)-8′, 11′, 14′-pentadecatriene]-*p*-hydroquinone, structure 1, which is exuded by sorghum roots. The presence of the compound was chemically imaged with pigments such as methylene blue. The use of methylene blue suggested that structure 1 was exuded along the entire surface of the root for long periods. This exudation and the inherent instability of structure 1 together establish an apparent steady state concentration gradient of the germination stimulant around the sorghum root. The *Striga* seed must be exposed to micromolar concentrations of 1 for ≥5 hours before high germination percentages were observed. Such a requirement for a long term exposure to a steady state concentration of an inherently labile, exuded compound would provide an extra degree of resolution to signal detection and host commitment in *Striga* parasitism.**

Symbiotic and parasitic organisms require the precise integration of the physiological and biochemical processes of two separate individuals. The parasitic plants, a diverse group that occurs in at least 20 different families (18), typify many host-pathogen interactions in that the development program of the parasitic plant is dependent on its host. Therefore, the study of the molecules exchanged between the two organisms provides a unique opportunity to identify and further investigate not only host-pathogen signals but also signals involved in eucaryotic development.

Most of the known parasitic plants are hemiparasites, photosynthetic and capable of maturing to seed-set without a host. Species of *Aureolaria* (false foxglove), *Agalinis* (Gerardia), and *Castilleja* (Indian paintbrush) are some of the more common members. The holoparasites, on the other hand, frequently are nonphotosynthetic, *e.g. Epifagus virginiana* (beechdrops) and *Conopholis americana* (squawroot), but even those that are photosynthetic, *e.g. Striga asiatica* (witchweed), have an absolute requirement of host attachment for continued maturation.

These holoparasites are, therefore, more dependent on their host than hemiparasites and host recognition becomes a critical element (16). For example, *Epiphagus* and *Conopholis* attack only beech and red oak trees, respectively, while *S. asiatica* is restricted to members of the grass family (15, 17, 20, 24). The mechanism controlling this host recognition has been proposed to be based on signal molecules unique to the host, and a great deal of effort has focused on the identification of germination stimulants for *S. asiatica* (1, 2, 7–9, 12, 27–30). Yet, only recently has a *Striga* germination stimulant been isolated from a natural host (4, 5).

Once germinated, *Striga* is irreversibly committed to an association with the host. The small seed size (100 μm diameter) and limited food supply (5) contribute to restricting parasitic competence to about 5 d. Therefore, to be optimally effective, the signal molecule most provide not only information about the presence of the host, but also spatial information that restricts germination to a zone proximal to the host. The exclusive germination of *Striga* proximal to its host has been documented repeatedly (2). We now present evidence that this zone of germination can be defined by the inherent chemical reactivity of a single compound.

MATERIALS AND METHODS

Sorghum Germination

Seeds of *Sorghum bicolor* (L.) Moench cv IS8768 or cv Dabar (10 g) were surface-sterilized with a 1.6% solution of sodium hypochlorite (100 mL) for 20 min and then washed three times with sterile, twice-distilled water (100 mL). The seeds were transferred aseptically onto moist filter paper in ten disposable Petri dishes (100 × 15 mm) and wrapped with parafilm to prevent evaporation. The sorghum was grown in the dark for 4 d before subsequent transfer.

Agar Media Preparation

A 1% aqueous agar solution (10 g Difco Bacto/1 L) was prepared and autoclaved for 20 min. After cooling for 20 min, the agar solution was either poured directly into disposable Petri dishes (50 mL × 20 dishes) or mixed with the required additives (*e.g.* methylene blue [40 mg/L, 1×10^{-4} M] and sodium ascorbate [100 mg/L, 5×10^{-4} M]) and then poured and allowed to cool until it started to gel.

[1] Supported in part by National Institutes of Health grant GM 33585, The Frasch Foundation, and the Dow Chemical Company.

[2] Present address: Dow Chemical Company, Midland, MI 48674.

Methylene Blue Visualization

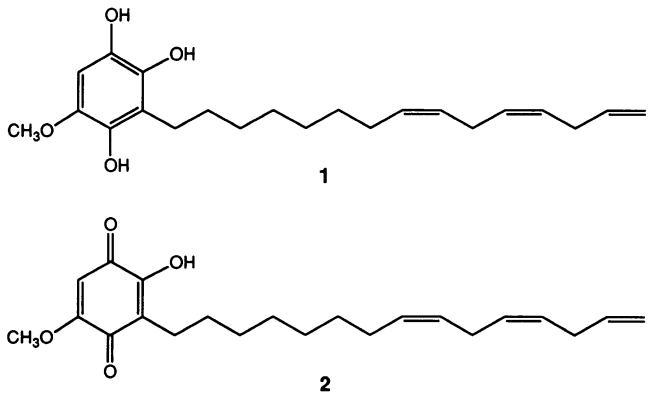
Four-day-old etiolated seedlings (cv IS8768) were transferred to plates containing the gelling agar as prepared above. The sodium ascorbate served as a buffering agent and to complex metals that could catalyze autoxidation processes. The plates were photographed after approximately one week.

