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Prochloraz for control of fungal pathogens of cultivated mushrooms

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Abstract

A wettable powder of prochloraz manganese complex 50% a.i. satisfactorily controlled the four major pathogens of *Agaricus bisporus* and *A. bitorquis: Verticillium fungicola* var. *fungicola,* the cause of 'dry bubble', *Mycogone perniciosa,* the cause of 'wet bubble', *Cladobotryum dendroides,* the cause of cobweb disease and *V.fungicola* var. *aleophilum,* the cause of brown spots. The results were obtained in trials in trays with the *Agaricus* species, which were inoculated with the pathogens.

The product was not toxic to both *Agaricus* species in the effective dosages and the taste of the mushrooms was not affected by prochloraz formulations. Application of 1.5 g a.i. m^{-2} nine days after casing is preferred, also in view of the residue levels. A 45% emulsifiable concentrate of the product without manganese was slightly toxic to mushrooms in a lower dosage than the wettable powder.

Additional keywords: mushroom diseases, dry bubble, wet bubble, cobweb disease, mycoparasites, brown spots, *Agaricus bisporus, Agaricus bitorquis, Verticillium fungicola* var. *fungicola, V.fungicola* var. *aleophilum, Mycogone perniciosa, Cladobotryum dendroides,* chlorothalonil, benzimidazole fungicides.

Introduction

Benzimidazole fungicides, registered for use in Dutch mushroom growing since 1973, were very effective for the control of fungal pathogens of the cultivated mushroom, *Agaricus bisporus* (Lange) Imbach (Snel and Fletcher, 1971; Holmes et al., 1971; Gandy, 1972; Fletcher et al., 1975). Fungal pathogens of *A. bisporus* are *Verticillium fungicola* (Preuss) Hassebr. var. *fungicola* (syn. *V. malthousei* Ware; for nomenclature see Gams, 1971, and Gams and Van Zaayen, 1982), the cause of 'dry bubble' (for description of symptoms see Ware, 1933); *Mycogone perniciosa* Magn., the cause of 'wet bubble' (Magnus, 1888) and *Cladobotryum dendroides* (Bull. ex Merat) W. Gams & Hoozemans, the cause of cobweb disease (Flachs, 1939/1940). A pathogen of *A. bitorquis* is *V. fungicola* var. *aleophilum* Gams & Van Zaayen, causing brown spots (Van Zaayen and Gams, 1982). The largest economic loss in mushroom growing is due to 'dry bubble' in *A. bisporus.*

In the Netherlands, about one year after registration of the benzimidazole compounds and thiophanate-methyl for use in mushroom growing, strains of *V. fungicola*

var. *fungicola* resistant to these chemicals were noticed (Bollen and Van Zaayen, 1975). Similar observations in English mushroom farms have been reported by Gandy and Spencer (1974) and by Fletcher and Yarham (1976).

Since 'dry bubble' is the most widespread and most harmful disease, other fungicides were tested for the control of benzimidazole tolerant strains of *V. fungicola* var. *fungicola.* Chlorothalonil proved to be satisfactory (Gandy, 1972; Gandy and Spencer, 1976), though not as effective as the benzimidazole compounds before 1974. Chlorothalonil is registered for use in mushroom growing in the Netherlands since 1977 (Van Zaayen, 1977). The search for better fungicides, however, was continued because chlorothalonil did not completely control 'dry bubble' and because dependance on only one fungicide implies the risk that the pathogen might produce strains which are resistant to it. British research workers considered prochloraz (Fletcher, 1981) or a carbendazim/maneb mixture to be more effective than chlorothalonil for control of 'dry bubble' disease; they reported the reduction of mushroom yield by one of the formulations of prochloraz (Fletcher and Hims, 1981; Gandy and Spencer, 1981).

We investigated the activity of prochloraz in controlling three fungal pathogens of *A. bisporus* and one of *A. bitorquis* under Dutch growing conditions. We also tested the toxicity of the fungicide to mushrooms and the possibility of an aberrant taste. The results are described in the present paper.

