Stimulation of spore germination of wood-decomposing Hymenomycetes by carbon dioxide

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Abstract. — The effect of carbon dioxide on the spore germination of 23 wood-decomposing *Hymenomycetes* was investigated by introducing 1 %, 5 %, and 20 % carbon dioxide — air mixtures into desiccators, in which spores were germinated on Hagem agar. Compared to ordinary air, low concentrations (1 % and 5 %) markedly stimulated the germination rate of several species. Species growing in living tree stems were stimulated most effectively *(Spongipellis borealis, Polyporus squamosus, Pholiota aurivella* and *Fomitopsis annosa)*. Stimulation took place at different pH values and seems to be independent of the acidifying action of carbon dioxide. Ecological aspects of the stimulation are discussed.

Introduction

Spores of wood-decomposing *Hymenomycetes* are often produced in great quantities, and a freshly cut exposed wood surface may receive spores within minutes, as experiments with *Fomitopsis annosa* have indicated (Rishbeth 1959). As the wood surface receives also spores of many other fungi as well as bacteria, the conditions at the wood surface evidently have a selective effect on the invading fungal population. In addition, tree wounds may remain susceptible to infection for only a relatively short time due to the formation of resin, tyloses, and the effects of other micro-organisms. For instance, spores of *Fomes igniarius* have been found to germinate on wounds of *Populus* only during the first 4 days (Manion & French 1968).

One of the many factors active at the wood surface is carbon dioxide. Living and decomposing wood contains considerable amounts of carbon dioxide which often exceed the concentration in the air by 100—500 times, (Thacker & Good 1952, Jensen 1969), and decomposing wood continuously produces CO_2 (Klingström 1965, Good, Basham & Kadzielawa 1968). Evidently carbon dioxide diffuses from the wood and affects the fungal spores at the surface. The present experiments were devised to investigate the effects of carbon dioxide on the germination of some wood-decomposing *Hymenomycetes*.

Methods

Spore deposits of wood-decomposing fungi were obtained by keeping a freshly collected basidiocarp above a sterile 9 cm petri dish for 4—12 hours. Dishes with spore deposits

were kept at +5°C, and used for experiments within 6 months of storage. As many wood-decomposing fungi require external carbon supply for germination (WALKINSHAW & SCHELD 1965, FRIES 1966, MANION & FRENCH 1969), Hagem agar was used as a standard medium (composition: glucose 5 g, malt extract 5 g, KH_2PO_4 0.5 g, $MgSO_4$ 7 H_2O 0.5 g, NH_4Cl 0.5 g, $FeCl_3$ 1 % solution 0.5 ml, agar 15 g, dist. H_2O 1000 ml). This medium, on which the mycelia of the species investigated grew well, proved to be superior to plain water agar. A spore suspension in sterile water was spread over the surface of the agar in a 5 cm petri dish which was placed, upside down in 10 l desiccator. The desiccators were flushed with 100 l carbon dioxide-air mixtures prepared by Aga Oy, Kilo, from 150 atm. pressure containers, and immediately closed. 1 %, 5 %, and 20 % carbon dioxide-air mixtures were used. The amount of gas was checked by an air-flow meter made by VEB Prüfgerätewerk, Medingen. At suitable intervals a dish was taken out of the desiccator in order to follow the germination process, and the desiccator containing the remaining dishes was flushed with 100 l of the same gas mixture and immediately closed. The germination was terminated by placing a few drops of formaldehyde on the opposite lid of the dish. In each dish 1000 spores were counted. A spore was regarded as having germinated when the germination tube was more than half the length of the spore. In the case of rapidly growing species, the mycelia from the spores which germinated first made the counting inaccurate within a short time, and the main emphasis was laid upon the initiation of the germination, and FULLER's method (1967) for cumulative germination percentage proved to be too laborious. Control dishes in ordinary air were kept under similar conditions in closed desiccators, but no flushing was done. The experiments were carried out at room temperature (20—24°C). The results are presented in Table 1. The significance test is based on the fact that

$$\mu = \frac{p_1 - p_2}{\sqrt{\frac{2p\,100 - p)}{n}}}$$

where p_1 and p_2 are percentages of germination, $p = \frac{1}{2}(p_1 + p_2)$ and $n = 100$, is approximately normally distributed.

Interaction of pH and carbon dioxide

As carbon dioxide in higher concentrations tends to acidify the cultural medium, the effect of pH on the action of carbon dioxide on spore germination was investigated by germinating spores on agar of different pH values and in different concentrations of carbon dioxide. A buffer, prepared from 0.1 M Na_2HPO_4, 0.1 N HCl, and 0.1 M KH_2PO_4 was added to Hagem agar of double concentration in the ratio of 1 : 1. The results are given in Fig. 1.

