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Growth inhibition of Agaricus bisporus and associated thermophilic species by fungicides used in wheat cultivation

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Summary — Eight fungicides used on wheat were checked for their toxic effect in vitro on the mycelial growth of 3 strains of the edible mushroom Agaricus bisporus and 3 isolates of thermophilic fungi active in mushroom composting. At concentrations < 2 ppm, captan, maneb and chlorothalonil were not found to be toxic. Only tebuconazole significantly limited the radial growth of A bisporus mycelium. The IC50 values of benomyl, carbendazim, flusilazol, prochloraz and tebuconazol on mycelial growth of thermophilic fungi ranged from 0.1-0.7 ppm. The presence of fungicide residues in wheat straw may therefore affect the process of mushroom composting.

mushroom compost / fungicide residue / Agaricus bisporus / Torula thermophila / Humicola grisea var thermoidea

INTRODUCTION

The cultivation of edible fungi currently constitutes the largest controlled application of microbial technology for profitable conversion of waste lignocellulosic residues from agriculture and forestry (Wood, 1989). Large quantities of straw are used for the production of the edible button mushroom, Agaricus bisporus. We estimate that in France, 500 000 tons of wheat straw are mixed yearly with horse manure and composted for the production of 1 200 000 tons of substrate for A bisporus cultivation. Composting process conditions wheat straw and horse manure by managing a natural succession of microorganisms to produce a substrate which is selective for the growth of mushroom mycelium. Among these microorganisms, ther-
mophilic fungi appear to be important for compost selectivity. *Torula thermophila* and *Humicola grisea var thermoidea* (synonyms of *Scytalidium thermophilum*) (Straatsma et al, 1991), have been shown to be the most abundant thermophilic fungi in mushroom compost (Ross and Harris, 1983; Straatsma et al, 1989).

Fungicides are used during intensive wheat production in Europe and certain other parts of the world. Significant quantities of residues in grain or straw can result from the application of fungicides during cultivation, but little information is available on possible effects of fungicides used during wheat cultivation on mushroom production.

In a previous study, we showed that fungicide application during wheat cultivation may have an effect on the chemical composition and potential degradability of wheat straw (Savoie et al, 1992). Moreover, as previously mentioned, fungicide residues have been detected in straw. We found carbendazim and prochloraz residue concentrations of < 0.1 ppm but flusilazol residues of ≈ 0.2-0.3 ppm in wheat straw. The aim of the present study was to investigate the toxicity of low concentrations of fungicides on both *A. bisporus* and thermophilic fungi responsible for compost selectivity in order to determine whether fungicide residues in straw could be directly or indirectly responsible for changes in mushroom yields. The *in vitro* effects of some fungicides used during wheat cultivation on mycelial growth were determined in 3 strains of *A. bisporus* and thermophilic fungi isolates from composts.

### MATERIALS AND METHODS

Three commercial strains of *A. bisporus* were used throughout the study: B62 and B98 from the Le Lion Co (France) and 1 Dutch hybrid strain Horst-U3 from Somycel (France). Thermophilic fungi were isolated from commercial mushroom compost by the method of colony isolation and spore suspension (Olivier and Guillaumes, 1976). These strains were taken from our laboratory collection and were obtained from a single spore propagule. Two *Torula thermophila* isolates were tested: T1 and T2, and 1 isolate of *Humicola grisea var thermoidea*: H1.

Eight fungicides were taken into consideration (table I). Initial fungicide solutions were made up in either water for captan, flusilazol, prochloraz or ethanol for benomyl, carbendazim, maneb, chlorothalonil and tebuconazol. Water was used for the dilutions from the initial solutions for all fungicides, with 5 ml fungicide solution per 495 ml medium. Fungicide solutions were sterilized by filtration (0.2 μm) and added to the agar medium at a temperature of 50 °C prior to pouring into Petri dishes.

Each Petri dish contained 20 ml medium with different fungicide concentrations. The *A. bisporus* strains were grown on Cristomalt media (Difal®) (Olivier and Guillaumes, 1976) and maintained at 25 °C. The thermophilic fungi isolates were grown on Raper's agar medium (20 g glucose, 2 g bactopeptone, 2 g yeast extract, 0.5 g MgSO4, 0.5 g KH2PO4, 1 g K2HPO4, 20 g agar, 1 l H2O), and incubated at 48 °C. The Petri dishes were inoculated with agar plugs (5 mm diameter) cut from the periphery of the actively growing mycelial colony precultured on the same medium without fungicide.

The effects of each fungicide were determined in separate experiments. Eight different final concentrations were used: 0, 0.02, 0.05, 0.1, 0.2, 0.5, 1, 2 ppm.

**Table I.** Characteristics of the fungicides tested and maximum authorised residues (MAR) in cereal grain a or straw b.

