

# Role of Ethylene in the Germination of the Hemiparasite *Striga hermonthica*<sup>1</sup>

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## ABSTRACT

Seed germination of the hemiparasitic angiosperm *Striga hermonthica* (Del.) Benth is elicited by compounds present in the root exudates of the host plant. Although a variety of compounds can substitute for the host-derived signal, the mechanism through which these act is unknown. In the present study, an inhibitor of ethylene biosynthesis, aminoethoxyvinyl glycine, was found to inhibit germination. Addition of an intermediate in ethylene biosynthesis, 1-aminocyclopropane-1-carboxylic acid, was found to override this inhibition and to act as a substitute for the host-derived signal. 2,5-Norbornadiene, an inhibitor of ethylene action, was also found to inhibit germination. Ethylene is rapidly produced by *Striga* seeds after treatment with host root exudates. These results are consistent with a model for *Striga* seed germination in which host-derived signals and other compounds act by eliciting the synthesis of ethylene and in which ethylene itself initiates the biochemical changes leading to germination.

Seed germination in many species of parasitic angiosperms is unique in so far as it is triggered by compounds released from the roots of host plants (14). Seeds of the hemiparasitic angiosperm *Striga hermonthica* (Del.) Benth require a period of imbibition (2–14 d) at temperatures about 30°C, a process known as conditioning, before they have the potential to germinate. Germination occurs, however, only in response to compounds present in exudates from host roots; conditioned seeds simply kept moist do not germinate (16, 18). The first naturally occurring germination stimulant to be characterized was the sesquiterpenoid, strigol, present in root exudates of the false host, cotton (5, 6). More recently the host plant, sorghum, has been shown to exude a dihydroquinone (2-hydroxy-5-methoxy-3-[(8'Z,11'Z)-8',11',14'-pentadecatriene]-p-hydroquinone which is a very potent stimulant of germination (4, 15). A third compound capable of stimulating *Striga gesnerioides* (Willd.) Vatke seed has been identified in exudates of cowpea roots; this compound consists of a xanthine ring, an unsaturated C<sub>12</sub>-carboxylic acid, and a dipeptide of glycine and aspartic acid (10, 20). Several other compounds

are reported to be active in stimulating the germination of *Striga* seed (21), albeit at higher concentrations than those at which root exudate compounds are active. Stimulants identified include kinetin, zeatin, abscisic acid, scopoletin, inositol, methionine, and ethylene. The specificity and mode of action of the germination signals remain unclear (18).

Although the requirement for host derived signals to trigger germination may be unique in parasitic plants such as *Striga*, many of the compounds which illicit this response *in vitro* also promote or enhance the germination of other angiosperms. Ethylene in particular stimulates the germination of many species (2, 7, 12, 13, 19). In this study we examine the hypothesis that ethylene is the germination stimulant for *Striga* seeds and that host derived germination signals trigger germination by eliciting the biosynthesis of ethylene.