It is evident that some interaction between pH and carbon dioxide stimulation occurs, especially in *Coprinus micaceus* and in *Spongipellis borealis*. On the other hand, the marked stimulation of carbon dioxide cannot, as a whole, be attributed to the action of pH, since spores of *Pholiota aurivella* had, in 41 hours, scarcely germinated in ordinary air at any of the pH values. Conversely, in carbon dioxide abundant germination had set in. In all the other experiments, Hagem agar of pH 5.5 was used as a standard medium.

Results

Table 1 shows the results of the present experiments. The most rapid germination was obtained in many cases in 1 % or 5 % carbon dioxide, and the difference between ordinary air and carbon dioxide is often statistically significant. Broadly speaking there seems to exist a certain correlation between the amount of stimulation and the habitat. Especially promoted are species which grow in nature within living trunks of

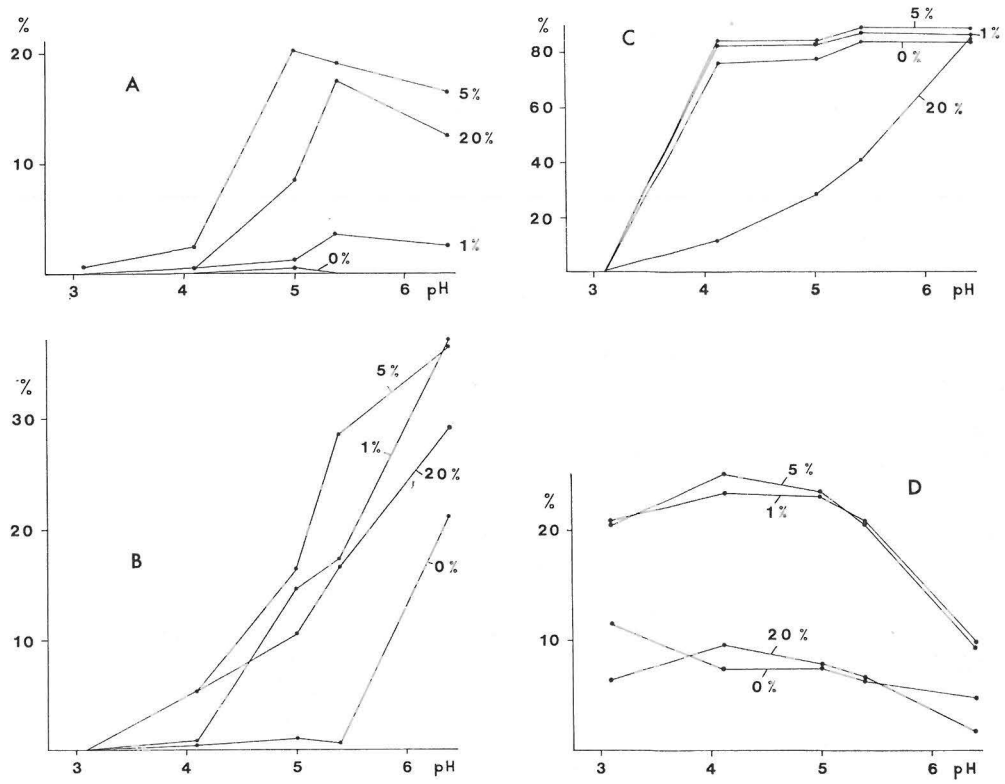

Fig. 1. The effect of carbon dioxide and pH on the germination of a: *Pholiota aurivella,* b: *Coprinus micaceus,* c: *Fomes fomentarius,* and d: *Spongipellis borealis.* Horizontal axis: pH, vertical axis: percentage of germination.

trees *(Polyporus squamosus, Fomitopsis pinicola, Pholiota aurivella* and *Spongipellis borealis).* 20 % carbon dioxide in the air was in most cases inhibitive, a remarkable exception being *Pholiota aurivella.* Weaker decomposers, like the *Mycena* species were already inhibited in 5 % carbon dioxide. As a comparison three soil fungi, *Lepista nuda, Collybia dryophila,* and *Trichoderma viride* were taken, of which the first-named was slightly stimulated by CO_2, and the last-named clearly inhibited.

Discussion

A stimulation of spore germination of fungi by carbon dioxide is found in several species (Cochrane 1958, Tabak & Cooke 1968). A few species require this gas for the intiation of germination, e.g. *Aspergillus niger* (Yanagita 1957), *Chaetomium globosum* (Buston et al. 1966) and *Penicillium griseofulvum* (Fletcher & Morton 1970). Among wood-decomposing species, the spores of *Schizophyllum commune* do not grow in atmospheres totally devoid of CO_2, although in ordinary air they germinate readily (Hafiz & Niederpruem 1963).

