Letter to the Editor

Endogenous Inhibitors of Spore Germination

David Gottlieb

Department of Plant Pathology, University of Illinois, Urbana 61801.

The presence of endogenous inhibitors of spore germination in fungi has long been known. Inhibitors have been demonstrated in uredospores of *Puccinia graminis tritici* (1, 2, 16) and other rusts, in conidia of *Erysiphe graminis, Glomerella cingulata* (6, 9) and *Peronospora tabacina* (14), in teliospores of *Ustilago tritici* (13), and in spores of many other fungi. In contrast, they have not been demonstrated in *U. nuda* or *U. avenae* (13). The recent isolation and identification of the self-inhibitors of uredospores of *Uromyces phaseoli* and *P. graminis tritici* as derivatives of cinnamic acid have put these studies on a sounder footing and indicate that we are indeed working with the natural inhibitors (3, 10, 11). Similarly, the self-inhibitor from spores of *Dictyostelium discoides* has now been isolated and characterized as 2-dimethylamino-6-oxypurine riboside (A. S. Sussman & C. Bacon, *personal communication*).

With this splendid progress, however, comes the pertinent question of the natural function of these self-inhibitors in the development of the spore. Though there is no hard evidence in this area, an hypothesis must be drawn if only to guide us into investigations on the question. A recent survey on spore germination has led me to the conclusion that the metabolism of spores is not significantly different from that of vegetative hyphae (7), though some mechanisms are operating which are unnecessary for vegetative growth. These might involve, for example, enzyme activities which weaken the cell wall prior to germ tube formation. The same basic respiratory metabolic systems are present in the spores, and if some enzymes are lacking they are synthesized during spore incubation (7). Similarly, the synthetic machinery is ready to function and produce macromolecules or other simpler metabolites from relatively small molecules already stored in pools or available from exogenous sources.

The inference from such data is that the spore is ready to germinate and needs no triggering mechanism to start the process. Instead, a more likely hypothesis is that the spore has some regulatory substance incorporated in it which prevents its metabolic activities from proceeding at more than a minimal rate. The removal of this compound would then allow the germination processes to proceed. The presence of endogenous auto-inhibitor in the spore and its removal or, more likely, its dilution by water to a level below a critical concentration would meet the properties expected for such a regulator. How do the data fit this concept?

First, water is the sine qua non for spore germination, except in a very few species such as some of the powdery mildew fungi. It promotes germination best when spores are suspended in the liquid phase, though it is also effective in the vapor phase. Water, of course, has many functions in the cell but our only interest in this context is in its role in diluting the inhibitor or removing it. One is aware of the presence of the inhibitor in the spore only when the spores are present in high concentration. Under these conditions the volume of water is not sufficient to dilute the inhibitor below the critical level, and there is a positive correlation between concentration of spores and percent inhibition (1, 2, 5, 6). Second, the inhibitor can be extracted from the spores by water, and germination goes on readily when these same spores are suspended in fresh water (1, 5). But if either fresh spores or previously extracted spores are placed in the extract, germination is inhibited. In very dilute suspensions of spores, such as *P. graminis tritici*, the water is of sufficient volume that the inhibitor readily diffuses out and is now at a concentration too low to be effective.

In *Erysiphe graminis* even spores in chains as short as two spores germinated less than did single isolated spores. Though the explanation given was greater permeability, it can also be interpreted as a dilution-concentration effect (8). Germination in some spores takes place even in the absence of liquid water. In such conditions one can visualize the uptake of water from the vapor phase and a consequent internal dilution of the inhibitor.

It is difficult to formulate a reasonable hypothesis for the role of self-inhibitors in the germination of spores after they have been disseminated. In most situations spores do not land on living or nonliving substrates in concentrations high enough to be self-inhibitory when water becomes available for germination. A special case might be proposed for situations in which the volume of water around the spores is very small, and environmental conditions might favor the rapid evaporation of the water. Under such conditions the spores might begin to germinate but then, with the disappearance of water, die. In this system even a small spore concentration might be self-inhibitory and preserve the viability of the spores until more favorable conditions occur.

On the other hand, there is a more attractive hypothesis - that the main role of the self-inhibitor is to prevent the germination of spores while they are still in or part of the fruiting body. The presence of the endogenous inhibitor in the fruiting body would be very important and even necessary for the spores' reproductive role. The fruiting body is the place where the spores are in highest concentration. Even under conditions of high humidity or the presence of free water germination is rarely observed. In these situations the inhibitor that diffuses from the spores would be in high concentration and would thus

prevent germination. If no such endogenous inhibitor were present, germination would take place and the potential of the fungus for multiplication or dispersal would be reduced. The spores would not be readily disseminated, if at all, for the germ tubes could become interwoven and spores would no longer be free to be readily carried away by wind or water. Their viability would be in a precarious state because germinated spores are more sensitive to drying, high temperatures and other deleterious environmental influences than are nongerminated spores.

Unfortunately, not all the observations fit neatly into this concept. Some fungi have been reported to lack self-inhibitors so that a systematic study of a wider range of species will have to be made (13). There is also a body of literature which indicates that the self-inhibitory effect can be offset by a wide range of chemicals whose structures bear no direct relationship to those of the two rust inhibitors we now know (2, 12). The actions of these chemicals must nevertheless be rationalized into the general scheme. Another bothersome phenomenon is that some powdery mildew spores do not require water to germinate, thus denying the involvement of the dilution role of water in that fungus (4, 15). As indicated earlier, the dilution by water is internal in the spore and could be caused by the direct absorption of water in its gaseous phase rather than by the absorption of liquid water.

The hypothesis that the endogenous germination inhibitors play an important role in the preservation of the spore until conditions are appropriate for germination and dissemination does fit most of the available data we now have. Furthermore, at the minimum, it furnishes us with a framework which will allow us to plan more critical research.

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