## MATERIALS AND METHODS

### Plant Materials, Growth Conditions, and Chemicals

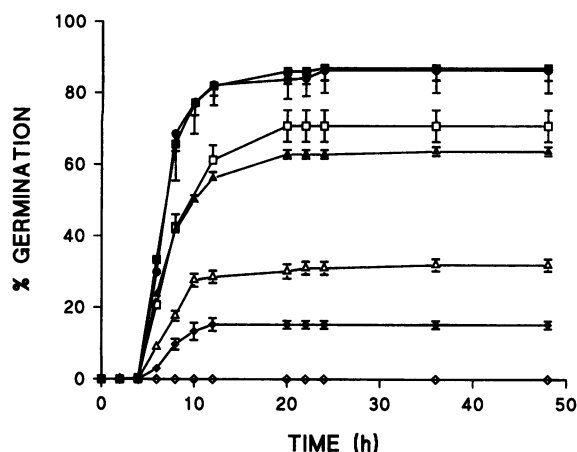
Seeds of *Striga hermonthica* (Del.) Benth were collected from Wad Medani, Sudan, in 1989. Seeds were surface-sterilized by washing in a 5% v/v solution of sodium hypochlorite (10–14% w/v available chlorine). Conditioning of seed took place on two sterile circles of Whatman No. 1 filter paper, saturated with sterile double-distilled deionized water, in 9 cm perspex Petri dishes incubated at 33°C in the dark for 7 d. Unconditioned seeds were treated as above except incubation was for 24 h only. Throughout the conditioning period the filter papers were saturated with sterile double distilled deionised water. For host root exudate collection, seeds of *Sorghum bicolor* (L.) Moench cv CSH-1 were surface-sterilized as above and germinated on moist sterile Whatman No. 1 filter paper at 33°C, after 48 h the germinated seeds were placed on nylon grids suspended over 500 cm<sup>3</sup> plastic beakers containing a 20% Long Ashton solution (11), so that the radicles were immersed in the solution. Plants were grown in a controlled environment (day temperature 35°C, night temperature 20°C, 16-h day length, light intensity at bench level of 103 Wm<sup>-2</sup>). Four days later, three plants were transferred to 50 cm<sup>3</sup> of sterile double-distilled deionized water and incubated at 20 to 25°C in the dark for 24 h; this solution was used as germination stimulant. All chemicals were of analytical grade or higher. AVG<sup>4</sup> and ACC were purchased from Sigma while NDE was purchased from Aldrich.

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<sup>4</sup> Abbreviations: AVG, aminoethoxyvinyl glycine; ACC, 1-aminocyclopropane-1-carboxylic acid; NDE, 2,5-norbornadiene.



**Figure 1.** Effect of AVG on *Striga* germination. Seeds were incubated in host root exudate containing different concentrations of AVG: ●, 0  $\mu\text{M}$ ; ■, 1  $\mu\text{M}$ ; □, 5  $\mu\text{M}$ ; ▲, 10  $\mu\text{M}$ ; △, 50  $\mu\text{M}$ ; ◆, 100  $\mu\text{M}$ ; ◇, 1 mM. Error bars indicate  $\pm$  SD ( $n = 12$ ).

### Measurement of Germination

Test solutions (200  $\mu\text{L}$ ) and *Striga* seeds (10/well) were added to flat-bottomed polystyrene microtiter plates (Sterilin, Middlesex, UK) at time zero and incubated in the dark at 33°C. Each sample was replicated 12 times (*i.e.* a total of 120 seeds). Incubation media included various concentrations and combinations of host root exudate, AVG and ACC. For tests involving NDE the seeds (10/vial) were placed in 1.95  $\text{cm}^3$  silicone rubber sealed vials (Life Science Laboratories Ltd., Luton, UK) together with 200  $\mu\text{L}$  exudate. Gas samples of 10 or 100  $\mu\text{L}$  of NDE stock were immediately injected, and the vials were rotated end to end at 1 revolution  $\text{s}^{-1}$  in an incubator at 33°C. NDE stock was prepared by injecting 18  $\mu\text{L}$  of NDE liquid into a 8.87  $\text{cm}^3$  silicone rubber sealed vial; this was left for 1 h at room temperature before use. Sterile double-distilled deionized water controls were included in all experiments. For measurement of ethylene-stimulated germination, seeds (10/vial) were placed in gas-tight vials as detailed for NDE above; with 400  $\mu\text{L}$  sterile double-distilled deionized water replacing host root exudate. Immediately after sealing, 100  $\mu\text{L}$  of 0.1% ethylene were bubbled through the water using a gas-tight Hamilton syringe and the vials incubated as above. Addition of NDE or ethylene took place at time zero. Germination counts based on radicle protrusion were made at intervals up to 48 h after the last treatment. All experiments were repeated at least three times and found to be reproducible. Results presented are a typical set of twelve replications. Mean percentages and standard deviations were calculated from arcsine transformed data.