Based on the experiments presented above, the stimulatory effect of carbon dioxide concentrations higher than in ordinary air on the spore germination of certain wood-decomposing *Hymenomycetes* seems to be so pronounced that in certain cases it may have ecological significance in the infection process and could be regarded as a developmental adaptation to the compact structure of wood, which prevents ventilation. Similarly the mycelial growth is often stimulated in this ecological group of fungi (Thacker & Good 1952, Hintikka & Korhonen 1970,

Table 1. The effect of carbon dioxide on the germination of wood-decomposing fungi. Asterisks indicate statistically significant stimulation compared with ordinary air (0.03 %).

Species	Time hours	Percentage of germination in air and in different CO_2-air mixtures 0.03 %	1 %	5 %	20 %
Armillariella mellea	16 h	6.3	5.5	4.8	4.8
	22 h	14.2	13.7	13.9	7.8
	40 h	26.5	20.6	20.9	17.2
Collybia dryophila	66 h	6.9	4.9	3.0	0.0
Coprinus micaceus	17 h	63.2	70.6**	69.5**	63.0
	24 h	64.3	79.7***	81.6***	69.3*
	41 h	84.2	85.7	81.2	75.8
Fomes fomentarius	21 h	84.8	87.4	88.6*	58.7
	48 h	93.4	95.2	95.4	84.1
Fomitopsis annosa, conidia from an aseptic culture *F. pinicola*	22 h	25.6	30.2*	27.7	10.1
	16 h	60.4	92.7***	95.8***	91.8***
Gymnopilus penetrans	24 h	0.3	1.2*	1.7**	1.6**
	41 h	0.3	0.9	2.6***	2.4***
Hirschioporus abietinus	19 h	10.2	11.3	8.2	6.4
	42 h	10.9	18.6***	16.1***	12.1
Hypholoma capnoides	6 h	20.6	25.8**	39.2***	9.8
	12 h	70.5	75.8**	74.9*	64.5
	18 h	70.8	76.0**	74.6	72.8
	24 h	80.7	80.0	78.6	78.6
H. fasciculare	12 h	11.6	16.3**	10.9	0.7
	18 h	16.7	23.8***	16.2	7.7
	24 h	31.3	32.7	24.5	15.7
H. sublateritium	12 h	64.4	72.5***	65.3	10.6
	18 h	67.3	67.2	65.9	58.8
	24 h	78.6	82.3*	66.9	62.1
Kuehneromyces mutabilis	12 h	30.0	61.0***	49.7***	13.4
	18 h	64.8	75.1***	77.9***	39.9
	24 h	79.2	72.5	77.8	41.0
Lepista nuda	66 h	0.6	0.7	1.9*	0.6
	90 h	1.0	1.4	2.4*	2.1*
Mycena galericulata	47 h	17.6	11.2	13.5	14.2
M. inclinata	65 h	25.0	11.6	14.1	0.3
	90 h	23.5	16.2	19.1	2.0
M. maculata	47 h	1.5	2.2	2.7	1.6
	65 h	3.5	3.1	4.4	4.7
Pholiota aurivella	41 h	0.0	1.4***	13.3***	11.6***
	47 h	0.3	3.7***	29.0***	29.6***
P. lenta	41 h	3.2	3.0	4.9	0.2
P. squarrosa	90 h	0.8	1.0	1.7	1.4
	122 h	0.8	1.0	3.0***	5.4***
Polyporus squmosus	66 h	11.4	62.9***	21.7***	2.6
	90 h	19.1	—	27.4***	18.6
	122 h	23.7	—	33.5***	38.0***
Piptoporus betulinus	21 h	31.2	39.3***	36.4*	18.5
	48 h	43.8	47.0	48.7*	46.9
Psathyrella spadicea	18 h	1.9	1.0	0.7	0.0
	24 h	—	8.3	6.0	0.3
	28 h	15.6	14.9	10.5	1.5
Pycnoporus cinnabarinus	72 h	3.5	3.8	2.8	0.6
Spongipellis borealis	24 h	0.6	1.5*	0.9	0.1
	33 h	2.6	12.3***	19.6***	7.8***
	44 h	7.2	36.9***	42.3***	29.0***
Trichoderma viride	18 h	14.5	3.7	0.0	0.0
	42 h	—	—	99.8	99.9

see also JENSEN 1967). The actual concentration of carbon dioxide as well as that of oxygen on the surface of wood is not readily determinable because changes in the temperature and air pressure may cause spores to be sucked into tracheas and tracheids, where higher concentrations of CO_2 may prevail.

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