# Sensitivity of *Peronospora hyoscyami* f. sp. *tabacina* to Carbon Dioxide, Compared to that of *Botrytis cinerea* and *Aspergillus niger*

A. M. Svircev, W. E. McKeen, and J. W. Berry

Graduate student, professor, and graduate student, Plant Sciences Department, The University of Western Ontario, London, Ontario, Canada N6A 5B7.
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## ABSTRACT

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Carbon dioxide concentrations as low as 0.8% significantly reduced germination of conidia of *Peronospora hyoscyami* f. sp. *tabacina*. Most inhibited conidia germinated if they were returned to air after 2 hr of treatment with $CO_2$. Germ tubes produced by conidia while in the presence of increased amounts of $CO_2$ were morphologically altered. The mitochondria remained shrunken and the outer mitochondrial membrane remained contorted for a much longer time during germination in 3% $CO_2$ than during germination in air. Five and 15% $CO_2$, respectively, were required to reduce germination of *Botrytis cinerea* and *Aspergillus niger* significantly.

While studying conidia of *Peronospora hyoscyami* f. sp. *tabacina* (6,13) we found that germination was erratic when the spores were placed in incubators containing tubes of yeast, *Saccharomyces* sp. Carbon dioxide was found to be the inhibitor. Several researchers (2,3,12) have reported the inhibitory effect of carbon dioxide on fungi. However, Stover and Freiberg (12) found that increasing the $CO_2$ content of soil increased the number of propagules of *Fusarium oxysporum* f. sp. *cubense*.

The object of this investigation was to determine the effects in vitro of various concentrations of $CO_2$ on the germination of conidia of *P. hyoscyami* f. sp. *tabacina, Botrytis cinerea,* and *Aspergillus niger.*

## MATERIALS AND METHODS

*P. hyoscyami* f. sp. *tabacina* was isolated from infected tobacco during the 1979 epidemic in Ontario. *B. cinerea* Pers. ex. Fr. (Accession 790) and *A. niger* v. Tiegh. (Accession 979) were obtained from the Plant Sciences Culture Collection of The University of Western Ontario.

Conidia of the blue mold fungus were washed from a fresh lawn of conidiophores on a leaf that had been heavily inoculated 5 or 6 days previously. The suspension was then centrifuged for 5 min at 1,000 *g* to obtain a loose pellet. The supernatant was discarded, and water was added to suspend the conidia at a concentration of $10^4$ spores per milliliter. Drops of the suspension were placed on 1.5% water agar in petri dishes. The petri dishes with lids ajar were placed in the test chamber, an incubator (30 × 30 × 30 cm).

Immediately, a portion of air equal to that to be replaced by $CO_2$/air mixtures was evacuated from the test chamber, and the reduced pressure was measured with a manometer. The desired amount of $CO_2$ was admitted, as measured with a mercury manometer, from either a 5% $CO_2$ and 95% filtered air tank or a 100% $CO_2$ tank (Canox Co. Ltd., London, Ontario, Canada). The tank of 5% $CO_2$/95% filtered air was used for obtaining concentrations of less than 2.5% $CO_2$ in the test chamber. Oxygen concentrations were always >17.5% even when 15% $CO_2$ was used. The time from harvesting the conidia until $CO_2$ was adjusted required 15 min. Control plates were kept in an incubator and all experiments were conducted at 19 C.

After 2.5, 5.5, 11.5, and 17.5 hr the conidia were treated with acid fuchsin in lactophenol to prevent further development.

A conidium was considered germinated when the germ tube exceeded one half the diameter of the conidium. Ten areas, containing 1,000 conidia (total), were counted in both the control and test plates. The data presented are means of three replicated experiments.