Materials and methods

Prochloraz-Mn-complex wettable powder 50% a.i. (Sportak 50 WP) and prochloraz emulsifiable concentrate 45% a.i. (Sportak 45 EC) were provided by FBC Ltd, Cambridge, U.K. and Aseptafabriek BV, Delft, the Netherlands. Times and quantities of application are mentioned in Tables 1 to 4. In trial 6 the emulsifiable concentrate was used; in all other trials the wettable powder was applied.

The pathogens were obtained from diseased fruit-bodies of *A. bisporus* or *A. bitorquis.* Isolate R_1 of *V. fungicola* var. *fungicola* (CBS 733.74) was isolated in 1974 from mushrooms taken from benomyl-sprayed crops in a farm where the fungicide had failed to control *V. fungicola* (Bollen and Van Zaayen, 1975). Isolates of *M. perniciosa* and *C. dendroides* were recently obtained from diseased mushrooms *(A. bisporus). V. fungicola* var. *aleophilum* (CBS 357.80) was isolated in 1979 from *A. bitorquis* with brown spots (Van Zaayen and Gams, 1982). Conidia for inoculation were obtained from mycelium that was usually grown on mushroom tissue agar (MTA; Schisler et al., 1968) at 25 °C for 2 to 3 weeks. Mycelium of *C.dendroides* was grown on a mixture of spent mushroom compost with 5% oatmeal (pH 6.0) at 25 °C for 2 to 3 weeks.

Trials in mushroom growing-rooms. Mushrooms of *A. bisporus* and *A. bitorquis* were grown as described by Bollen and Van Zaayen (1975), and Van Zaayen and Van der Pol-Luiten (1977), respectively. The growing temperatures were 25 °C during mycelial growth and 16-18 °C during harvesting for *A. bisporus,* and 30 °C during mycelial growth and 25 °C during harvesting for *A. bitorquis.* In the trials, different varieties of *A. bisporus* and variety Horst K32 of *A. bitorquis* were used. After casing, fungicides were sprayed (in 1 liter water per m^2) onto the surface of the casing soil. See for further details Tables 1 to 4.

Fig. 1. Wet bubble in *A. bisporus* after inoculation with an aleuriospore suspension of *Mycogone perniciosa.*

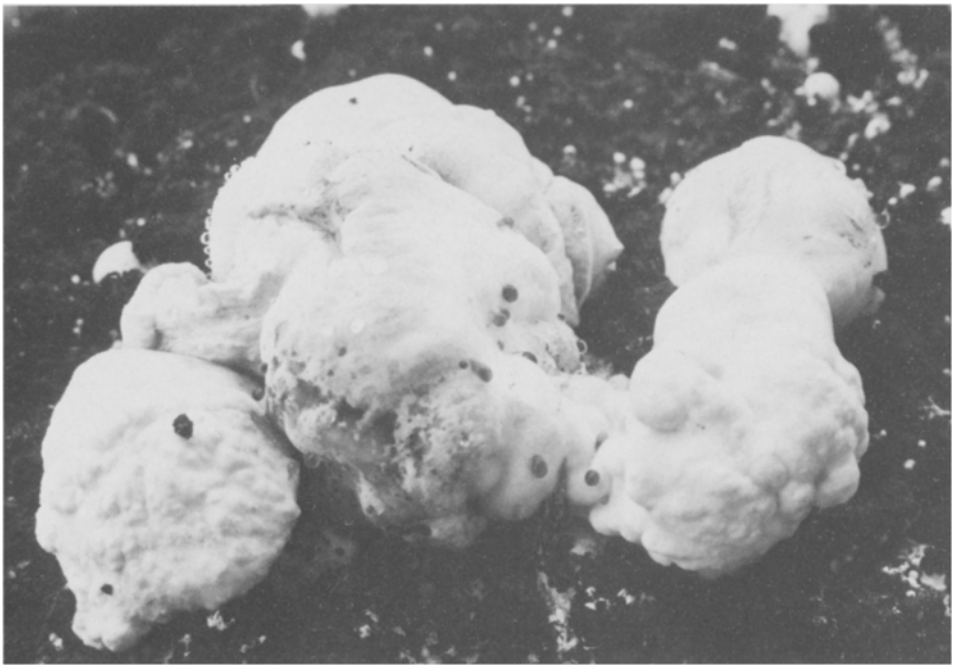

Fig. 1. Natte mollen in A. bisporus na inoculatie met een aleuriosporensuspensie van Mycogone perniciosa.