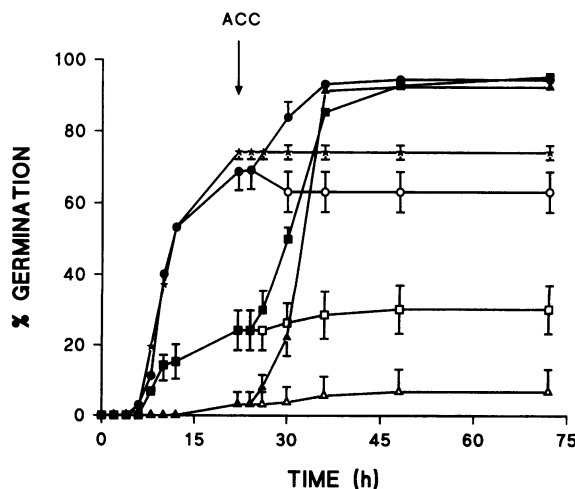
### Gas Analysis

For each sample 30 mg of seed were surface-sterilized and conditioned as above, washed with sterile double-distilled deionized water and transferred to a 8.87- $\text{cm}^3$  vial containing a final test solution volume of 1.5  $\text{cm}^3$ . Each vial was placed in the incubator for 1 min for temperature equilibration before being sealed as above and rotated end to end at 1

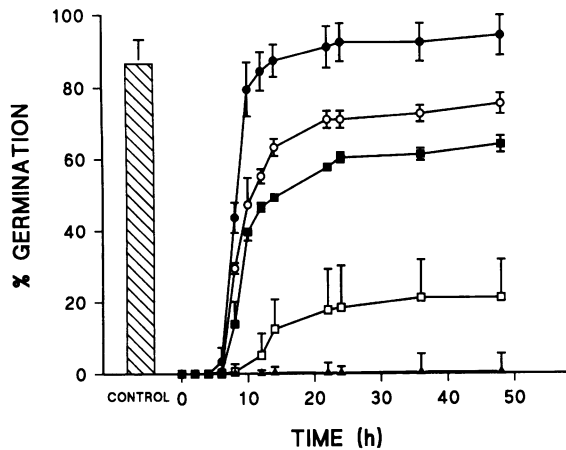
revolution  $\text{s}^{-1}$ . At intervals, 5  $\text{cm}^3$  of the head-space were withdrawn from a vial, using a gas-tight Hamilton syringe, for ethylene measurement. Values were corrected for dilution due to sampling method and new vials were used for each sampling time. Concentrations were determined by gas chromatography using a Poropak R (80–100 mesh) column and a flame ionization detector. Flow rates and temperatures were set as previously described (9).

### RESULTS AND DISCUSSION

The germination of *S. hermonthica* seeds triggered by root exudates from sorghum roots was detectable after 6 h and had reached its maximum by 20 to 24 h (Fig. 1). Germination was found to be inhibited by concentrations of AVG greater than 5  $\mu\text{M}$  and was completely inhibited at 1 mM (Fig. 1). AVG had little effect on the time course of germination, but altered the final percentage germination. The addition of ACC to seeds incubated for 24 h with AVG brought about a rapid increase in germination, a response to ACC being observed within 6 h (Fig. 2). The germination of ACC treated seeds reached its maximum 24 h after the addition of ACC. Concentrations of ACC above 100  $\mu\text{M}$  were found to stimulate germination of *Striga* seeds and at 50 mM ACC gave levels of germination comparable with those obtained with root exudate alone (Fig. 3). Germination triggered by sorghum root exudates was also found to be inhibited by NDE (Fig. 4), which is known to inhibit ethylene mediated responses (17). NDE stock at 10  $\mu\text{L}$ /vial reduced germination by over 50% although increasing the concentration of NDE 10-fold resulted in no further reduction. Conditioned seeds stimulated with ethylene showed a similar response to those stimulated by host root exudate (Fig. 5). Seeds which had imbibed for only 24 h also responded to ethylene treatment although there was a 12-h lag period before germination (Fig. 5).