For electron microscopy, conidia were fixed for 30 min in fresh cold (over ice) mixtures of equal parts of 4% glutaraldehyde and 2% osmium tetroxide buffered in 0.1 M sodium cacodylate at pH 6.8. The material was rinsed in fresh buffer and postfixed for 30 min in 2% osmium tetroxide. After fixation the material was rinsed in water, stained in 5% uranyl magnesium acetate for 30 min at room temperature, dehydrated in a graded acetone series, and infiltrated with Epon-Araldite (5). Sections cut with a diamond knife on a Porter-Blum ultramicrotome (Sorval MT-2) were mounted on copper grids and stained with lead citrate (10).

## RESULTS

In a chamber containing 15% $CO_2$ there was no germination of conidia of *P. hyoscyami* f. sp. *tabacina* until the 17th hour (Table 1). These germ tubes were shorter and thicker than the germ tubes in the controls.

At 1.3% $CO_2$ after 2.5 hr germination was 51% of control. Even at 0.8% $CO_2$ germination was reduced by 10% at the end of 2.5 hr, and this reduction was statistically significant. The 1.3 and 0.8% $CO_2$ tests produced germ tubes that were reduced in thickness and curled when compared to the controls (Figs. 1 and 2).

Germination of *B. cinerea* was 100% of the control in 1.3% $CO_2$ (Table 2). Initially, germination in 5 and 7.5% $CO_2$ was retarded significantly ($P < 0.05$) but reached 100% after 11.5 and 17.5 hr, respectively. Fifteen percent $CO_2$ completely prevented germination of conidia of *B. cinerea* for 17.5 hr. *A. niger* germination was not significantly inhibited at 1.3, 5.0, and 7.5% $CO_2$ up to 17.5 hr. Fifteen percent $CO_2$ for 17.5 hr reduced germination of conidia of *A. niger* significantly ($P < 0.05$) to 92% of the control.

In 7.5 and 15% $CO_2$ after 17.5 hr, germ tubes of *B. cinerea* and *A. niger* were considerably distorted and shorter than the germ tubes of the controls.

Conidia of *P. hyoscyami* f. sp. *tabacina, B. cinerea,* and *A. niger* resumed normal germination rates and morphology when removed from a $CO_2$-enriched atmosphere.

The pH of a thin layer of distilled water over agar was not lowered below pH 6.5 after exposure to 5% $CO_2$ for 3 hr. Good germination of conidia of *P. hyoscyami* f. sp. *tabacina* occurred on

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agar at pH 5.5, and the germ tube was straight and normal in thickness. Therefore, the lowering of the pH by $CO_2$ was not a factor in the inhibition of germination.

Mitochondria of *P. hyoscyami* f. sp. *tabacina* changed much more slowly from a shrunken to a swollen state during germination in increased concentration of $CO_2$ than in normal air (0.03% $CO_2$). In 3% $CO_2$ when the germ tube was 50 $\mu$m in length, the mitochondria (Fig. 4) were still shrunken and had a contorted outer membrane and resembled mitochondria in conidia that had been germinated in air when the germ tube was only 5 $\mu$m in length. When the germ tube of a conidium germinated in air was 50 $\mu$m in length, the mitochondria (Fig. 3) had smooth outer membranes. Thirty germ tubes of each type were examined.

## DISCUSSION

Conidial germination of *P. hyoscyami* f. sp. *tabacina* was retarded at considerably lower $CO_2$ concentrations (0.8% $CO_2$) than previously reported in fungi (2). In contrast, conidia of *B. cinerea* and *A. niger* required high concentrations of $CO_2$ to induce an inhibitory effect on spore germination. Wells and Urota (14) found that concentrations less than 32% $CO_2$ stimulated germination of *Fusarium roseum* and levels higher than 30% were required to inhibit conidial germination of *Alternaria tenuis*. However, conidial germination of *Rhizopus stolonifer, B. cinerea*, and *Cladosporium herbarum* was inhibited 90% in 16% $CO_2$.

Because *P. hyoscyami* f. sp. *tabacina* is an obligate parasite it was impossible to grow the hyphae in controlled $CO_2$ atmosphere, and thus the work was restricted to in vitro germination studies. However, if germination of conidia is affected by $CO_2$ to the same degree as hyphal growth, then comparisons may be made with other fungi.