Methylene Blue Titration

Sorghum exudate (4, 5) (1.0 mg) dissolved in 50% methanol/water containing 0.5% acetic acid (100 μL) was reduced by sonication over coarse zinc filings (5 min). The mixture was filtered through a cotton plug, and the cotton was washed with water (900 μL) to give a 3×10^{-4} M solution of 2-hydroxy-5-methoxy-3-[(8′Z, 11′Z)-8′, 11′, 14′-pentadecatriene]-*p*-hydroquinone (structure 1). An aqueous solution of methylene blue (10^{-3} M) was added until the the blue color persisted. The exudate reduced 0.96 equivalents of methylene blue. In a control, the sorghum exudate was omitted from the reduction reaction and no methylene blue was reduced. The methylene blue reaction was extracted with methylene chloride; the extract was dried over magnesium sulfate and the solvent was removed *in vacuo*. This residue was redissolved in chloroform-d_1 and analyzed by ^{1}H-NMR (5). This procedure established that 2-hydroxy-5-methoxy-3-[(8′Z, 11′Z)-8′, 11′, 14′-pentadecatriene]-*p*-quinone, structure 2, was produced cleanly and in good yields under these reaction conditions.

Spatial Control of Germination

Agar plates were prepared as above but the sodium ascorbate was omitted since *Striga* requires an acidic environment for optimal germination rates. The methylene blue functioned as an effective fungicide for long-term experiments and was added to the agar. Four-day-old, etiolated seedlings (cv IS8768, and cv Dabar) were transferred to the gelling agar. *Striga* seeds were pipetted into the still gelling agar as a 1 cm wide swath perpendicular to the sorghum root. Distances from the root were measured with a ruler and lines parallel to the root were drawn on the Petri dish at 0, 0.3, 0.5, 0.8, 1.0, and 1.5 cm. The germination percentages were determined by counting the number of seeds which germinated within each zone and dividing by the total number of seeds. Six replicates were typically run simultaneously and four plates were included in the averaged germination data.



Structures 1 and 2. 1: 2-hydroxy-5-methoxy-3-[(8′Z, 11′Z)-8′,11′,14′-pentadecatriene]-*p*-hydroquinone. 2: 2-hydroxy-5-methoxy-3-[(8′Z, 11′Z)-8′,11′,14′-pentadecatriene]-*p*-quinone.

Direct UV Analysis of the Agar (13)

Four-day-old etiolated sorghum seedlings (cv Dabar) were transferred to plates containing a 1% solution of gelling triterated agar and allowed to solidify. The plates were loosely sealed with parafilm to prevent evaporation and placed in the dark at 26°C for two additional weeks. The agar was cut parallel to the sorghum root (0–0.5, 0.5–1, 1–1.5, 1.5–2, and 2–2.5 cm) and removed with a pipette. Each section, starting with 0 to 0.5 cm section, was removed before the next section was cut so as to cause minimal disruption of the agar matrix. The agar was homogenized and transferred to a quartz UV cell, and the UV spectrum was recorded between 200 and 900 nm. The absorbance of the quinone plus hydroquinone at 280 nm was integrated as an estimate of their concentration.

Preparation of Sorghum Exudate

Sorghum seeds were germinated as above and grown for 7 d. The entire seedling root was dipped for 3 s in methylene chloride containing 1% acetic acid and the extract was evaporated *in vacuo*. The exudates (1.8 mg/g seeds for Dabar and 0.52 mg/g seeds for IS8768) were weighed and divided into 100 μg portions, separated into vials, and stored desiccated at 4°C.

For assay, a 100 μg portion of the root exudate was dissolved in 500 μL methylene chloride and 50 μL was placed in each of 10 vials. The methylene chloride was evaporated under a stream of nitrogen, and 1 mL of sterile, twice-distilled water was added to give a 28 μM solution of the hydroquinone. The vials were capped and sonicated for 3 min. Further dilutions were made from these 28 μM solutions as required. The solutions of root exudate were pipetted into microtiter wells containing pretreated *Striga* seeds. For the 2,6-di-*tert*-butyl-4-hydroxytoluene (BHT) stabilized assays, 1 μL of a 3.1 mM solution of BHT in DMSO was added to the aqueous solutions of hydroquinone. The seeds were incubated for 24 h at 27°C, and the percent germination was recorded. At least five replicate runs were recorded for each data point.

The quinone was purified by HPLC on Zorbax ODS as described previously (4, 5). Reduction to 1 typically involved dissolving the quinone (100 μg) in acetic acid (100 μL) containing 1 mg of tin amalgam and warming the mixture to about 30°C for 3 min before filtering through glass wool under N_2. The filtrate was dried *in vacuo*, and the residue was redissolved in CH_2Cl_2 and refiltered. This filtrate was dried again *in vacuo* and this time taken up in 1 mL of H_2O for assay.

