agronomie (1993) 13, 407-412

Plant pathology

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

N Chalaux [1]*, JM Savoie [2], JM Olivier [2]

[1] *INRA, Centre technique du champignon;*
[2] *INRA, Station de recherches sur les champignons, centre de Bordeaux, BP 81, F33883 Villenave-d'Ornon, France*

(Received 17 February 1993; accepted 3 March 1993)

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 tebuconazol significantly limited the radial growth of *A bisporus* mycelium. The IC_{50} 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*

Résumé — Inhibition de la croissance d'*Agaricus bisporus* et d'espèces thermophiles associées par des fongicides utilisés dans la culture du blé. *La paille de blé est le constituant principal des composts qui servent de substrat de culture pour le champignon de Paris* (Agaricus bisporus). *L'utilisation de fongicides lors de la culture du blé peut avoir des conséquences sur la production de champignon de Paris si les résidus présents dans les pailles sont actifs. La toxicité de huit fongicides utilisés contre les maladies fongiques du blé a été testée* in vitro *pour trois souches d'*A bisporus *et pour trois isolats de champignons thermophiles utiles au compostage et à l'électivité du compost : deux* Torula thermophila *et un* Humicola grisea *var* thermoidea. *À des concentrations inférieures à 2 ppm le captane, le manèbe et le chlorothalonil n'étaient pas toxiques. La croissance radiale d'*A bisporus *n'a été fortement limitée que par le tébuconazole. Les valeurs de CI50 du bénomyl, du carbendazime, du flusilazole, du prochloraze et du tébuconazole pour la croissance mycélienne des champignons thermophiles étaient comprises entre 0,1 et 0,7 ppm. La présence de résidus de fongicides dans les pailles de blé peut donc avoir des conséquences sur le compostage, et donc indirectement sur la culture du champignon de Paris.*

***compost de champignonnière / résidus de fongicides* / 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-

* Correspondance et tirés à part

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 $MgSO_4$, 0.5 g KH_2PO_4, 1 g K_2HPO_4, 20 g agar, 1 l H_2O), 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].

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

MAR: see in Anonymous (1992).

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 benomyl, 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 IC_{50} value was interpolated from this regression (Köller *et al*, 1991). The IC_{50} value was the fungicide concentration (in ppm) which inhibited mycelial growth by 50%. When the IC_{50} 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 IC_{50} 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 IC_{50} 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 benomyl 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 IC_{50} 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. IC_{50} 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	>2	>2	>2
Flusilazol	2.05	>2	>2	0.26	0.22	0.24
Tebiconazol	1.25	1.07	1.04	0.10	0.17	0.06
Prochloraz	>10	>10	>10	0.32	0.20	0.11

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.

Fungicide	Agaricus bisporus			*Thermophilic fungi*		
	U3	*B62*	*B98*	*T1*	*T2*	*H1*
Benomyl	71.0	74.6	82.2	0	0	0
Carbendazim	36.6	29.4	59.7	0	0	0
Captan	100 [a]	100	100	100	93.7	82.6
Chlorothalonil	88.8	94.6	100	90.7	100	100
Maneb	82.3	84.8	95.2	100	100	100
Flusilazol	48.7	73.9	88.6	0	0	0
Tebuconazol	24.4	23.1	30.0	0	0	0
Prochloraz	92.2	100	100	12.7	0	0
Prochloraz 10 ppm	68.9	83.0	100	0	0	0

[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.

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, *ie* 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_{50} values for prochloraz were > 10 ppm. Our strains were more tolerant than those used by Gandy (1981) who estimated IC_{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, *ie* 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-

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*, 1989). Savoie *et al* (1992) observed concentrations of carbendazim and prochloraz < IC_{50} values determined in the present study, but concentrations of fluzilazol of 0.25–0.32 ppm were measured. The IC_{50} 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

Thanks are expressed to INRA and the CTC French Technical Center for Mushroom Growers (Saint-Paterne-Racan, France) for their financial support.