Durbin (3) found that hyphal linear growth of *Phytophthora cinnamoni* was not inhibited, but linear growth of *P. cactorum* was

TABLE 1. Effect of various concentrations of carbon dioxide and time of exposure on germination of conidia of *Peronospora hyoscyami* f. sp. *tabacina* at 20 C

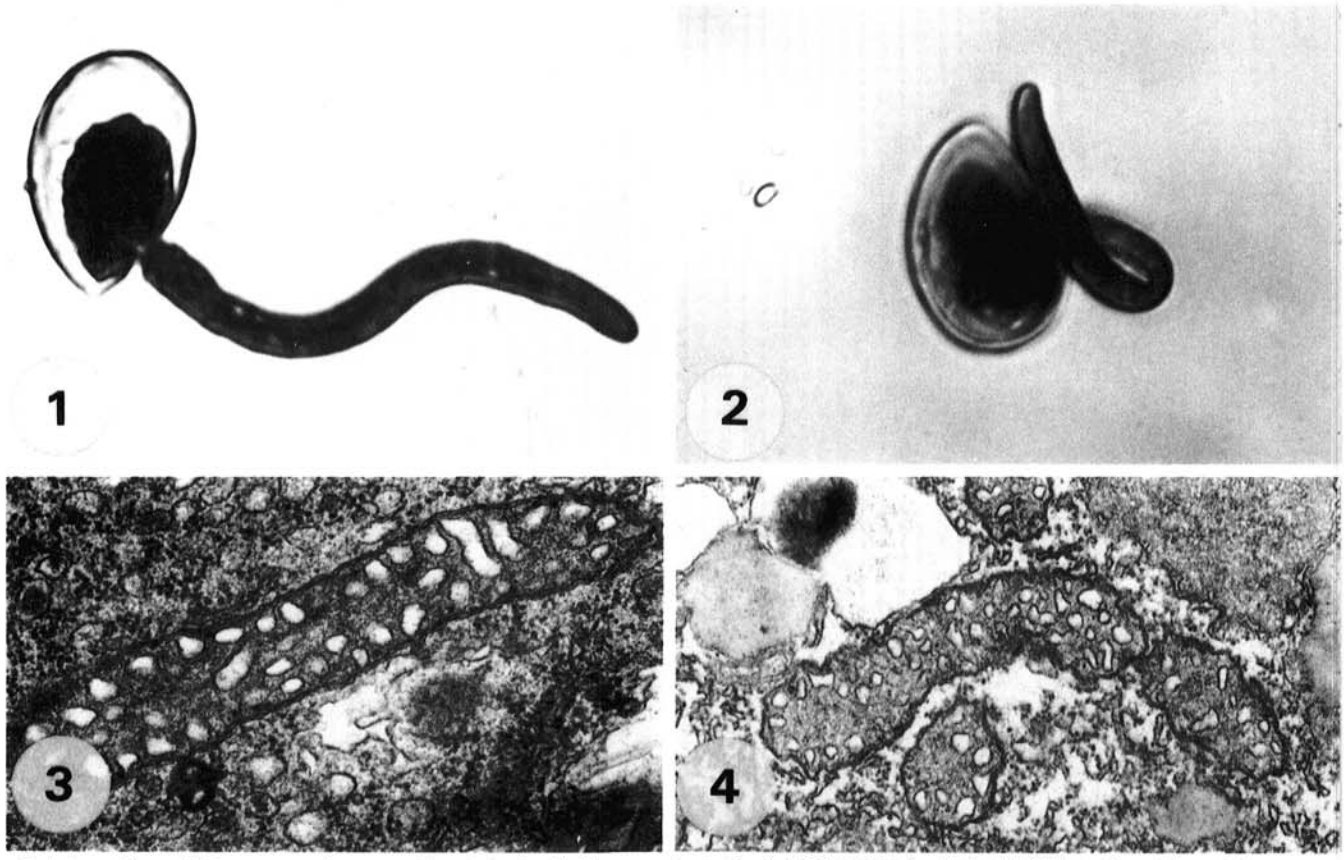
| Exposure (hr) | Germination at indicated $CO_2$ concentration (%)[y] | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0.5 | 0.6 | 0.8 | 1.3 | 5 | 7.5 | 15 |
| 2.5 | 103[z] a | 100 a | 90 | 51 | 35 | 10 | 0 |
| 5.5 | | | 100 a | 84 | 43 | 27 | 0 |
| 11.5 | | | 100 a | 87 | 59 | 30 | 0 |
| 17.5 | | | | 100 a | 64 | 33 | 9 |