Pretreatment of *Striga asiatica* Seeds

Striga seeds were surface-sterilized (4) by sandwiching them between two 25-mm polypropylene Millipore filters (10 μg pore size) in a 25-mm Swin-lock holder connected to a 10-

mL luer-lock syringe. Sterilization was accomplished by washing with each of the following solutions sequentially: (a) 10 mL of a solution containing 100 μL Tween 20 in 250 mL; (b) sterile water (2 × 10 mL); (c) 10 mL of a 0.16% solution of sodium hypochlorite for 5 min; and (d) sterile water (4 × 10 mL). The seeds were then transferred aseptically to a 125 mL Erlenmeyer flask and covered with 50 mL of water. Over 90% of the seeds responded to the germination stimulant after 2 weeks of incubation at 27°C.

RESULTS

Parasite-Host Response

In an attempt to quantify the *Striga* seed response to the host, seedlings from two distinct sorghum cultivars were imbedded in an agar gel containing *Striga* seeds. The germination percentage was measured as a function of distance from the host root surface (Fig. 1). The data are expressed as the sum of the germination percentages from four separate experiments, each experiment represented by a differently shaded area. In each cultivar, the percent germination was high (>90%) within 5 mm of the host root but dropped off and became more variable at greater distances. The *Striga* seeds responded in the same way at all positions along the sorghum root surface tested. Maximal germination was reached within 2 d.

Germination Stimulant

Sorghum seedlings have been shown to exude large quantities of organic material as part of their exudate (19). This material, which can be selectively removed by dipping the entire root of the young seedlings for 3 s in organic solvents, contained a 1:1 mixture of structures 1 and 2, together amounting to about 95% of the mass of the organic exudate (5). Freshly collected material induced quantitative *Striga* seed germination. However, as the concentration of 1 in the exudate fell, the biological activity was correspondingly reduced (Fig. 2). Varying the concentration of the quinone, structure 2, over a large concentration range had no effect on the activity of the exudate. When the purified quinone was chemically reduced to structure 1 and added to samples containing suboptimal levels of the exudate, the level of the inducing activity corresponded to the concentration of the added hydroquinone.

Imaging of the Hydroquinone

Since the hydroquinone appeared to be the essential component of the exudate in the induction of *Striga* germination, methods were required for specifically localizing hydroquinone production along the sorghum root surface. Methylene blue, a commercially available heterocyclic pigment, can be reduced electrochemically ($E_0' = +20$ mV at pH 7.6) (6) or with chemical reductants like sodium dithionite. The hydroquinone, structure 1, reacted stoichiometrically with methylene blue to give the visibly transparent $2e^-$ reduced form. In the presence of O_2, the reduced dye autoxidized back to the blue form (Scheme 1) and structure 2 was isolated intact. Therefore, in the presence of O_2, this pigment can be used to investigate the persistence of the production of structure 1.

