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Short communication

Germination and appressorial formation by uredospores of *Uromyces viciae-fabae* exposed to inhibitors of polyamine biosynthesis

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Abstract

An examination was made of the effects of three polyamine biosynthesis inhibitors on germination and appressorium formation by uredospores of the bean rust fungus *Uromyces viciae-fabae* on artificial membranes. The ornithine decarboxylase inhibitor α-difluoromethylornithine had no effect on uredospore germination, even when used at 2mM, whereas appressorium formation was reduced by 63% at 0.5 mM and by 99% at 2mM. Methylglyoxal bis(guanylhydrazone), an inhibitor of S-adenosylmethionine decarboxylase, reduced germination when used at 0.025 mM, and at this concentration, appressorium formation was completed prevented. Uredospore germination was unaffected by as much as 3 mM cyclohexylamine, an inhibitor of spermidine synthase, while appressorium formation was reduced at 1mM and completely prevented at 3.3 mM. These results support previous suggestions that inhibitors of polyamine biosynthesis exert their main effect on the early stages of fungal development on the leaf surface.

Abbreviations: CHA = cyclohexylamine; DFMO = α-difluoromethylornithine; MGBG = methylglyoxal bis(guanylhydrazone).

Inhibitors of polyamine biosynthesis have been shown to reduce fungal growth [Rajam and Galston, 1985; West and Walters, 1989] and to reduce infection of a number of plants by pathogenic fungi [Rajam *et al.*, 1985; Walters, 1986]. Indeed, the ornithine decarboxylase (ODC) inhibitor α-difluoromethylornithine (DFMO), has been shown to control infections by rust and powdery mildew fungi in both glasshouse and field experiments [e.g. Weinstein *et al.*, 1987; West and Walters, 1988; Havis *et al.*, 1992]. A number of authors have shown that although DFMO can reduce rust or powdery mildew infection when applied either as a pre-inoculation or post-inoculation treatment, its efficacy is greatest when applied post-inoculation. West and Walters [1988] have thus shown that DFMO gave best control of powdery mildew infection of barley seedlings when applied 3 d after inoculation. These authors also obtained similar results with the S-adenosylmethionine decarboxylase (AdoMetDC) inhibitor methylglyoxal bis(guanylhydrazone) (MGBG) and the spermidine synthase inhibitor cyclohexylamine (CHA). These results suggest that such inhibitors exert their effects on the leaf surface, especially since DFMO applied to roots or lower leaves of barley seedlings only gave partial control of mildew infection [Walters and Kingham, 1990]. Although Rajam *et al* [1989] showed that DFMO reduced uredospore germination and germ tube growth in *Uromyces phaseoli*, no other data exist on the effects of inhibitors of polyamine biosynthesis on germination, or on appressorium formation, in phytopathogenic fungi. This paper reports on the effects of DFMO, MGBG and CHA on germination and appressorium formation in uredospores of *Uromyces viciae-fabae* on artificial membranes.

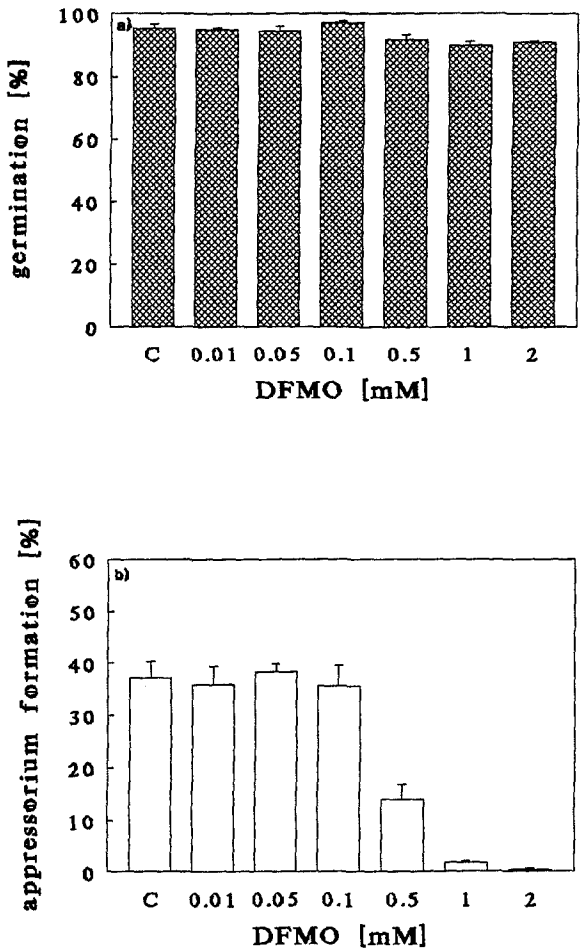

Fig. 1. Effects of different concentrations of DFMO on germination (a) and appressorium formation (b) by uredospores of *U. viciae-fabae* on artificial membranes. Bars indicate standard deviations.