[y] Percent germination of treated as compared to control (85% germination in control). Based on 1,000 conidia per observation in each of three trials.
[z] Numbers followed by letter "a" are not statistically different from the control; all others are significantly different according to a *Z*-test, $P = 0.05$.

TABLE 2. Effect of various concentrations of carbon dioxide and time of exposure on germination of conidia of *Botrytis cinerea* and *Aspergillus niger*

| Fungus | Exposure (hr) | Germination at indicated $CO_2$ concentration (%)[y] | | | |
|---|---|---|---|---|---|
| | | 1.3 | 5 | 7.5 | 15 |
| *B. cinerea* | 2.5 | 100[z] a | 40.4 | 0 | 0 |
| | 5.5 | 100 a | 83.8 | 40 | 0 |
| | 11.5 | 100 a | 100 a | 95 | 0 |
| | 17.5 | 100 a | 100 a | 100 a | 0 |
| *A. niger* | 17.5 | 100 a | 100 a | 97 a | 92 |

[y] Percent germination of treated as compared to control (90 and 98% germination of *B. cinerea* and *A. niger*, respectively). Based on 1,000 conidia per observation in each of three trials.
[z] Numbers followed by letter "a" are not statistically different from the control according to a *Z*-test, $P = 0.05$.




**Figs. 1–4.** A conidium of *Peronospora hyoscyami* f. sp. *tabacina* that has germinated in air (0.03% $CO_2$) for 2.5 hr (×1,200). **2,** A conidium of *Peronospora hyoscyami* f. sp. *tabacina* that has germinated in 0.8% $CO_2$ for 2.5 hr. The germ tube is curled and has a smaller diameter than the one germinated in air (Fig. 1) (×1,200). **3,** A mitochondrion in a conidium that has germinated in air for 2.5 hr (×36,000). **4,** A mitochondrion in a conidium that has germinated in 3% $CO_2$ for 2.5 hr. The outer membrane is contorted (×36,000).

inhibited in 20% $CO_2$. Mitchell and Zentmyer (7) found that growth of *Phytophthora capsici, P. citrophthora, P. palmivora,* and *P. parasitica* was reduced by two thirds of control at 15% $CO_2$.

The inhibition phenomenon of $CO_2$ on fruiting formation (1) and spore germination (8,9) in *Agaricus bisporus* have been correlated with the impairment of Krebs cycle activity. Rast et al (8,9) suggested that $CO_2$ induces specific inhibition of the enzyme succinate dehydrogenase. Although direct comparisons cannot be made between $CO_2$ inhibition of spore germination and hyphal growth, the site of inhibition may be similar, ie, the enzyme succinate dehydrogenase.

Germ tubes of *P. hyoscyami* f. sp. *tabacina* formed in 0.8% $CO_2$ were curled and reduced in diameter. At higher concentrations germ tubes of *P. hyoscyami* f. sp. *tabacina* (>3% $CO_2$), *B. cinerea* (>7.5% $CO_2$), and *A. niger* (>15% $CO_2$) became swollen, convoluted, and reduced in length.

Macko and Fuchs (4) found that apical swelling developed on the germ tubes of uredospores of *Puccinia striiformis* when $CO_2$ concentration was 5%. Higher $CO_2$ concentrations resulted in extrusion of cell contents.