In trials 1 to 3 the efficacy of prochloraz-Mn-complex was tested. The trials were in nine replicates, in isolated growing-rooms for disease experiments. To prevent other growing-rooms of the Experimental Station from becoming contaminated, precautions were observed as described by Dieleman-van Zaayen (1972). Trays with a growing area of 0.27 m^2 were inoculated with the fungal pathogens by atomizing a conidial suspension at a rate of c. 1.5×10^6 conidia per m^2 in trials 1 and 2 and c. 1.5×10^7conidia per m^2 in trial 3. This was done 8 to 9 days after casing. Conidia were obtained from cultures grown on MTA at 25 °C. Spore suspensions of *M. perniciosa* predominantly contained aleuriospores; the few phialospores were not counted. During inoculation and for about 1 h afterwards, uninoculated (control) trays were covered with plastic sheets and ventilation and circulation in the growing-room were switched off to prevent contamination. As inoculation with conidia of *C. dendroides* did not lead to infection in earlier trials (see also Flachs, 1939/1940), growing-trays were inoculated in the centre, two days after casing, with mycelium of *C. dendroides.* At the time of inoculation the mycelium had completely covered a spent compost/oatmeal mixture in Petri-dishes; each tray was inoculated with the content of one Petri-dish.

In trials 4 to 6 the toxicity of prochloraz and a possible influence on the mushroom taste were tested. The trials were in ten replicates unless otherwise stated, in produc-

Fig. 2. Cobweb disease in *A. bisporus,* after inoculation with mycelium of *Cladobotryum dendroides.*

Fig. 2. A. bisporus met spinnewebschimmel, na inoculatie met mycelium van Cladobotryum dendroides.

tion rooms in trays with a growing area of 0.35 m^2. Mushrooms from the first flush of *A. bisporus* and *A. bitorquis* were tasted as freshly blanched and as canned products by a panel of taste experts.

Mushrooms appeared 19 to 21 days after casing. For the next 5 weeks they were harvested every 2 to 3 days and weighed; the yields were adjusted for 100 kg compost per m^2 (cut mushrooms). Diseased mushrooms were not weighed but counted. The yields and the numbers of diseased mushrooms were processed by analysis of variance.

Residues of prochloraz in the mushrooms were determined by FBC Ltd, Cambridge, U.K.

Results

Trials 1 to 3. In the plots spawned with *A. bisporus,* inoculated with conidial suspensions of *V. fungicola* var. *fungicola* and not treated with prochloraz, brown spots on the mushroom caps occurred in the first week of harvesting. Deformed fruit-bodies ('dry bubble') were usually present from the second week of harvesting onwards. Inoculation of *A. bitorquis* with conidial suspensions of *V. fungicola* var. *aleophilum* resulted in brown-spotted mushrooms. Severely mis-shapen fruit-bodies ('wet bubble') appeared, from the first week of harvesting onwards, after inoculation

Table 1. Effect of prochloraz[1] and chlorothalonil on yield of *A. bisporus* (variety Le Lion B92) and on incidence of dry bubble, after inoculation with c. 1.5×10^6 conidia of *Verticillium fungicola* var. *fungicola,* isolate R_1 (trial 1).

Fungicide	Total quantity applied (g a.i. m^{-2})	Application	Inoculated	Average yield[2] (kg m^{-2})	Infected mushrooms[2] (average number per tray)
none	0	—	—	19.0	6
none	0	—	+	12.9**	163**
chlorothalonil	4.5 (s.a.)[3]	at casing and 2 weeks later	+	15.8**	70**
prochloraz	1.5 (s.a.)	9 days after casing and after first flush	+	17.4	3
prochloraz	1.5	9 days after casing	+	17.9	5
prochloraz	1.5	at casing	+	18.4	13
prochloraz	3.0 (s.a.)	at casing and 2 weeks later	+	16.4**	2
prochloraz	1.5 (s.a.)	at casing and 2 weeks later	+	17.9	3
prochloraz	1.5 (s.a.)	at casing and after first flush	+	17.0**	6

[1] Sportak 50 wettable powder.
[2] Double asterisks indicate values significantly different from untreated, uninoculated control at the 1% level.
[3] s.a. = split application.

