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Striga asiatica, "witchweed", an obligate parasitic plant which attaches to the roots of corn, Sorghum, and other grasses, causes severe damage to crop yields around the world. The seeds of this parasite require a germination stimulus,[1] and once germinated Striga survives for less than 2 weeks in the absence of a host. In vitro, Striga seeds germinate only within a 0.75-cm zone of the roots of both corn and Sorghum[2] and produce roots that are no more than ~3 mm in length. Several years ago, the sesquiterpene strigol was isolated from cotton, a nonhost plant, and found to be a potent germination stimulus for Striga[3]. However, a stable sesquiterpene which could accumulate in the soil may stimulate germination at too great a distance for host attachment.[4] We describe here the identification of the first germination stimulant for Striga from the root exudate of a natural host and provide an explanation of how this compound would define the distance away from the host root at which Striga germination occurs.

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Chemical Regulation of Distance: Characterization of the First Natural Host Germination Stimulant for *Striga asiatica*

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Striga asiatica, "witchweed", an obligate parasitic plant which attaches to the roots of corn, *Sorghum*, and other grasses, causes severe damage to crop yields around the world. The seeds of this parasite require a germination stimulus,[1] and once germinated *Striga* survives for less than 2 weeks in the absence of a host. In vitro, *Striga* seeds germinate only within a 0.75-cm zone of the roots of both corn and *Sorghum*[2] and produce roots that are no more than ~3 mm in length. Several years ago, the sesquiterpene strigol was isolated from cotton, a nonhost plant, and found to be a potent germination stimulus for *Striga*.[3] However, a stable sesquiterpene which could accumulate in the soil may stimulate germination at too great a distance for host attachment.[4] We describe here the identification of the first germination stimulant for *Striga* from the root exudate of a natural host and provide an explanation of how this compound would define the distance away from the host root at which *Striga* germination occurs.

Sorghum bicolor (L.) Moench cv. IS 8768 seeds (10 g) were grown aseptically on moist filter paper in the dark at 27 °C for 7 days. The roots were dipped in 0.5% HOAc/CH_2Cl_2 (100 mL) for 2 s,[5] and the extract was evaporated in vacuo to give 15 mg of a biologically active crude exudate.[6] The ^{1}H NMR spectrum

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(1) Egley, G. H.; Dale, J. E. *Weed Sci.* **1970**, *18*, 586. Worsham, A. D.; Moreland, D. E.; Klingman, G. C. *J. Exp. Bot.* **1964**, *15*, 556. Okonkwo, S. N. C. *Am. J. Bot.* **1966**, *53*, 679.

(2) Riopel, J. L.; Lynn, D. G.; Baird, V.; Chang, M. *Striga asiatica* (*Witchweed*), Cooperative Research and Control Programs in the United States, monograph; Weed Science of America, in press.

(3) Cook, C. E.; Whichard, L. P.; Wall, M. E.; Egley, G. H.; Coggon, P.; Luhan, P. A.; McPhail, A. T. *J. Am. Chem. Soc.* **1972**, *94*, 6198. Cook, C. E.; Whichard, L. P.; Turner, B. M.; Wall, E.; Egley, G. H. *Science (Washington, D.C.)* **1966**, *154*, 1189.

(4) In vitro experiments in agar show that strigol can stimulate germination at great distances from the point of addition.

(5) Netzly, D. H.; Butler, L. G. *Crop Sci.* in press. Netzly, D. H.; Butler, L. G.; Chang, M.; Lynn, D. G. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1986**, *45*, 1566.

(6) *Striaga asiatica* seeds were obtained from Eplee, R. E., U.S.D.A. Witchweed Methods Development Laboratory, Whiteville, NC, and handled under quarantine control at Chicago, IL. Seeds were preconditioned as described by: Nickerent, D. C.; Musselman, L. J.; Riopel, J. L.; Eplee, R. E. *Ann. Bot.* **1979**, *43*, 233. For each assay, fractions were exposed to 30–50 2-week-old preconditioned seeds in 1 mL of distilled H_2O at 25 °C in the dark, and the percentage of seeds germinating were counted 36 h later under a dissecting microscope.