Sietsma et al (11), working with the basidiomycete, *Schizophyllum commune*, showed that $CO_2$ changed the fungal wall composition during the growth period.

The fact that conidia of *P. hyoscyami* f. sp. *tabacina* germinated normally when they were removed from high $CO_2$ concentrations and placed in air indicates that the effect of $CO_2$ is one of retardation rather than toxicity.

Electron micrographs of $CO_2$-treated germ tubes of *P. hyoscyami* f. sp. *tabacina* and controls demonstrated a structural difference in the mitochondria. The fact that mitochondria did not become turgid with a smooth outer membrane as quickly as they normally did when the conidium was germinated in air indicates that mitochondrial activity was reduced by increased amounts of $CO_2$. The effect of $CO_2$ on the mitochondria becomes significant if we consider that it is the site of action of the enzymatic reactions involved in the Krebs cycle.

Our work has shown that the conidia of *P. hyoscyami* f. sp. *tabacina* are more sensitive to elevated levels of $CO_2$ than conidia of other fungi. This sensitivity is reflected in structural changes.

Fungal walls of taxonomic groups vary in chemical composition; nonetheless, the $CO_2$-enriched atmosphere probably has an effect on cell wall synthesis in all groups.

## LITERATURE CITED


1. Bachofen, R., and Rast, D. 1968. Carboxylierungs-reaktionen in *Agaricus bisporus*. III. Pyruvat und phosphoenolpyruvat als $CO_2$-acceptoren. Arch. Microbiol. 60:217-234.
2. Brown, W. 1922. On the germination and growth of fungi at various temperatures and in various concentrations of oxygen and of carbon dioxide. Ann. Bot. 365:257-283.
3. Durbin, R. D. 1959. Factors affecting the vertical distribution of *Rhizoctonia solani* with special reference to $CO_2$ concentration. Am. J. Bot. 46:22-25.
4. Macko, V., and Fuchs, E. 1970. Effect of carbon dioxide on uredospore germ tubes of *Puccinia striiformis*. Phytopathology 60:1529-1530.
5. McKeen, W. E. 1971. Woronin bodies in *Erysiphe graminis* D.C. Can. J. Microbiol. 17:1557-1570.
6. McKeen, W. E., and Svircev, A. M. 1981. Early development of *Peronospora tabacina* in the *Nicotiana tabacum* leaf. Can. J. Plant Pathol. 3:145-158.
7. Mitchell, D. J., and Zentmyer, G. A. 1971. Effects of oxygen and carbon dioxide tensions on growth of several species of *Phytophthora*. Phytopathology 61:787-791.
8. Rast, D., Greuter, B., Lendenmann, J., and Zobrist, P. 1976. The biochemistry of dormancy and germination in *Agaricus bisporus* spore. Mushroom Sci. 9:59-75.
9. Rast, D., Stauble, E. J., and Zobrist, P. 1976. The Krebs cycle and control of dormancy in the *Agaricus bisporus* spore. New Phytologist 76:469-477.
10. Reynolds, E. S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell. Biol. 17:208-212.
11. Sietsma, J. H., Rast, D., and Wessels, J. G. H. 1977. The effect of carbon dioxide on fruiting and on degradation of cell-wall glucan in *Schizophyllum commune*. J. Gen. Microbiol. 102:385-389.
12. Stover, R. H., and Freiberg, S. R. 1958. Effect of carbon dioxide on multiplication of *Fusarium* in soil. Nature 181:788-789.
13. Svircev, A. M., and McKeen, W. E. 1982. The haustorium of *Peronospora hyoscyami* f. sp. *tabacina* in the susceptible tobacco leaf cell. Can. J. Plant Pathol. 4:119-128.
14. Wells, J. M., and Urota, M. 1970. Germination and growth of five fungi in low oxygen and high-carbon dioxide atmospheres. Phytopathology 60:50-53.