Tabel 1. Invloed van prochloraz[1] en chloorthalonil op de opbrengst van A. bisporus (ras Le Lion B92) en op het optreden van droge mollen, na inoculatie met isolaat R_1 van Verticillium fungicola var. fungicola.

of *A. bisporus* with aleuriospore suspensions of *M. perniciosa* (Fig. 1). Inoculation with mycelium of *C. dendroides* caused a decreased yield since the development of pinheads and mushrooms was inhibited by the quickly growing white, later pink mycelium of this fungus (Fig. 2).

Inoculation with each pathogen resulted in a significant reduction of mushroom yield and a significantly higher number of diseased mushrooms in comparison with those of the uninoculated control trays (Tables 1 to 3). Prochloraz-Mn-complex in a dosage of 1.5 to 3 g m^{-2} (in one application or as a split application at various times after casing to the inoculated trays) always caused a significant increase of yield and decrease of infected mushrooms, as compared with the inoculated, untreated trays (Tables 1 to 3). Mushroom yields and numbers of infected mushrooms on inoculated trays treated with prochloraz-Mn-complex usually equalled those of uninoculated control trays and often were not significantly different from those of such trays. The occurrence of diseased mushrooms in the uninoculated control trays demonstrates

Table 2. Effect of prochloraz[1] on yield of *A. bisporus* (variety Somycel 53) and on incidence of dry bubble, wet bubble or cobweb disease, after inoculation with *V. fungicola* var. *fungicola* (isolate R_1), *Mycogone perniciosa* and *Cladobotryum dendroides*, respectively (trial 2).

Pathogen (inoculum density)	Prochloraz, total quantity applied (g a.i. m^{-2})	Application	Average yield[2] (kg m^{-2})	Infected mushrooms[2] (average number) per tray)
control (not inoculated)	0	—	21.2	6
V. fungicola var. *fungicola* (1.5×10^6 conidia m^{-2})	0	—	12.3**	247**
	1.5	9 days after casing	19.1	6
	1.5 (s.a.)[3]	at casing and 2 weeks later	19.4	2
	1.5	6 days after casing	20.0	10
M. perniciosa (1.5×10^6 aleurio-spores m^{-2})	0	—	4.3**	442**
	1.5	9 days after casing	19.3	5
C. dendroides (mycelium)	0	—	8.3**	+[5]
	1.5	9 days after casing	11.0**[4]	0[5]

[1] Sportak 50 wettable powder.
[2] Double asterisks indicate values significantly different from untreated, uninoculated control at the 1% level.
[3] s.a. = split application.
[4] Value also significantly different from untreated, with *C. dendroides* inoculated control at the 1% level.
[5] + = symptoms of cobweb disease; 0 = no symptoms.

Tabel 2. Invloed van prochloraz [1] op de opbrengst van A. bisporus (ras Somycel 53) en op het optreden van droge mollen, natte mollen en spinnewebschimmel, na inoculatie met resp. isolaat R_1 van V. fungicola var. fungicola, Mycogone perniciosa en Cladobotryum dendroides.

that infection by the fungal pathogens, particularly *Verticillium* and *Mycogone*, could not be kept restricted to the inoculated trays only. Diseased fruit-bodies on the uninoculated trays were found in the last weeks of the crops mainly. The infection is principally due to conidial dispersion during watering and circulation. In the experimental design the plots were distributed at random. Hence, control trays often adjoined inoculated ones.

Yields of *A. bitorquis* were abnormally low in trial 3 (Table 3), probably due to a compost that was unfit.

The incomplete control of 'dry bubble' by chlorothalonil is apparent from Table 1.

Table 3. Effect of prochloraz[1] on yield of *A. bitorquis* (variety Horst K32) and on incidence of brown spots after inoculation with c. 1.5×10^7 conidia of *Verticillium fungicola* var. *aleophilum* (trial 3).