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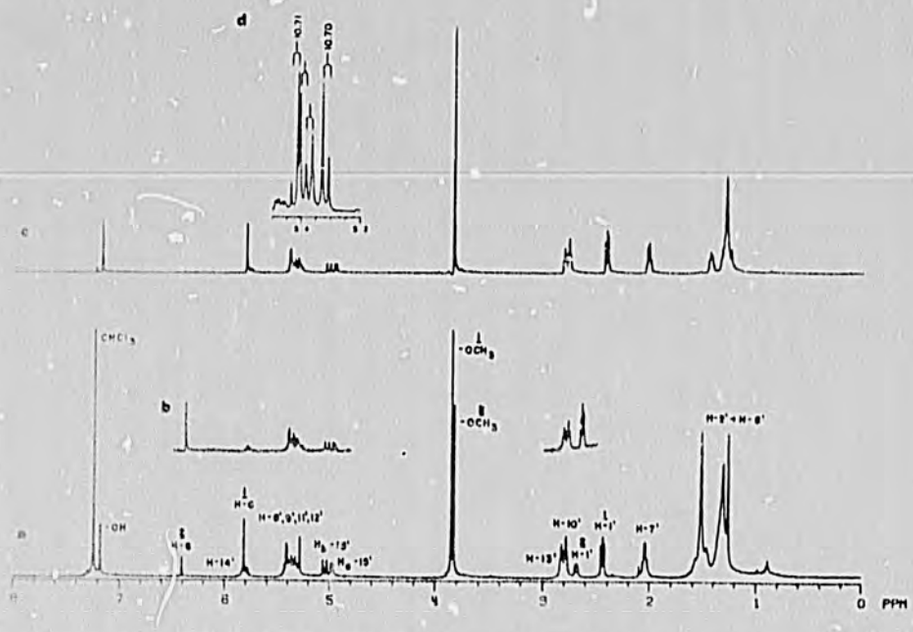


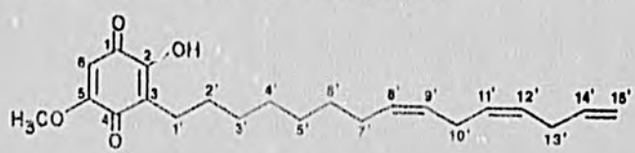
Figure 1. ^{1}H-NMR (500 MHz) spectra in 500 μL of $CDCl_3$ of (a) *Sorghum* crude exudate (1 mg), (b) hydroquinone **2** obtained by reducing **1** (1 mg) with zinc dust/HOAc, and (c) quinone **1** (4 mg), (d) triple-frequency irradiation of H-7′, -10′, and -13′ simplified the vinylic region to two AB patterns with J = 10.7 Hz.

showed it to be remarkably clean (Figure 1a), and its HPLC trace (Zorbax ODS, 4.6-mm i.d. × 25 cm, 88.0% MeOH/11.4% H_2O/0.6% HOAc, 1.5 mL/min, 280 nm) contained two major components (**1** and **2**) eluting at 6.9 and 7.6 min, respectively. When >99% pure, **1** did not induce germination of Striga. The biological activity of the exudate was related directly to the concentration of **2**. This compound was very unstable and on standing was rapidly converted to **1**. The germination stimulant was, therefore, isolated (9 mg) and characterized in its more stable form.

The UV spectrum (CH_2Cl_2) contained transitions at 283 (4.22), 288 (4.30), and 412 nm (2.68) suggesting a 2,5-hydroxylated benzoquinone.[7] EI-MS (70 eV, 200 °C) further supported this assignment with a molecular ion at m/z 358.2128, $C_{22}H_{30}O_4$ (calcd 358.2145), and a major benzylic cleavage[8] which resulted in ions at m/z 167, 168, and 169 (base peak) corresponding to Q^+, QH^+, and QH_2^+ respectively. ^{1}H NMR[9] (Figure 1c) showed the presence of a quinonoid proton (H-6, δ 5.81, 1 H, s), a methoxy group (δ 3.84, 3 H, s), a hydroxyl substituent (δ 7.19, 1 H, s, D_2O exchangeable; ν (CH_2Cl_2) 3380 cm^{-1}, br), and a C-15 side chain containing three double bonds. A terminal olefin was evident upon irradiation of H-10′ and H-13′ (H-14′, δ 5.80, 1 H, m, J = 5.5, 10.2, and 17.1 Hz; H_a-15′, δ 4.97, 1 H, dd, J = 1.7 and 10.2 Hz; H_b-15′, δ 5.03, 1 H, dd, J = 1.7 and 17.1 Hz).[10] The other four vinylic protons appeared as a multiplet at δ 5.3–5.4. Irradiation of this region and H-14′ unambiguously distinguished the methylenes at 10′ and 13′ (H-10′, δ 2.78, 2 H, dd, J = 5.5 and 5.9 Hz; H-13′, δ 2.82, 2 H, dd, J = 5.1 and 5.5 Hz)[11] and permitted the assignment of H-7′ (δ 2.04, 2 H, dt, J = 6.6 and 6.9 Hz). The remaining signals corresponded to H-1′ (δ 2.43, 2 H, t, J = 7.6 Hz) and the H-2′ to H-6′ methylenes (δ 1.2–1.4, 10 H, m).