Figure 3 is a photograph of a typical plate resulting from

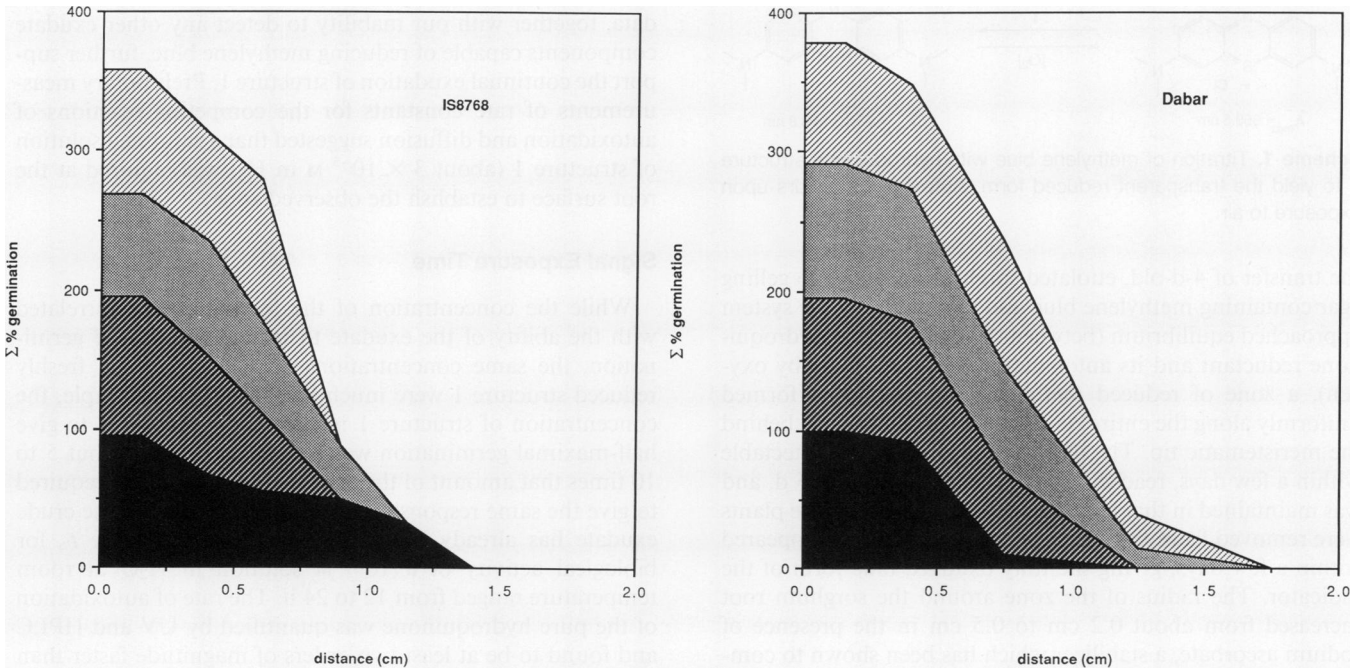


Figure 1. *Striga* germination is plotted as a function of distance from the root of two sorghum cultivars, IS8768 and Dabar. *Striga* seeds were suspended in agar around a sorghum seedling, and germination was recorded in zones of 0.2 to 0.5 cm increments parallel to the sorghum root. Germination was recorded 24 and 48 h after placing the sorghum seedling into the agar. Each shaded region between two graphed lines represents a separate experiment recorded at 48 h, hence the data are summed vertically.

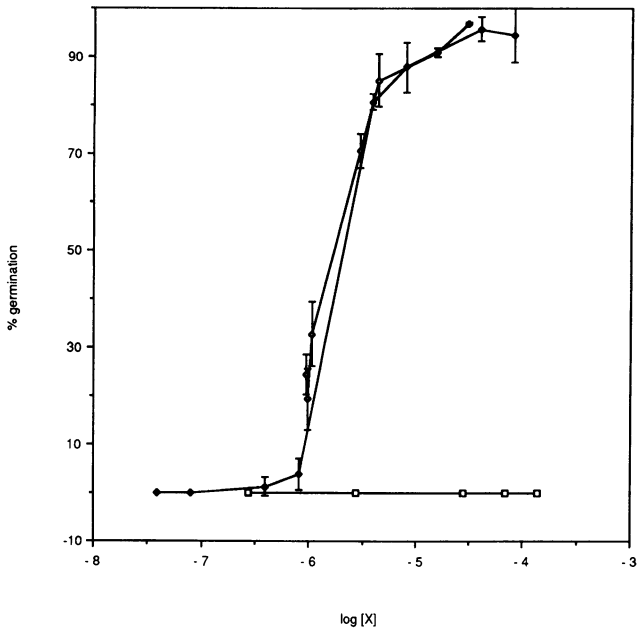
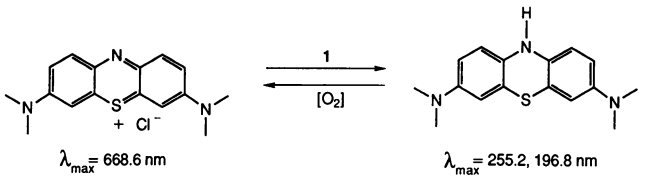


Figure 2. Germination activity of sorghum root exudate relative to the hydroquinone concentration (♦) along with the addition of either synthetic hydroquinone structure 1 (◇) or purified quinone structure 2 (□). The admixture experiments used a fixed amount of inactive exudate (about 0.1 μM structure 1). Relative and absolute concentrations of the hydroquinone or quinone [X] were determined by integration of the aromatic proton in the ^{1}H NMR spectrum of the mixture. Experimental points represent the average of five assays.



Scheme 1. Titration of methylene blue with hydroquinone structure 1 to yield the transparent reduced form. Autoxidation occurs upon exposure to air.

the transfer of 4-d-old, etiolated sorghum seedlings to gelling agar containing methylene blue and ascorbate. As the system approached equilibrium (between the exudation of hydroquinone reductant and its autoxidation to the quinone by oxygen), a zone of reduced methylene blue indicator formed uniformly along the entire root surface, beginning just behind the meristematic tip. The clear, reduced zone was detectable within a few days, reached a steady state after about 5 d, and was maintained in that form for at least 1 month. If the plants were removed from the agar, then the clear zone disappeared within a few days, giving the fully oxidized blue form of the indicator. The radius of the zone around the sorghum root increased from about 0.2 cm to 0.5 cm in the presence of sodium ascorbate, a stabilizer which has been shown to complex pro-oxidants (10). These results were consistent with methylene blue detection of a steady exudation of structure 1 around the sorghum root surface.

The total concentration of the quinone and hydroquinone

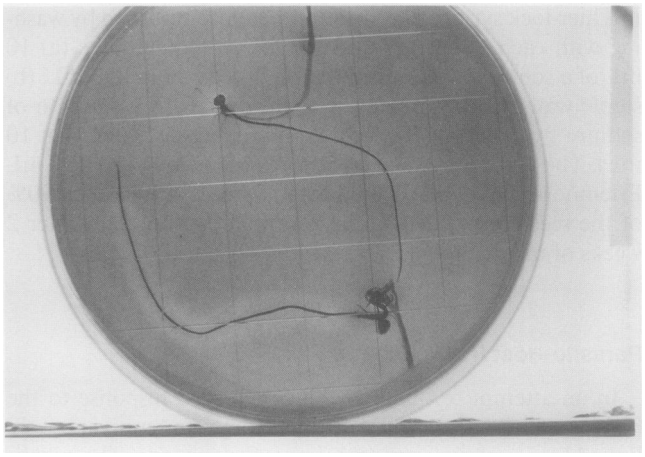


Figure 3. Photograph of 10-d-old sorghum roots grown 6 d in agar media containing methylene blue and sodium ascorbate. Although there is some variability in the intensity of the white zone about the roots, in general the zone is uniformly distributed along the length of each root up to the meristematic tip. Removal of the root results in loss of the zone. Freshly collected exudate will titrate a solution of methylene blue stoichiometrically with hydroquinone concentration, and exudate depleted of hydroquinone will not react with methylene blue.