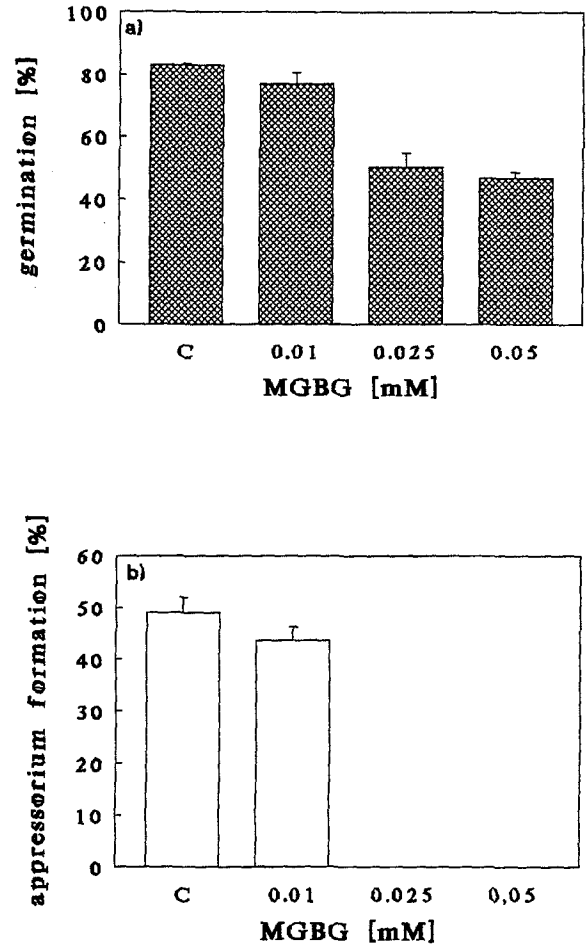

Fig. 2. Effects of different concentrations of MGBG on germination (a) and appressorium formation (b) by uredospores of *U. viciae-fabae* on artificial membranes. Bars indicate standard deviations.

Broad bean plants (*Vicia faba* L. cv Bunyards Exhibition) were grown in a heated glasshouse and inoculated with the rust fungus *Uromyces viciae-fabae* (Pers.) Schroet., as described previously [Walters, 1986]. Uredospores were collected from infected plants 18 d after inoculation, stored at 4 °C until required, and were always used within 1 month of collection. Immediately before use, uredospores were given a heat shock at 45 °C for 3 min. Dry uredospores (10 mg) were then dusted onto polyethylene membranes (1.5 cm^2) using a settling tower (30 × 30 × 30 cm). The membranes were plastic bags (200 gauge) scratched with a fine wire brush four times in two directions. Each membrane was then floated, spore-side down, in a Petri dish containing 5 ml of distilled water or inhibitor solution at pH 5.6. After 8 h, the germlings were stained with Commassie brilliant blue-lactophenol solution and examined under a light microscope (Leitz DM RB with photoautomat, Leica UK). Germination and appressorium formation were assessed by counting 100 germlings in three separate experiments, and data were analysed using Student's t-test.

In control treatments, germination of uredospores of *U. viciae-fabae* ranged between 80% ad 96%, while appressorium formation ranged between 35% and 55% (Figs 1–3; Fig. 4a). DFMO had little effect on uredospore germination, even when used at 2 mM. On the other hand, appressorium formation was reduced by 63% at 0.5 mM DFMO and by 99% at 2 mM DFMO ($P < 0.01$; Fig. 1; Fig. 4 b). MGBG produced a significant reduction in uredospore germination when used