The olefin stereochemistry could not be readily assigned by 2D NMR experiments because of spectral overlap. However, a triple frequency irradiation experiment (H-7′, H-10′, and H-13′) reduced it to two isolated AB patterns with coupling constants of 10.7 Hz (Figure 1d). The cis olefin geometry, suggested by the small coupling constant, was confirmed by a hetereonuclear correlation experiment[12] which identified two γ-gauche interactions for C-10′ (δ 25.6) relative to one for C-13′ (δ 31.5). In addition, a 2.7% NOE[13] enhancement of H-7′ was observed upon irradiation of H-10′, unequivocally confirming a *Z* configuration about both double bonds.

Both NOE difference and 2D-exchange experiments demonstrated that the quinonoid proton (H-6) was adjacent to the methoxy substituent (22% enhancement). The rest of the substitution pattern was evident from the UV and IR spectra[7] and the high-field-shifted ^{13}C NMR chemical shifts of the carbonyl resonances (δ 182.8, 181.6). These spectroscopic data identified compound **1** as the 2-hydroxy-5-methoxy-3-[(8′*Z*,11′*Z*)-8′,11′,14′-pentadecatriene]-*p*-benzoquinone.



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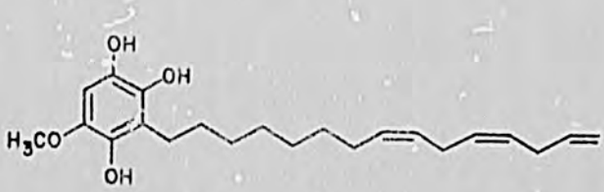

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The identity of the germination stimulant (**2**) was established from the ^{1}H NMR spectrum of the crude root exudate, which

(7) Chang, M.; Lynn, D. G. *J. Chem. Ecol.* **1986**, *12*, 561.

(8) Ogawa, H., Natori, S. *Chem. Pharm. Bull. (Tokyo)* **1968**, *16*, 1709; Beaumont, P. C.; Edwards, R. L. *J. Chem. Soc. C* **1969**, 2398.

(9) Spectra obtained at 500 MHz in $CDCl_3$ on a home-built console, an Oxford magnet, and an NTC 1280 computer.

(10) Upon irradiation (C_6D_6) of H-10′ and H-13′, the multiplet at δ 5.80 simplified to two doublets with J = 10.2 and 17.1 Hz.

(11) Irradiation of the vinylic region simplified H-13′ from a double doublet to a doublet (J = 5.5 Hz), H-10′ from a double doublet to a singlet, and H-7′ from a quartet of triplets to a triplet (J = 6.9 Hz). Irradiation of H-14′ collapsed H-13′ to a doublet (J = 5.1 Hz), while H-10′ remained unchanged.

(12) Bax, A. *J. Magn. Reson.* **1983**, *52*, 330. Bax, A. *J. Magn. Reson.* **1983**, *53*, 517. Rutar, V. *J. Am. Chem. Soc.* **1983**, *105*, 4095.

(13) Preirradiation (selective 180°) of H-10′ was followed after a delay (5 s) by a 90° observed pulse. The spectrum (400 transients) was acquired simultaneously with a spectrum in which one selective pulse was 1.5 ppm downfield, and the two spectra were computer subtracted to observe the enhancements.

contained additional signals (28%, δ 6.38, 1 H, s; δ 3.78, 3 H, s; δ 2.64, 2 H, t) that could be attributed to the hydroquinone form of **1** (Figure 1a). These signals disappeared as the crude exudate lost biological activity. Upon reduction of **1** with zinc dust[14] or tin amalgam,[15] the presence of the hydroquinone **2** in the crude exudate was confirmed (Figure 1b). The presence of **2** was further demonstrated by EI-MS analysis of the silylated crude exudate showing an ion at m/z 576 (m/z 360 + 3 Me_4Si) and by direct silylation of the second eluting component collected from HPLC under a N_2 atmosphere.

The ability of the crude root exudate to stimulate *Striga* germination is related directly to the concentration of **2**. When the concentration of the hydroquinone dropped below 10^{-7} M, the exudate no longer possessed activity.[16] However, the biological activity could be quantitatively recovered by adding synthetic hydroquinone **2** back to the inactive exudate. The ability of *Striga* to recognize this labile hydroquinone allows it to commit itself to a host through germination only within the distance through which **2** can diffuse before being oxidized. This report documents the first characterization of a *natural host-derived germination stimulant* for *Striga* and demonstrates the biological commitment of this parasite to a *transient species* that can define viability of and distance to a potential host. The generality of this mechanism and the reasons for the exudation of such molecules from host plants are currently under investigation.

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(16) Chang, M.; Netzly, D. H.; Butler, L. G.; Lynn, D. G., in preparation.