around 2-week-old roots was determined directly by measuring the ultraviolet spectrum of the agar cut from specific zones around the host root. The concentration fell from about 10^{-6} M close to the root to 10^{-7} M in the zone from 1 to 1.5 cm. There was no evidence for a buildup of either the quinone or the hydroquinone around the roots consistent with the finding that both compounds gradually decompose in H_2O. These data, together with our inability to detect any other exudate components capable of reducing methylene blue, further support the continual exudation of structure 1. Preliminary measurements of rate constants for the competing reactions of autoxidation and diffusion suggested that a saturated solution of structure 1 (about 3×10^{-5} M in H_2) was required at the root surface to establish the observed zone.

Signal Exposure Time

While the concentration of the hydroquinone correlated with the ability of the exudate to induce *Striga* seed germination, the same concentrations of HPLC-purified, freshly reduced structure 1 were much less active. For example, the concentration of structure 1 in the exudate required to give half-maximal germination was 8×10^{-6} M (Fig. 1), but 5 to 10 times that amount of the pure hydroquinone was required to give the same response. The oxidative lability of the crude exudate has already been documented (5), and the $t_{1/2}$ for biological activity of a 10^{-5} M solution in H_2O at room temperature ranged from 12 to 24 h. The rate of autoxidation of the pure hydroquinone was quantified by UV and HPLC and found to be at least two orders of magnitude faster than the exudate.

In an effort to prolong the exposure period, the initially added exudate was removed and replaced with fresh material every 2 h for an 8 h total exposure time (Table I). At 3×10^{-6}

Table I. *Sorghum (cv Dabar) Root Exudate Assayed at Suboptimal Levels Shows Improved Activity when Replaced or Stabilized with an Autoxidation Inhibitor*

Conditions	Hydroquinone	
	10 μg/mL	1 μg/mL
	% germination[a]	
Unreplaced	89 ± 7	16 ± 15
Replaced every 2 h for 8 h		65 ± 5
Unreplaced, with 1 μg/mL BHT[b]	96 ± 5	89 ± 6

[a] Percent germination was recorded after 24 h. [b] BHT was added as a 1 mg/mL solution in DMSO.

M hydroquinone, 65% of the seeds germinated in the replacement assay, whereas in the single exposure experiment only 16% germinated. Chemical stabilization of the hydroquinone also increased the germination percentages. The addition of an autoxidation inhibitor, BHT, (3 μM) together with 3 μM hydroquinone raised the germination percentage to almost 90%.

Since both chemical stabilization and repeated additions of the exudate increased the *Striga* seed response, we assumed that the exposure time of *Striga* to the germination stimulant was an important component of the activity. In order to test for such a specific time-dependence, *Striga* seeds were exposed to the exudate from each of two sorghum cultivars for various times, washed three times with distilled H_2O, and then monitored for germination 24 h after the start of the experiment (Fig. 4). With exposure times of less than 2 to 4 h, there was very little germination. However, with exposures of greater than 8 h, the seeds germinated at a high percentage. The exposure time necessary to induce half-maximal germination, T_{50}, was approximately 5 h for both cultivars.

Exudate Models

Assuming that the entire germination response could be controlled by the hydroquinone, attempts were made to model the germination zone around the sorghum roots. A hole bored in the center of an agar plate served as a well for the hydroquinone solution. Pretreated *Striga* seeds were dispersed around the well, and a grid, marked on the bottom of the Petri plate, was constructed similar to that used around the sorghum roots. The well was then filled with a solution of freshly reduced hydroquinone, BHT (an inhibitory of autoxidation), and the detergent Tween 20. The *Striga* seeds responded only within a defined zone which developed within 1 d and did not change over the next 2 d (Fig. 5).

The maximal germination occurred within 5 mm of the well and, as with the sorghum roots, very little or no germination was observed at distances ≥1 cm. High concentrations of all three components were used to ensure insignificant depletion of the source and to maintain a constant gradient of structure 1. There was an obvious reduction in the overall germination percentages. This reduction is attributed to the high concentration of the unnatural stabilizer, BHT. Without the BHT, low and variable germination percentages were observed, and it is anticipated that the germination zone will