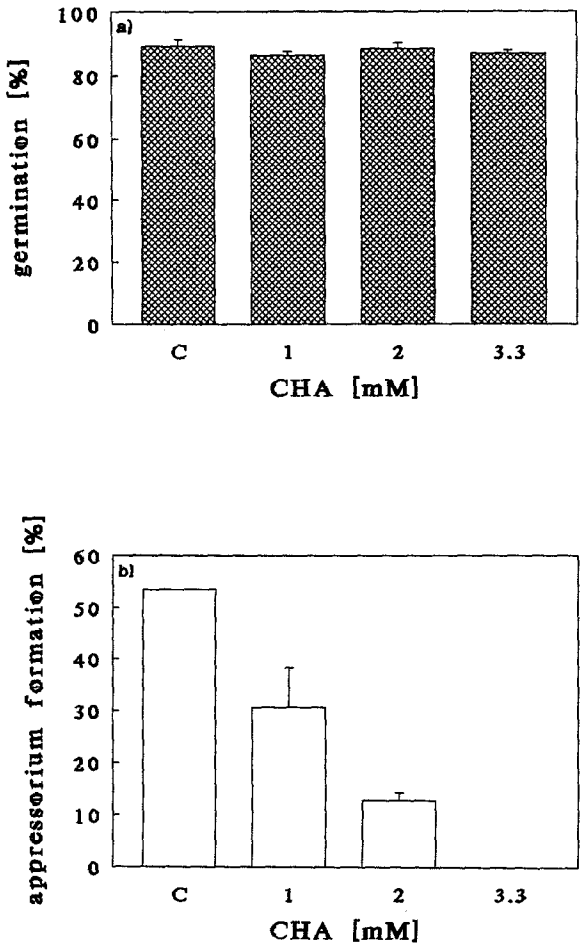

Fig. 3. Effects of different concentrations of CHA on germination (a) and appressorium formation (b) by uredospores of *U. viciae-fabae* on artificial membrances. Bars indicate standard deviations.

at concentrations as low as 0.025 and 0.05 mM, and at these concentrations appressorium formation did not occur ($P < 0.001$; Fig. 2; Fig. 4 c). Uredospore germination was not significantly affected by treatment with up to 3.3 mM CHA, although appressorium formation was significantly reduced by all concentrations of CHA examined, and was completely prevented at 3.3 mM ($P < 0.01$; Fig. 3; Fig. 4 d).

The lack of any effect of DFMO on germination of uredospores of *U. viciae-fabae* is suprising in view of the results obtained using uredospors of *U. phaseoli*. In that work, Rajam *et al.* [1989] found that 1 mM DFMO reduced uredospore germination by 36%. As far as we are aware, no data exist for the effects of MGBG or CHA on uredospore germination. MGBG reduced germination when used at 0.025 mM, although this effect may be only partly related to a perturbation of polyamine biosynthesis, given that MGBG also inhibits respiration [Janne *et al.*, 1985]. The lack of any effect of CHA on germination is also surprising, since this compound is known to inhibit spermidine synthase and spermidine is the most common polyamine in fungi [Walters, 1995].

In contrast to the effects on germination, the inhibitors all reduced appressorium formation by germlings of *U. viciae-fabae*. This represents the first report on the effects of polyamine biosynthesis inhibitors on appressorium formation by plant pathogenic fungi and supports previous observations that these inhibitors exert their main effect against the developing germling on the leaf surface. It will now be of great interest to determine whether these inhibitor-induced reductions in appressorium formation are associated with changes in polyamine biosynthesis in developing germlings.

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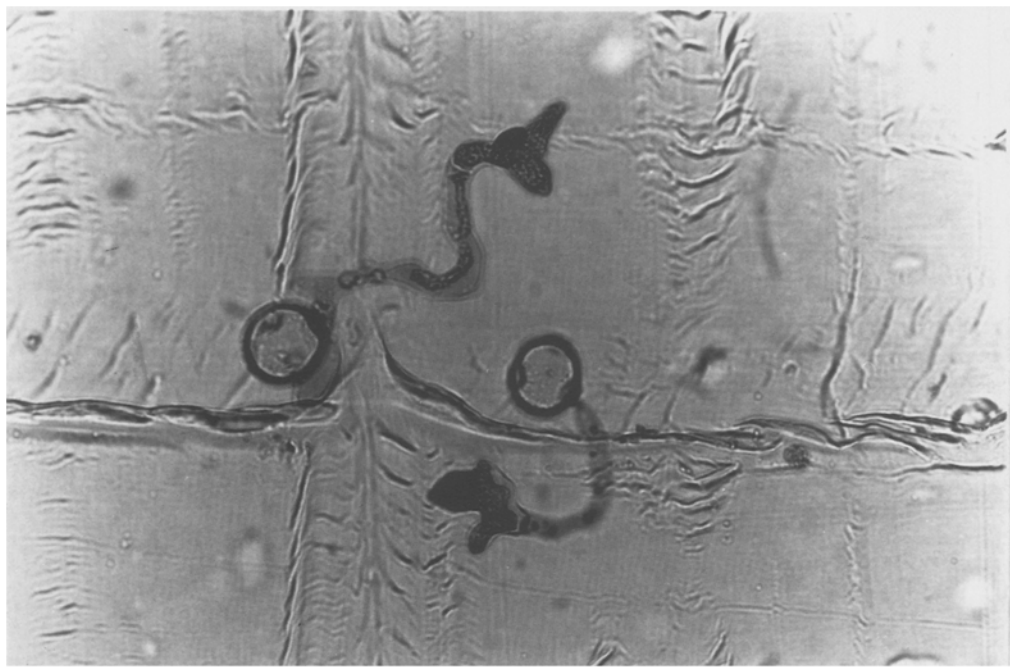
a