REFERENCES

Anonymous (1992) *Index Phytosanitaire 1992*. Acta, 75595 Paris Cedex 12, France

Challen MP, Elliott TJ (1985) The *in vitro* responses to a range of fungicides of two strains of the mushroom *Agaricus bisporus* and the pathogen *Verticillium fungicola*. *Mycopathology* 90, 161-164

Challen MP, Rao BG, Elliott TJ (1991) Transformation strategies for *Agaricus*. *In: Proc 1st Int Semin Mushroom Sci* (van Griensven LTLD, ed) Pudoc, Wageningen, 129-134

Fermor TR, Grant WD (1985) Degradation of fungal and actinomycete mycelia by *Agaricus bisporus*. *J Gen Microbiol* 131, 1729-1737

Fletcher JT, Drakes GD, Talent CJW (1975) The control of wet bubble disease of mushrooms caused by *Mycogone perniciosa*. *Ann Appl Biol* 79, 35-41

Fletcher JT, Hims MJ, Hall RJ (1983) The control of bubble diseases and cobweb disease of mushrooms with prochloraz. *Plant Pathol* 32, 123-131

Gandy DG (1981) Sensitivity of mushroom strains to fungicides. *Mushroom Sci* 11, 473-483

Gandy DG (1985) Bacterial and fungal diseases. *In: The Biology and Technology of the Cultivated Mushroom* (Flegg PB, Spencer DM, Wood DA, eds) John Wiley and Sons Ltd, Chichester, 261-277

Gandy DG, Spencer DM (1981) Fungicide evaluation for control of dry bubble, caused by *Verticillium fungicola* on commercial mushroom strains. *Sci Hortic* 14, 107-115

Köller W, Parker DM, Reynolds KL (1991) Baseline sensitivities of *Venturia inaequalis* to sterol demethylation inhibitors. *Plant Dis* 75, 726-728

Laborde J, Houdeau G, Bes B, Olivier JM, Delpech P (1989) Indoor static composting: description of the process; analysis. Main results. *Mushroom Sci* 12, 457-469

Miller FC (1991) Biodegradation of solid wastes by composting. *In: Biological Degradation of Wastes. 1. Waste Material Disposal* (Martin AM, ed) Elsevier Sci Publ Ltd, Essex, 1-30

Nair NG, Baker HJ (1978) Studies on the control of wet bubble disease of mushrooms with benzimidazoles. *Aust J Res* 29, 545-553

Nair NG, Macauley BJ (1987) Dry bubble disease of *Agaricus bisporus* and *Agaricus bitorquis*, and its control by prochloraz–manganese complex. *N Z J Agric Res* 30, 107-116

Newman RH, Savidge M (1969) Mancozeb dust – a breakthrough in mushroom disease control. *MGA Bull* 232, 161-162

Olivier JM, Guillaumes J (1976) Étude écologique des composts de champignonnières. *Ann Phytopathol* 8, 277-283

Peake R (1972) Benlate – the fungicide for mushrooms. *MGA Bull* 267, 127-129

Ross RC, Harris PJ (1983) The significance of thermophilic fungi in mushroom compost preparation. *Sci Hortic* 20, 61-70

Savoie JM, Chalaux N, Olivier JM (1992) Variability in straw quality and mushroom production: importance of fungicide schedules on chemical composition and potential degradability of wheat straw. *Bioresour Technol* 41, 161-166

Snel M, Fletcher JT (1971) Benomyl and thiabendazole for the control of mushroom diseases. *Plant Dis Rep* 55, 120-121

Straatsma G, Gerrits JPG, Augustijn MPAM, Op Den Camp HJM, Vogels GD, Van Griensven LJLD (1989) Population dynamics of *Scytalidium thermophilum* in mushroom compost and stimulatory effects on growth rate and yield of *Agaricus bisporus*. *J Gen Microbiol* 135, 751-759

Straatsma G, Gerrits JPG, Gerrits TM, Op Den Camp HJM, Van Griensven LJLD (1991) Growth kinetics of *Agaricus bisporus* mycelium on solid subtrate (mushroom compost). *J Gen Microbiol* 137, 1471-1478

Van Zaayen A, Van Adrichem JCJ (1982) Prochloraz for control of fungal pathogens of cultivated mushrooms. *Neth J Plant Pathol* 88, 203-213

Wood D (1989) Mushroom biotechnology. *Int Indust Biotechnol* 9, 5-8

Wuest PJ, Cole HJr (1970) Effect of three fungicides on vegetative growth of *Verticillium malthousei* and *Agaricus bisporus* isolates. *Phytopathology* 60, 1320-1321

Yoder JB, Sinden JW, Hauser E (1950) Experience with zinc ethylene bisdithiocarbamate as a fungicide in mushroom cultivation. *Mushroom Sci* 1, 100-108