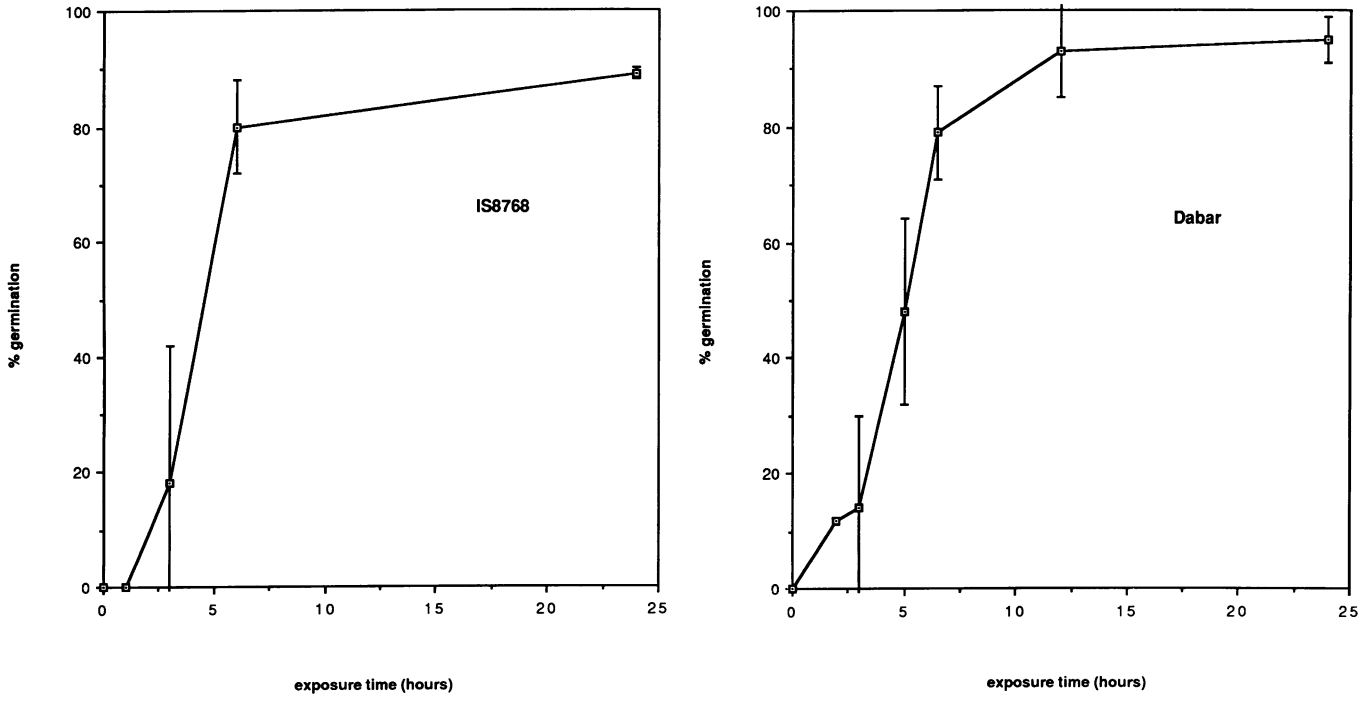


Figure 4. Effect of exposure time to root exudates of sorghum cultivars Dabar and IS8768 on the germination of *S. asiatica*. Seeds were exposed to solutions of exudate (3×10^{-5} M), the stimulant was removed at varying times, and the seeds washed 3 times and placed in 1 mL, twice-distilled sterile water. Percent germination was recorded after 24 h. Half-maximal germination is reached after 5 h for Dabar and after 4.5 h for IS8768.

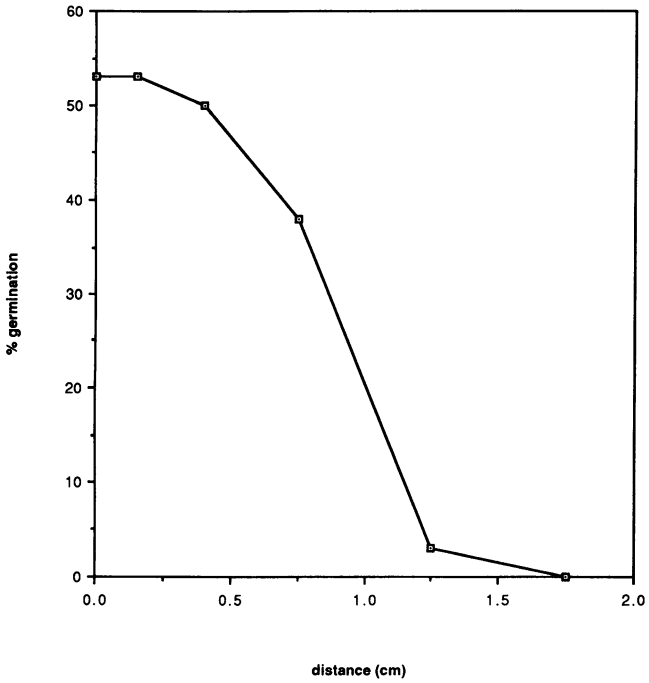


Figure 5. *In vitro* mimic of root exudation. Synthetic hydroquinone (3×10^{-5} M) containing BHT (4.5×10^{-6} M) and Tween 20 (1.2×10^{-6} M) were placed in a well in the center of an agar plate containing *Striga* seeds. The plot shows the average of three agar plates. Germination was counted up to 2 cm away from the well in zones of 0.2 to 0.5 cm increments. Germination was recorded 24 h after placing the materials in the well.

be more accurately modeled once what is assumed to be the natural stabilizer has been identified.