<table>
<thead>
<tr>
<th>Fungicide class</th>
<th>Active ingredient</th>
<th>Trade name</th>
<th>MAR (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzimidazoles</td>
<td>Benomyl</td>
<td>Benlate</td>
<td>0.5 a</td>
</tr>
<tr>
<td></td>
<td>Carbendazim</td>
<td>Bavistine</td>
<td>0.5 a</td>
</tr>
<tr>
<td>Phthalimides</td>
<td>Captan</td>
<td>Phytocape 83</td>
<td>–</td>
</tr>
<tr>
<td>Phthalic derivatives</td>
<td>Chlorothalonil</td>
<td>Daconil 500 flowable</td>
<td>0.02 a</td>
</tr>
<tr>
<td>Dithiocarbamates</td>
<td>Maneb</td>
<td>Dithane M 22</td>
<td>2 a</td>
</tr>
<tr>
<td>Ergosterol</td>
<td>Flusilazol</td>
<td>Olymp 10 EC</td>
<td>0.1 b</td>
</tr>
<tr>
<td>Biosynthesis</td>
<td>Tebuconazol</td>
<td>Horizon</td>
<td>2 b, 0.05 a</td>
</tr>
<tr>
<td>Inhibitors</td>
<td>Prochloraz</td>
<td>Sportak 45</td>
<td>0.05 b</td>
</tr>
</tbody>
</table>

Two ppm is the maximum tolerated amount of tebuconazol residue in France in cereal straw (Anonymous, 1992). For other fungicides, the maximum amounts of residues tolerated are given for grains, but they are < 2 ppm (table I). For prochloraz and benzomyl, both used in the mushroom industry, we investigated concentrations up to 10 ppm and 2.5 ppm respectively. Agar-media without fungicide but with ethanol added to the medium at a concentration equivalent to the quantity added in the assay with fungicide solution was used as a control for the effect of ethanol.

For each fungicide concentration, 6 dishes were inoculated with each strain. Each colony was measured across 2 diameters at right angles after incubation for 15 d with A. bisporus, or for 3 d with thermophilic fungi. The relation between relative growth, expressed as the ratio of mean diameter in assay in the presence of fungicide to mean diameter in the control without fungicide, and the log fungicide concentrations was calculated. The IC50 value was interpolated from this regression (Köller et al, 1991). The IC50 value was the fungicide concentration (in ppm) which inhibited mycelial growth by 50%. When the IC50 were > 2 ppm the means of colony diameter with 2 ppm fungicide were compared to the control means using the t-test.

RESULTS AND DISCUSSION

Ethanol up to the maximum concentration used during our assays (1% of the agar medium), had no effect on mycelial growth (results not shown).

The fungicides maneb, captan and chlorothalonil were characterized by IC50 values > 2 ppm for both A. bisporus and thermophilic fungi (table II), indicating a low sensitivity. The range of fungicide concentrations chosen was too low to calculate their IC50 values, but mycelial growth inhibitions at 2 ppm were never >18% for the strain x fungicide combinations tested (table III). Consequently, residues of these fungicides at low concentrations in wheat straw are less likely to have significant consequences on composting and on mushroom growth. However, chlorothalonil had been shown to induce some toxicity problems in mushroom mycelial growth in vitro at concentrations between 0.5–2 ppm (Gandy, 1981; Gandy and Spencer, 1981; Fletcher et al, 1983; Challen and Elliott, 1985). A. bisporus strains used in the latter experiments were different from our strains. In our experiment, when radial growths with 2 ppm fungicide were compared with controls, reductions in growth were < 16% and < 18% with chlorothalonil and captan respectively. So, the tested strains which are usually cultivated today in Europe seem to be more tolerant in vitro to phthalimides than older commercial strains used in previous studies.

In the past, dithiocarbamates such as maneb were routinely used in mushroom cultivation. Treatments with such fungicides have not produced any evidence of damage to mushroom at any stage of its cultivation (Yoder et al, 1950; Newman and Savidge, 1969). This is in agreement with our observations on the low growth-inhibiting effects of maneb.

The fungicides prochloraz, flusilazol and benzomyl had limited effects on A. bisporus growth between 0 and 2 ppm but were more toxic for thermophilic fungi (tables II, III).

Our results agree with some previous work in which the IC50 values measured in vitro were from 10–54 ppm for different strains of A. bisporus (Snel and Fletcher, 1971; Gandy, 1981; Nair and Macauley, 1987). Since mycelial growths

| Table II. IC50 values (ppm) of fungicides for the radial growth of different strains of A. bisporus and isolates of thermophilic fungi. |
|---|---|---|---|---|---|
| Fungicides | Agaricus bisporus | Thermophilic fungi |
| | U3 | B62 | B98 | T1 | T2 | H1 |
| Benomyl | >2.5 | >2.5 | >2.5 | 0.31 | 0.31 | 0.43 |
| Carbendazim | 1.04 | 1.36 | >2 | 0.57 | 0.68 | 0.70 |
| Captan | >2 | >2 | >2 | >2 | >2 | >2 |
| Chlorothalonil | >2 | >2 | >2 | >2 | >2 | >2 |
| Maneb | >2 | >2 | >2 | 0.26 | 0.22 | 0.24 |
| Flusilazol | 2.05 | >2 | >2 | 0.10 | 0.17 | 0.06 |
| Tebiconazole | 1.25 | 1.07 | 1.04 | 0.32 | 0.20 | 0.11 |
| Prochloraz | >10 | >10 | >10 | | | |
may be related to mushroom yields (Laborde et al., 1989), rapid growth in compost is a necessary but not sufficient step for fruiting. Benomyl, carbendazim and prochloraz appeared to cause few toxicity problems to mushroom crops when used early during mushroom cultivation (Gandy and Spencer, 1981; Fletcher et al., 1983) at concentrations < 100 ppm (Nair and Backer, 1978), but they may be slightly toxic when applied later in the crop cycle, i.e., during harvesting (Van Zaayen and Van Adrichem, 1982). However, strains showed different levels to tolerance to fungicides. Obtaining new strains of A. bisporus resistant to fungicides which are active on fungal antagonists (Challen et al., 1991) therefore presents a challenge.