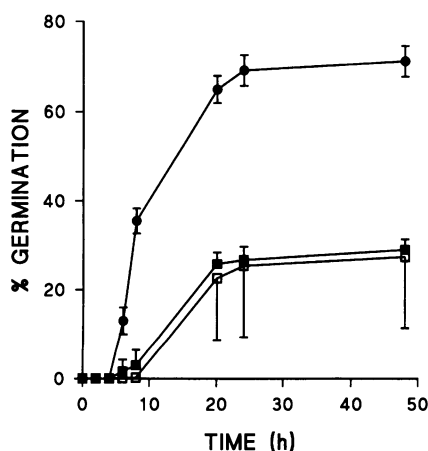
**Figure 2.** Overriding AVG germination inhibition by addition of ACC. Seeds were incubated for 24 h in host root exudate containing either 0.1  $\mu\text{M}$  AVG (●), 50  $\mu\text{M}$  AVG (■), or 1 mM AVG (▲) before addition of either 50 mM ACC (closed symbols) or 100  $\mu\text{M}$  ACC (open symbols). Control treatment, ★, was of host root exudate only. Error bars indicate  $\pm$  SD ( $n = 12$ ).



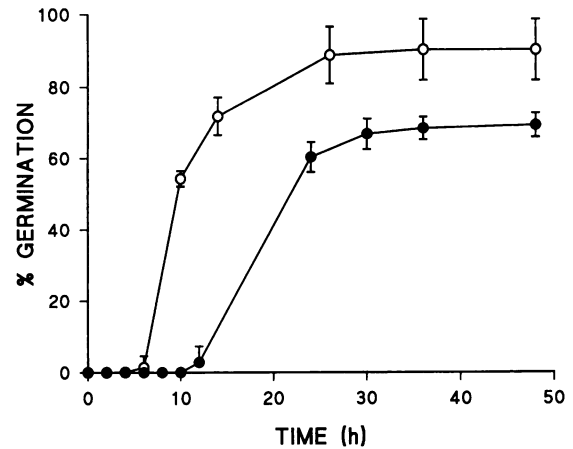
**Figure 3.** Effect of ACC on *Striga* germination. ACC concentrations were: ●, 50 mM; ○, 10 mM; ■, 5 mM; □, 1 mM; ▲, 100 μM. Control bar represents germination with host root exudate at 48 h. Error bars indicate ± SD ( $n = 12$ ).

Sterile double-distilled deionized water controls gave zero germination.

*Striga* seed germination triggered by sorghum root stimulants is blocked by an inhibitor of ethylene biosynthesis (AVG) which has been shown by Adams and Yang (3) to inhibit ACC synthase. This inhibition is completely overridden by an intermediate of ethylene biosynthesis (ACC) which is the product of the reaction inhibited by AVG. Moreover, ACC can not only override the AVG inhibition of germination but it can also substitute for host root stimulant in promoting the germination of dormant *Striga* seeds. Similar responses to AVG and ACC have been observed with *S. hermonthica* seed from the same region, collected in 1981 and 1987. These results suggest that the mode of action of the germination stimulants exuded by sorghum roots could be indirect, through activation of the synthesis of ethylene. Con-



**Figure 4.** Effect of NDE on *Striga* germination. Seeds were incubated in host root exudate with NDE added at two concentrations as described in "Materials and Methods," ■, 10 μL/vial; □, 100 μL/vial. Control treatment, ●, was of host root exudate. Error bars indicate ± SD ( $n = 12$ ).