Prochloraz, total quantity applied (g a.i. m^{-2})	Application	Inoculated	Average yield[2] (kg m^{-2})	Infected mushrooms[2] (average number per tray)
0	—	—	11.3	3
1.5	9 days after casing	—	11.8	2
0	—	+	7.8**	80**
1.5	9 days after casing	+	11.4	21
1.5 (s.a.)[3]	at casing and two weeks later	+	11.6	16
1.5	6 days after casing	+	10.5	28**

[1] Sportak 50 wettable powder.
[2] Double asterisks indicate values significantly different from untreated, uninoculated control at the 1% level.
[3] s.a. = split application.

Tabel 3. Invloed van prochloraz[1] op de opbrengst van A. bitorquis (ras Horst K32) en op het optreden van bruine vlekken na inoculatie met Verticillium fungicola var. aleophilum.

Prochloraz-Mn-complex was very active and least toxic in a dosage of 1.5 g a.i. m^{-2}, applied once 9 days after casing or as a split application, i.e. just after casing and 2 weeks later (Tables 1 to 3). Split application of 3 g a.i. m^{-2} as well as application after the first flush resulted in a reduction in yield (Table 1).

Trials 4 to 6. Again, a decrease in yield was the result of a split application of 3 g a.i. m^{-2} of prochloraz-Mn-complex (Table 4). In trial 6 with prochloraz emulsifiable concentrate 45% a.i., a decrease in yield was caused by split application of 2.7 g a.i. m^{-2} and also, though less significant, by split application of 1.35 g a.i. m^{-2} (Table 4).

A panel of taste experts did not notice any difference in taste between untreated mushrooms and mushrooms from trays that had been treated with various dosages of prochloraz.

Residue analyses. From the results of residue analyses (Table 5) it is clear that a split application of 1.5 g a.i. m^{-2} prochloraz-Mn-complex with treatments at casing and after the first flush resulted in the highest residue level. However, if 1.5 g a.i. m^{-2} was applied 9 days after casing or as a split application, i.e. just after casing and 2 weeks later, the residue value was about one fourth of the above mentioned figure.

Discussion

The efficacy of prochloraz-Mn-complex in controlling the most frequent and damaging mushroom pathogens is accompanied by the absence of toxicity to cultivated

Table 4. Effect of prochloraz-Mn-complex[1] and prochloraz[2] on average yields of *A. bisporus* (varieties Somycel 53 in trial 4 and Horst U_1 in trial 6) and *A. bitorquis* (variety Horst K32 in trial 5, in eight replicates).

Treatment	Total quantity applied (g a.i. m^{-2})	Application	Average yield[3] (kg m^{-2})	
			A. bisporus (trial 4)	*A. bitorquis* (trial 5)
none	0	—	21.8	15.3
prochloraz-Mn-complex	1.5 (s.a.)[4]	at casing and 2 weeks later	21.0	16.3
	1.5	9 days after casing	21.3	15.6
	3.0 (s.a.)	at casing and 2 weeks later	19.3**	15.1
			(trial 6)	
none	0	—	19.3	
prochloraz	1.35 (s.a.)	at casing and 2 weeks later	16.9	
	1.35	9 days after casing	18.8	
	2.7 (s.a.)	at casing and 2 weeks later	15.5**	

[1] Sportak 50 wettable powder.
[2] Sportak 45 emulsifiable concentrate.
[3] Double asterisks indicate values significantly different from untreated control at the 1% level.
[4] s.a. = split application.

Tabel 4. Invloed van prochloraz-Mn-complex[1] en prochloraz[2] op de opbrengsten van A. bisporus (ras Somycel 53 in proef 4 en Horst U_1 in proef 6) en A. bitorquis (ras Horst K32 in proef 5, in 8 herhalingen).

Table 5. Results of residue analyses of mushrooms after treatment with prochloraz-Mn-complex (means of 4 replicates).

Fungicide	Total quantity applied (g a.i. m^{-2})	Application	Sampling time in days after the last treatment	Total prochloraz-derived residue[1] (mg kg^{-1} fresh material)
none	0	—	—	0.03 ± 0.02
prochloraz	1.5	9 days after casing	10	0.20 ± 0.08
prochloraz	1.5 (s.a.)[2]	at casing and 2 weeks later	5	0.19 ± 0.04
prochloraz	1.5 (s.a.)	at casing and after first flush	3	0.83 ± 0.28

[1] Measured as 2,4,6-trichlorophenol but expressed as a prochloraz-derived residue by correcting for the molecular weight factor (× 1.906).
[2] s.a. = split application.