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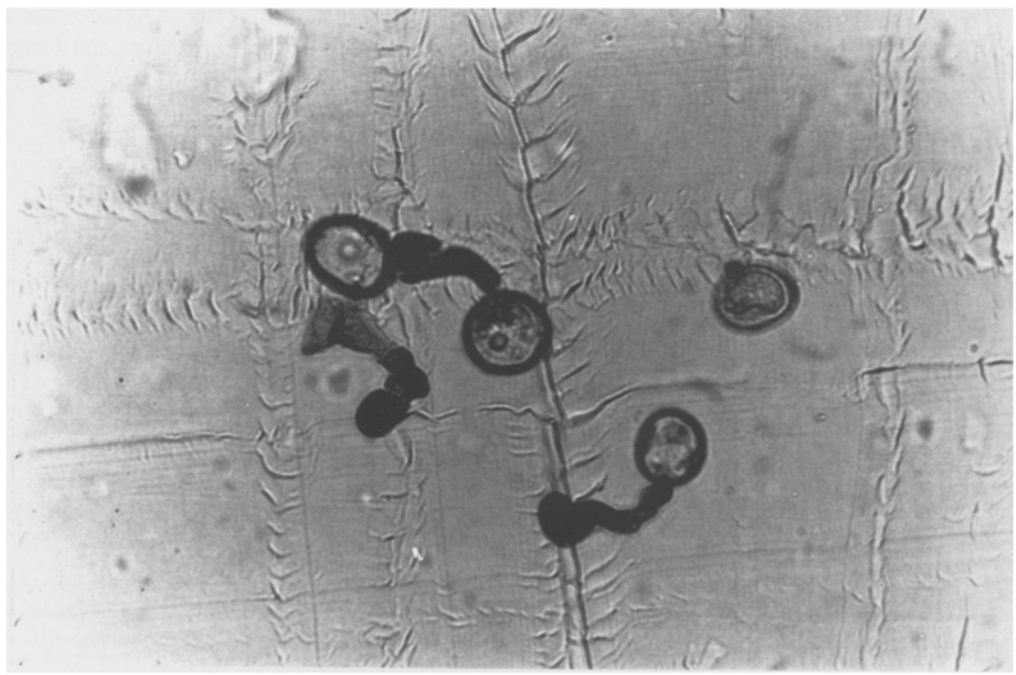
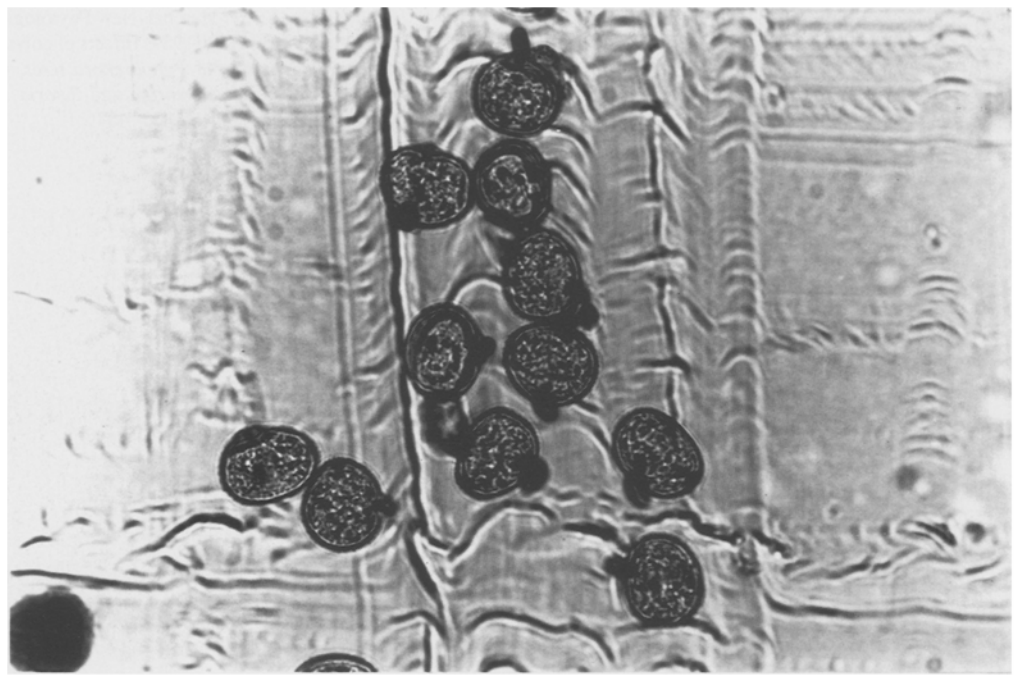


Fig. 4. Photomicrographs of germination and appressorium formation by uredospores of *U. viciae-fabae* on artificial membranes, × 500 (a) control (b) 2 mM DFMO (c) 0.025 mM MGBG (d) 3.3 mMCHA.

c



d

Fig. 4. Continued.

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