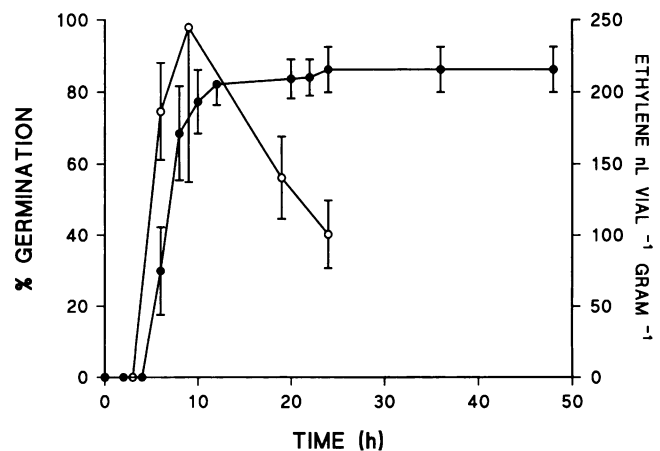


**Figure 5.** Time course of unconditioned, ●, and conditioned, ○, *Striga* seed germination in response to exogenous ethylene as detailed in the "Materials and Methods." Error bars indicate ± SD ( $n = 12$ ).

sistent with this suggestion is the observation that NDE also inhibits the germination of *Striga* seeds triggered by host root exudate. Silver ions in the form of silver thiosulfate also inhibit *Striga* seed germination (data not shown).

Further evidence for the endogenous role of ethylene is provided by the similarities between the response of seeds challenged with either host root exudate or exogenous ethylene. Unlike host root exudate, however, ethylene promotes germination of seeds not subjected to a prolonged conditioning period. This suggests that the conditioning process is not a prerequisite to ethylene perception or transduction of its biochemical effects.

Application of host root exudate induces the production of ethylene by *Striga* seeds within 6 h (Fig. 6), and production was at its maximum after 9 h incubation. As might have been anticipated, AVG substantially inhibited, while ACC greatly



**Figure 6.** Time course of *Striga* seed germination, ● ( $n = 12$ ), and ethylene production, ○ ( $n = 3$ ), by *Striga* seeds after incubation with host root exudate. Seeds were added to host root exudate at time zero. Germination and ethylene production were measured as described in "Materials and Methods." Error bars indicate ± SD.

**Table I.** Effect of AVG, ACC, and NDE on Ethylene Production

Ethylene production was measured as described in "Materials and Methods." For ACC, readings were first made at 6 h then the vials were reequilibrated with air at 33°C, returned to the incubator and sampled 24 h later. The control treatment was of host root exudate. Seeds were added to the test solution at time zero. Values are means of three replicates  $\pm$  SD.

Treatment	Ethylene Production		
	6 h	24 h	30 h
	<i>nL via<sup>-1</sup> g<sup>-1</sup></i>		
Control	186 $\pm$ 34	101 $\pm$ 24	
10 mM ACC	1082 $\pm$ 56		6256 $\pm$ 15
100 $\mu$ M ACC	Not detected		68 $\pm$ 18
1 mM AVG	Not detected		
50 $\mu$ M AVG	Not detected	15 $\pm$ 9	
NDE	274 $\pm$ 18		
Distilled H <sub>2</sub> O	Not detected	Not detected	

stimulated, ethylene production (Table I). NDE was found to slightly stimulate ethylene production by *Striga* seeds.

The production of ethylene by *Striga* seeds challenged with host root exudate is consistent with the hypothesis that the host derived trigger stimulates ethylene biosynthesis which then triggers *Striga* seed germination. Observations that the addition of ACC stimulates the germination of seeds in the absence of root exudate and that AVG inhibits germination suggests that the chemical stimulus from the host has its effect prior to ACC in the ethylene biosynthetic pathway.

We have also observed that the *Striga* seeds stimulated to germinate by ACC have very short radicles and seem thicker than those which germinate in response to host root exudate. This response to ACC may be due to inhibition of root elongation by the high concentrations of ethylene generated (1, 8).

Some of the chemicals present in sorghum root exudates which trigger germination of *Striga* seeds have been shown to have allelopathic properties, inhibiting the germination of other seeds (15). It is tempting to speculate that their effect on *Striga* seed germination represents a modification of the plant wound syndrome in which wounding, chemical or mechanical is accompanied by a burst of ethylene synthesis (2).

The results presented here are consistent with a model for the control of *Striga* seed germination in which a variety of chemical signals derived from host root exudates act through a common mechanism, namely the elicitation of ethylene biosynthesis and it is ethylene itself which initiates the biochemical cascade which results in germination.

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