Fungicide resistance among *Cladobotryum* spp. – causal agents of cobweb disease of the edible mushroom *Agaricus bisporus*

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A survey of fungicide resistance among isolates of the mushroom pathogens *Cladobotryum mycophilum* and *C. dendroides* Types I and II was undertaken, with respect to the active ingredients thiabendazole, carbendazim (benzimidazoles) and prochloraz manganese following an epidemic in Britain and Ireland in 1994/95. The majority of isolates (41/57) were strongly resistant to thiabendazole ($ED_{50} > 200$ ppm) and were exclusively *C. dendroides* Type II. All *C. mycophilum* and *C. dendroides* Type I isolates, and four *C. dendroides* Type II isolates, were weakly resistant to thiabendazole (ED_{50} 1–10 ppm). Thiabendazole-resistant *C. dendroides* Type II isolates were only weakly resistant to carbendazim (ED_{50} 2–10 ppm) and isolates which were weakly resistant to thiabendazole were carbendazim-sensitive ($ED_{50} < 1$ ppm), demonstrating a lack of complete cross resistance between these two benzimidazole fungicides. The ED_{50} values for all isolates with respect to prochloraz manganese ranged from 0.14 to 7.8 ppm. Benzimidazole resistance was considered to have been an important factor influencing the severity of the 1994/95 cobweb epidemic but 25% of isolates collected were benzimidazole sensitive.

Cladobotryum dendroides is more commonly known in the mushroom industry by its synonym, *Dactylium dendroides*, or as its teleomorph *Hypomyces rosellus*. It is responsible for the mushroom disease known as cobweb (Fletcher, White & Gaze, 1989). It produces a verticillately branched conidiophore bearing two-, three- and four-celled conidia. Colonies on mushroom casing are usually circular in appearance and can rapidly engulf adjacent mushrooms, causing rapid decay. Massive sporulation usually occurs once mushrooms are encountered and the dry spores, which are easily dislodged when watering the mushroom crop, are responsible for a brown spotting symptom on mushroom fruit bodies. Other *Cladobotryum* species have been recorded from cultivated mushrooms by various authors and include *C. multiseptatum*, *C. mycophilum*, *C. varium* (syn. *C. variospermum*) and *C. verticillatum* (Sinden, 1971; De Hoog, 1978; Sharma, Suman & Guleria, 1992). A number of *Hypomyces* spp. with *Cladobotryum* anamorphs have been isolated from members of the *Aphyllophorales* and *Agaricales* growing in the wild (Rogerson & Samuels, 1993, 1994). Some of these produce conidia and conidiophores which are similar to *C. dendroides* so it is possible that some unusual *Cladobotryum* species may occur on commercial mushrooms.

Outbreaks of cobweb disease on British mushroom farms were sporadic for many years with the disease being controlled using available fungicides (Fletcher *et al.*, 1989). In the early 1990s outbreaks became more frequent, and reached epidemic proportions in 1994/95. Although fungicide resistance was implicated, outbreaks of cobweb were already on the increase, and it was suggested that the warmer and moister environments in which mushrooms are now grown, compared with the early 1980s, may have been a contributory factor to the emergence of cobweb (Gaze, 1995). In the 1970s resistance to benzimidazole fungicides had developed in *Verticillium fungicola*, another mushroom pathogen, and a survey of 229 U.K. isolates indicated that 53% had ED_{50} values of > 50 ppm with respect to the fungicide benomyl (Fletcher & Yarham, 1976). This 1970s survey also reported that, of 24 *Cladobotryum* isolates tested, none grew at 5 ppm benomyl (or 1 ppm where tested), indicating that isolates were still sensitive to this fungicide. In the British mushroom industry benomyl has been largely replaced by the active ingredient carbendazim, which is a major primary breakdown product of benomyl (Hassall, 1990), and would be expected to have similar activity as benomyl against individual pathogens. Fletcher & Yarham (1976) demonstrated this for 10 *V. fungicola* isolates. In the 1980s benomyl, thiabendazole (and prochloraz manganese) were still effective against an isolate of *Cladobotryum* in a cropping experiment (Fletcher, Hims & Hall, 1983). In the same year, Lockley & Gay (1983) reported that an isolate from a farm using thiabendazole was capable of 15 and 0% growth at 50 and 100 ppm thiabendazole, respectively, while isolates from two other farms failed to grow at thiabendazole concentrations of 25 ppm or above (ED_{50} values for all isolates were $\leqslant 10$ ppm). In a later survey of 12 British farms in 1993, 14 *Cladobotryum* isolates from one farm were capable of growth at 2 and 20 ppm thiabendazole (ED_{50} not given), but only one of these isolates grew at 2 ppm

benomyl, which led the authors to conclude that resistance to thiabendazole but not benomyl had occurred on this one farm (Fletcher & Jaffe, 1993). These data confirm that some *Cladobotryum* isolates from British farms were capable of growth at 50 or 20 ppm thiabendazole in 1983 and 1993, respectively, but, in the absence of detailed baseline data, it is difficult to draw any firm conclusions as to whether isolates were becoming resistant; these observations may have simply reflected normal variation in the background level of sensitivity to benzimidazole fungicides within the *Cladobotryum* population as a whole. A survey was, therefore, undertaken to re-examine, in more detail, the resistance status of *Cladobotryum* isolates in Britain.