DISCUSSION

The root exudates of the grasses were shown many years ago to be very complex mixtures containing sugars, amino acids, organic acids, nucleotides, phenolics, and enzymes (22). Experimental evidence for specific functional roles of these exudates in the rhizosphere is generally lacking. However, Doberenier and Pedrosa (11) have discussed the evidence for the specific association between N_2-fixing bacteria and grasses. Recently, single components in the exudates of legumes have been shown to initiate the rhizobial gene expression necessary for symbiotic nodulation (21).

The seeds of the parasitic plant *Striga asiatica* germinate only when in close association with host plants (2). When planted in agar, sorghum roots induce germination along the entire length of the root, specifically within a 0.5 cm radius of the host surface. Exudates from sorghum roots collected with organic solvents are remarkably clean, containing only two major compounds, the hydroquinone structure 1 and a further oxidized product, structure 2. The germination activity correlates only with the concentration of structure 1.

Methylene blue proved to be an effective chemical imaging agent for the hydroquinone. The experiments with this indicator supported a continual release of structure 1 along the entire length of the sorghum seedling. The exudates of several sorghum varieties, maize and other *Striga* hosts reduced methylene blue, whereas those of several nonhosts did not (G Fate, DG Lynn, unpublished data). The generality of this methylene blue imaging procedure for characterizing root exudates is being further explored.

Since the half-maximal concentration for the induction of germination with fully stabilized hydroquinone is about 2×10^{-7} M, this level must be maintained at 0.9 cm from the roots of the Dabar cultivar and at 0.75 cm from the IS8767 cultivar (Fig. 2). Both the purified quinone and hydroquinone are unstable in dilute aqueous solution and, therefore, do not appear to accumulate to any significant degree. Direct UV analyses of the agar put an upper limit on the accumulated concentration of both quinone and hydroquinone which was consistent with the concentration required for the germination response. In addition, measurements of the rates of diffusion and autoxidation of 1 in agar suggest the establishment of a steady state within 2 d (13), again consistent with the timing for the germination response. The results from both the methylene blue and germination signal exposure time experiments suggest that the concentration of structure 1 does not vary from this steady state for extended periods of time, and the modeling results support this contention. The formation of the steady state gradient can therefore provide information not only about host presence but also about the spatial distance between host and parasite.

The extent to which these specific distances in agar reflect what occurs in soil is not known. Since *Striga* remains parasitically competent for no more than 5 d after germination and over that time period the radicle can grow no more than 5 mm in length, the observed distance control certainly provides a selective advantage for *Striga* host attachment. It is expected that decreased diffusion rates and increased autoxidation in soil would shorten the distance and provide an even greater selective advantage to *Striga*.

The findings that two sorghum cultivars exuded large quantities of structure 1 and that other grasses may exude related materials suggest that hydroquinone exudation is a general feature of *Striga*'s hosts. It is possible that such a metabolically expensive process could be defensive in nature. In that regard, related quinones have been shown to be metabolic toxins (26) serving as general phytoalexins (3). The autoxidation of the hydroquinone results in the generation of reactive and toxic oxygen intermediates. The bis-allylic methylenes of the side chain are also known to react with oxygen radicals. Related compounds containing both catechol and olefinic functionalities polymerize (23) and, in fact, formed the basis of the original lacquer industry. Such a polymerization reaction may generate other structures of relevance to the rhizosphere or a polymerization may even function to stabilize the soil around the roots. Any of these mechanisms could provide general protection for the sorghum roots.

A more appealing possibility would involve a specific role for these compounds. *Azospirillium* spp. and other nitrogen-fixing bacteria colonize many of the same species that *Striga* parasitizes (11). These exudates could induce specific root colonization by reducing the oxygen tension around the host root (most of these bacteria multiply and fix N_2 only at reduced O_2 pressures), serving as a specific reductant or ter-

minal electron acceptor for bacterial respiration, or even as a specific bacterial chemoattractant (11). Any specific role that these compounds might play awaits further investigations.

In such specific relationships, the distance between the two organisms can be the limiting factor defining the probability of a successful interaction. The concepts of diffusing chemical species defining distances have formed the basis of developmental theories for decades (14, 25). *Striga* represents a case where the chemistry of a single diffusing compound appears to define very accurately a distance critical to developmental maturation. The requirement of a precise exposure time before germination may serve to increase the accuracy or resolution of signal detection, a much needed feature for a developmental program that is functionally irreversible. Further studies of the mechanism for signal detection in *Striga* and the precise chemistry defining the steady state concentration should provide additional insight into signal transduction in general and a model system for understanding the relevance of diffusing species organizing developmental events between organisms.