Tabel 5. Resultaten van de residubepalingen in champignons na toepassing van prochloraz-mangaancomplex (gemiddelden van 4 herhalingen).

mushrooms in the effective dosage of 1.5 g a.i. m^{-2}. These properties would make the fungicide an outstanding product for use in the mushroom industry.

The low yield (11.0 kg m^{-2}) of trays with *A. bisporus,* inoculated with mycelium of *C. dendroides* and treated with 1.5 g a.i. m^{-2} prochloraz-Mn-complex, 9 days after casing (Table 2), is not caused by any infection but by omittance of an otherwise usual working procedure: 6 days after casing, the casing soil with mushroom mycelium usually is 'ruffled up', which has a favourable influence on the yield of *A. bisporus.* Because of the previous inoculation with mycelium of *C. dendroides,* this practice was omitted here. The yield of the inoculated and prochloraz treated plots, however, was significantly higher than that of the untreated, inoculated trays. Symptoms of the disease were present on the latter trays only.

British research workers achieved the best control of mushroom pathogens with three separate spray applications at 0.3 g a.i. m^{-2} of prochloraz-Mn-complex at each application, at 7, 21 and 35 days after casing (Fletcher et al., 1982). We tested various times of application of prochloraz. The product seemed slightly toxic when applied later in the crop, i.e. during harvesting (Table 1). Since earlier prochloraz application was not toxic, but effective for a long time and since such an application earlier in the crop was more desirable in view of the expected residues in mushrooms, it was investigated further. Although a split application of 1.5 g a.i. m^{-2} prochloraz-Mn-complex, i.e. just after casing and 2 weeks later, is as effective as an application of a similar dosage applied 9 days after casing, the latter is preferred because it involves less labour and it may give protection for a longer period.

Moreover, if resistance to the ergosterol-biosynthesis-inhibiting fungicides will develop under practical conditions, only strains with a low level of resistance are expected to emerge (De Waard and Fuchs, 1982). For this type of resistance Dekker (1982) advised to use higher dosages of the fungicide in order to kill the resistant strains.

Application 6 days after casing was tested since the fungicide could then be used just after 'ruffling up' of *A. bisporus* mycelium in the casing soil, but the effect was not always as good as that of the application 9 days after casing (Table 3).

The results of residue analyses confirmed, that prochloraz should not be applied during harvesting.

Prochloraz emulsifiable concentrate 45% a.i. was slightly toxic to mushrooms in a dosage of 1.35 g a.i. m^{-2}, whereas prochloraz-Mn-complex was not yet toxic in a dosage of 1.5 g a.i. m^{-2} (Table 4). Fletcher and Hims (1981) already mentioned the same diversity in toxicity between different formulations of prochloraz; they also preferred prochloraz-Mn-complex for use on the mushroom crop.

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Samenvatting

Prochloraz ter bestrijding van pathogene schimmels in de champignonteelt

Een spuitpoeder met 50% prochloraz als werkzaam bestanddeel (een prochloraz-mangaan-complex) gaf in de teelt van champignons *(A. bisporus* en *A. bitorquis)* een uitstekende bestrijding van de vier belangrijkste pathogene schimmels: *Verticillium fungicola* var. *fungicola* (de veroorzaker van 'droge mollen'), *Mycogone perniciosa* (de veroorzaker van 'natte mollen'), *Cladobotryum dendroides* (spinnewebschimmel) en *V. fungicola* var. *aleophilum* (de veroorzaker van bruine vlekken). Deze resultaten werden verkregen in proeven met kisten waarin de *Agaricus*-soorten, die geïnoculeerd werden met genoemde pathogenen, werden geteeld.

Het produkt was voor geen van beide *Agaricus*-soorten toxisch als het werd toegepast in de werkzame doseringen, en de champignonsmaak werd door het middel niet nadelig beïnvloed. De voorkeur wordt gegeven aan toepassing van 1,5 g werkzame stof per m^2, 9 dagen na het afdekken, mede op grond van de gevonden residuwaarden. Een 45% vloeibare formulering zonder mangaan was, in lagere doseringen dan het spuitpoeder, in lichte mate toxisch voor champignons.

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