At low concentrations (1 ppm), benomyl and other benzimidazoles can stimulate vegetative growth (Wuest and Cole, 1970) and increase mushroom yield (Peake, 1972; Nair and Baker, 1978). In agreement with Fletcher et al. (1975), we did not observe such a stimulatory effect in our experiments; this could be due to differences in the strains used for the experiments.

As observed by Nair and Macauley (1987), IC\textsubscript{50} values for prochloraz were > 10 ppm. Our strains were more tolerant than those used by Gandy (1981) who estimated IC\textsubscript{50} values to be from 8–14 ppm for prochloraz with different A. bisporus strains.

We measured concentrations of fungicide residues in straw and found them to be < 0.4 ppm (Savoie et al., 1992). Direct toxic effects of residues of most fungicides used during wheat cultivation on vegetative growth of A. bisporus cannot therefore be responsible for changes in mushroom yield. Tebuconazol and to a lesser extent carbendazim were the only fungicides with a toxic effect on both A. bisporus and thermophilic fungi (table II). The fungicide tolerance we observed in vitro was low when compared to the maximum authorized concentration of residues in straw, i.e., 2 ppm in France for tebuconazol. Therefore application of tebuconazol could be a limiting factor for the use of straw for compost preparation and mushroom cultivation. There are no specific data on fungicide degradation during mushroom composting; but in a review, Miller (1991) showed that composting can result in decomposition of hazardous materials. We can then postulate that tebuconazol and carbendazim may be partly degraded during composting but this and toxicity to thermophilic fungi have to be investigated in composting experiments.

Most of the fungicides investigated in this in vitro study caused inhibition of the thermophilic fungi which grow during composting. The inhibition by low concentrations of several fungicides may have both a negative effect if residues are present in straw during composting and a positive effect when fungicides are added in the com-

### Table III. Mycelial growth of A. bisporus and thermophilic fungi in the presence of 2 ppm fungicides. Radial growth expressed in percent of the radial growth in fungicide-free controls.

<table>
<thead>
<tr>
<th>Fungicide</th>
<th>Agaricus bisporus</th>
<th>Thermophilic fungi</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>U3</td>
<td>B62</td>
</tr>
<tr>
<td>Benomyl</td>
<td>71.0</td>
<td>74.6</td>
</tr>
<tr>
<td>Carbendazim</td>
<td>36.6</td>
<td>29.4</td>
</tr>
<tr>
<td>Captan</td>
<td>100,\textsuperscript{a}</td>
<td>100</td>
</tr>
<tr>
<td>Chlorothalonil</td>
<td>88.8</td>
<td>94.6</td>
</tr>
<tr>
<td>Maneb</td>
<td>82.3</td>
<td>84.8</td>
</tr>
<tr>
<td>Flusilazol</td>
<td>48.7</td>
<td>73.9</td>
</tr>
<tr>
<td>Tebuconazol</td>
<td>24.4</td>
<td>23.1</td>
</tr>
<tr>
<td>Prochloraz</td>
<td>92.2</td>
<td>100</td>
</tr>
<tr>
<td>Prochloraz 10 ppm</td>
<td>68.9</td>
<td>83.0</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Values of 100% indicate that means of mycelial growth with and without fungicide were not significantly different at the 0.05 level. In all other cases, differences were significant.
post at spawning. Straatsma et al (1991) observed that S thermophilum may have inhibitory effects on A bisporus, in addition to a growth-promoting effect. The inhibitory effects were suppressed by inactivation of S thermophilum with benomyl. According to our observations prochloraz, which is used by mushroom growers, may have the same positive effect at the beginning of mushroom cultivation. However, the presence of benomyl, carbendazim, prochloraz or fluzilazol residues during composting may limit colonization by these useful thermophilic fungi, resulting in composts with low selectively for A bisporus and which are consequently less productive (Ross and Harris, 1983; Fermor and Grant, 1985; Gandy, 1985; Straatsma et al, 1999). Savoie et al (1992) observed concentrations of carbendazim and prochloraz < IC50 values determined in the present study, but concentrations of fluzilazol of 0.25–0.32 ppm were measured. The IC50 values observed in vitro ranged from 0.22–0.26. Even if the sensitivity is generally higher in vitro than in situ, the risk represented by fungicide residues in wheat straw has to be taken into account in mushroom compost production. Work is in progress to confirm the conclusions of this study in experiments on composting.

ACKNOWLEDGMENTS

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