LITERATURE CITED

1. **Brown R, Edwards M** (1945) Effects of thiourea and allylthiourea on the germination of the seeds of *Striga lutea*. Nature **155:** 455–456
2. **Brown R, Johnson AW, Robinson E, Todd AR** (1949) The stimulant involved in the germination of *Striga hermonthica*. Proc Roy Soc Lond B Biol Sci **136:** 1–12
3. **Bundenburg DE, Jong HL, Klaar WJ, Vliegenthart JA** (1955) Glycosides and their importance in the wheat germ. Third International Brotkongress, Hamburg, pp 29–33
4. **Chang M** (1986) Isolation and characterization of semiochemicals involved in host recognition in *Striga asiatica*. PhD Thesis University of Chicago, Chicago
5. **Chang M, Netzly DH, Butler LH, Lynn DG** (1986) Chemical regulation of distance: characterization of the first natural host germination stimulant for *Striga asiatica*. J Am Chem Soc **108:** 7858–7860
6. **Clark WM** (1960) Oxidation Reduction Potentials of Organic Systems. Williams & Wilkins, Baltimore, pp 130–135, 422
7. **Cook CE, Whichard LP, Wall ME, Egely GH** (1966) Germination of witchweed (*Striga lutea* Lour.): isolation and properties of a potent stimulant. Science **196:** 1189–1190
8. **Cook CE, Whichard LP, Wall ME, Egely GH, Coggon P, Luhan PA, Mcphail AT** (1972) Germination stimulants. II. the structure of strigol-a potent seed germination stimulant for witchweed (*Striga lutea* Lour.). J Am Chem Soc **94:** 6198–6199
9. **Dale JE, Egley GH** (1971) Stimulation of witchweed germination by run-off water and plant tissues. Weed Sci **19:** 678–681
10. **Denisov ET, Khudyakov IV** (1987) Mechanism of action and reactivities of the free radicals of inhibitors. Chem Rev **87:** 1313–1357
11. **Dobereiner J, Pedrosa F** (1987) Nitrogen-Fixing Bacteria in Nonleguminous Crop Plants. Springer-Verlag, New York
12. **Egley GH, Dale JE** (1970) Ethylene,2-chloroethylphosphonic acid and witchweed germination. Weed Sci **18:** 586–589
13. **Fate GD, Lynn DG** (1990) Molecular diffusion coefficients: experimental determination and demonstration. J Chem Educ (in press)
14. **Gierer A, Meinhardt H** (1972) A theory of biological pattern formation. Kybernetik **12:** 30–39
15. **King LJ** (1966) Weeds of the World: Biology and Control. Interscience, New York
16. **Kuijt J** (1969) The Biology of Parasitic Flowering Plants. University of California Press, Berkeley
17. **Musselman LJ, Ayensu ES** (1984) Taxonomy and biosystematics of *Striga*. *In* ES Ayensu, H Doggett, RD Keynes, J Marton-Lefevre, LJ Musselman, C Parker, A Pickering, eds, *Striga*, Biology and Control. International Council Scientific Union Press, Paris, pp 37–45
18. **Musselman LJ** (1980) The biology of *Striga*, *Orobanche*, and other root-parasitic weeds. Annu Rev Phytopathol **18:** 463–489
19. **Netzley DH, Butler LG** (1986) Roots of sorghum exude hydrophobic droplets containing biologically active components. Crop Sci **26:** 775–778
20. **Nour J, Press M, Stewart G, Tuohy J** (1983) Africa in the grip of witchweed. New Scientist **9:** 44–48
21. **Peters NK, Frost JW, Long SR** (1986) A plant flavone, luteolin, induces expression of *Rhizobium meliloti* nodulation genes. Science **233:** 977–980
22. **Rovira AD** (1965) Plant root exudates and their influence upon microorganisms. *In* KF Baker, WC Snyder, eds, Ecology of Soil-borne Pathogens. University of California, Berkeley, pp 170–186
23. **Takada M, Oshima R, Yamauchi Y, Kumanotani J, Seno M** (1988) Coupling reactions of 4-*tert*-butyl-*o*-benzoquinone with olefinic compounds. J Org Chem **53:** 3073–3080
24. **Vasudeva Rao MJ, Musselman LJ** (1987) Host specificity in *Striga* spp. and physiological strains. *In* LJ Musselman, ed, Parasitic Weeds in Agriculture, Vol 1. CRC Press, Boca Raton, FL, pp 13–24
25. **Wolpert L** (1969) Positional information and the spatial pattern of cellular differentiation. J Theor Biol **25:** 1–47
26. **Wong SM, Pezzuto J, Fong HS, Farnsworth NR** (1985) Isolation, structural elucidation and chemical synthesis of 2-hydroxy-3-octadecyl-5-methoxy-1,4-benzoquinone (irisoquin), a cytotoxic constituent of *Iris miisouriensis*. J Pharm Sci **74:** 1114–1116
27. **Worsham AD, Moreland DE, Klingman GC** (1959) Stimulation of *Striga asiatica* (witchweed) seed germination by 6-substituted purines. Science **130:** 1654–1656
28. **Worsham AD, Klingman GC, Moreland DE** (1962) Promotion of germination of *Striga asiatica* seed by coumarin derivatives and the effects on seedling development. Nature **195:** 199–201
29. **Worsham AD** (1961) Germination of *Striga asiatica* (L.) Kuntze (witchweed) and studies on the chemical nature of the germination stimulant. PhD Thesis, North Carolina State University, Raleigh
30. **Worsham AD, Moreland DE, Klingman GC** (1964) Characterization of the *Striga asiatica* (witchweed) germination stimulant from *Zea mays* L. J Exp Bot **15:** 556–567