Stimulation of Germination of Polyporus dryophilus Basidiospores by Carbon Dioxide

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ABSTRACT

Germination of *Polyporus dryophilus* basidiospores was stimulated by gaseous emanations of other fungi. The stimulation appeared to be due to carbon dioxide since its removal prevented germination, and the addition of gaseous carbon dioxide stimulated germination. The mechanism of stimulation by the supplementary CO_2 did not appear to be related to a change in the pH of the medium or a reduction of other displaced atmospheric gases.

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Basidiospores of *Polyporus dryophilus* germinated in vitro when placed on an agar medium from which vegetative mycelium of the fungus had been removed (1). Germination was also stimulated by placing the spores on a separate medium in close proximity to, but not in contact with the vegetative mycelium of *P. dryophilus* (6). Of 50 fungi tested for their capacity to stimulate germination of *P. dryophilus* spores in this manner, the oak wilt fungus, *Ceratocystis fagacearum* (Bretz) Hunt was the most active. Because CO_2 is known to have diverse effects upon sporophore production, sporulation, and germination (1, 2, 9), we sought to establish whether this metabolic product might be responsible, in part, for stimulating germination of *P. dryophilus* basidiospores.

Four isolates of *P. dryophilus* var. *vulpinus* (Fries) Overh. used in this study were collected from quaking aspen (*Populus tremuloides* Michx.) in Minnesota. The *C. fagacearum* isolate was also obtained in Minnesota. The spores and sporophores of *P. dryophilus* were produced axenically in vitro according to techniques reported previously (8). Germination was studied microscopically on the surface of agar media. The water agar (WA) medium contained glass-distilled, deionized water and Difco-Bacto agar. Several volatile compounds, including ethylene and various esters and alcohols, at concentrations of 1 to 1,000 ppm, known to be produced by species of *Ceratocystis*, were included with spores of *P. dryophilus* to test their influence on germination. To determine if CO_2 was involved in the stimulation by *C. fagacearum*, the CO_2 was removed from the culture atmosphere by 40% KOH (w/v). In another experiment, a Mallcosorb filter (which absorbs CO_2) separated the culture atmosphere of *C. fagacearum* and the spores of *P. dryophilus*. The effects of supplementary CO_2 upon germination were estimated directly by exposing the *P. dryophilus* spores to several known concentrations of the gas. Compressed CO_2 was used to displace known amounts of sterile water from stoppered bottles; the amount of water displaced was assumed to equal the volume of CO_2 admitted into the atmospheres of the bottles, and thus several broad ranges of CO_2 concentrations were evaluated for their effects upon germination. Because the supplementary CO_2 of the *C. fagacearum* culture gases could have changed the pH value of the agar and thereby indirectly stimulated germination, several experiments were set up in which the WA medium was buffered with 0.2 M citrate-phosphate at several pH values. Another mechanism by which germination might be affected indirectly was through the displacement of other components by the supplementary CO_2. To test this possibility, spores were incubated in the presence of 30% CO_2-70% air, or in an air atmosphere supplemented with 10, 20, 30, or 40% N_2.

None of the esters or alcohols stimulated germination, while control spores in the presence of *C. fagacearum* did germinate. When CO_2 was removed by KOH or filter, germination was 9 and 0.9% respectively, compared to 38 and 28% respectively, for the controls. The results of several tests showed that a few spores germinated erratically at initial CO_2 concentrations of 5 to 15%, and up to 85% of the spores germinated in atmospheres containing 20 to 100% CO_2. Similar experiments showed that in the presence of about 30% CO_2, a few spores germinated after 3 days of exposure to CO_2, and that maximum germination occurred after 12 days of exposure to CO_2. Up to 39% germination resulted if the agar disks bearing the spores were replaced with 60 μ-thick wood slices. All of these experiments indicated that exposure of the spores to supplementary CO_2 stimulated germination beyond that which occurred in the absence of CO_2. The use of buffered media indicated that the stimulatory effects of CO_2 were due to the direct effect upon the spores and the CO_2 had little or no effect upon the pH of the medium. None of the spores germinated in any of the atmospheres enriched in nitrogen while 7 to 46% of the spores germinated in 30% CO_2.

Although many fungi were found to stimulate germination of the basidiospores of *P. dryophilus*, the greatest numbers of spores germinated when exposed to cultures of *C. fagacearum*. CO_2 appears to be a component of the stimulant since its absorption with either a filter or KOH reduced or eliminated that stimulation. The use of ethylene, alcohols, and esters failed to stimulate germination, and the mechanism by which *C. fagacearum* stimulated more germination than other microorganisms was not established.

CO_2 is clearly implicated in the stimulation of

germination, however, since concentrations of 5 to 100% for 3 to 14 days brought about germination of *P. dryophilus* basidiospores. In addition, when the spores of *P. dryophilus* were incubated either with gaseous emanations of *C. fagacearum* or in 30% CO_2 for as little as 3 days, they began to germinate.

The mechanism of action of CO_2 is not thought to be due to a change in the pH of the substrate, because CO_2 did not measurably change the pH of a buffered medium, and because a buffered medium without CO_2 did not stimulate germination. Similarly the mechanism does not appear to be due to a displacement of atmospheric components by supplementary gases because nitrogen failed to stimulate germination. CO_2 fixation as the mechanism of germination stimulation has been suggested with preliminary supporting data (4, 5, 7).

Naturally occurring concentrations of CO_2 from 2 to 25% have been found in the intercellular spaces of many tree species (3). This suggests that CO_2 can accumulate in woody tissue where it is a large proportion of the gaseous environment and makes the stimulation of germination ecologically feasible. Basidiospores could come in contact with an environment high in CO_2 in tree wounds, or in the presence of insects or microorganisms that could produce high CO_2 contents in a microenvironment.

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