MATERIALS AND METHODS

Fungicides

Commercial formulations of the three most commonly used fungicides in the British mushroom industry were used in this study (Table 1). Thiabendazole and carbendazim are both benzimidazole fungicides which bind to fungal beta-tubulins and inhibit microtubule function. Prochloraz manganese is a sterol C-14 demethylation inhibitor which impairs biosynthesis of ergosterol, an essential compound for the stability and functioning of lipoprotein membranes (Hassall, 1990). Freshly-made stock solutions were prepared to give specific concentrations of active ingredient in ppm. Volumes of stock solution were added to molten (50 °C) sterile culture media prior to pouring giving active ingredient concentrations ranging from 0.01 to 500 ppm depending on the experiment.

Isolates

Fifty-seven *Cladobotryum* isolates were screened (Table 2) of which 51 were obtained from various mushroom farms in Britain (England, Scotland and Wales). The remaining isolates were obtained from France (3), Northern Ireland (2) and the Republic of Ireland (1). Of the British isolates, 35 were obtained as single isolates from individual farms, five farms provided two isolates each and two farms provided three isolates each, giving a total of 42 farms. Isolate 5A was obtained in 1993 while all remaining isolates were obtained during 1995. All isolates were placed in the HRI culture collection in liquid nitrogen and were maintained on 2% malt agar slopes at 4° during the experimental period. Selected isolates were sent to IMI, Egham, Surrey, U.K. for identification.

Determination of fungicide activity against mycelial growth in vitro

Fungicide activity against mycelial growth *in vitro* was determined for each isolate with respect to the three fungicides thiabendazole, carbendazim and prochloraz manganese. Isolates were grown on malt extract agar (MEA, Oxoid, Basingstoke, England) in which active ingredients were incorporated at concentrations of 0, 1, 2, 10, 20 and 50 ppm. Three replicate Petri dishes were prepared for each isolate at each concentration of each active ingredient. Petri dishes were inoculated with a 5 mm diam. plug of mycelium which was taken from the growing edge of a 2–4 d old culture. Inoculum plugs were placed 10 mm in from the edge of a 90 mm diam. Petri dish and incubated at 25°. Radial growth across the Petri dish was measured daily. A daily radial growth rate (mm d^{-1}) was calculated using data from the linear phase, usually between days 2–4 or 3–7, depending on how fast individual isolates grew. When growth was slow, particularly on media containing high concentrations of fungicides, radial growth rate was calculated based on measurements taken at 3–7 d intervals over a period of 14 or 21 d. Growth response curves were then prepared for each isolate with respect to each of the three active ingredients by plotting radial growth rate against fungicide concentration. An ED_{50} category (i.e. the concentration range within which the linear growth rate was reduced by 50%) was assigned to each isolate for each active ingredient. Isolates were tested in groups of 5–10 as they were received from growers during the period from February to August 1995.

When all initial tests were completed, 13 isolates were selected for repeat testing but the range of concentrations used was extended based on the results from the initial tests. Thus, thiabendazole concentrations ranged from 1 to 500 ppm; carbendazim concentrations ranged from 0.01 to 64 ppm and prochloraz manganese concentrations ranged from 0.01 to 100 ppm. ED_{50} values were calculated for each isolate using two methods. For each of three replicate concentration series, the concentration which gave a 50% reduction in colony

Table 1. Details of three fungicide active ingredients used in mushroom crop protection.

	Fungicide active ingredient (a.i.)		
	Thiabendazole	Carbendazim	Prochloraz manganese
Trade name	Hymush[1]	Bavistin	Sporgon
Formulation (% a.i.)	60% WP	50% WG	46% WP
Supplier	Agrichem	BASF	Darmycel (Agrevo)
Standard dose[2] (g product m^{-2})	2.0	2.5	1.2 (× 2)
g a.i. m^{-2} ($\simeq$ 50 l casing)	1.2	1.25	0.55 (× 2)
Concentration in casing[3] mg a.i. kg^{-1} casing (ppm)	34.2	35.7	15.5 (× 2)

[1] No longer available for use on mushrooms in Britain.
[2] Refer to product labels for full details of fungicide application rates.
[3] Based on an average casing bulk density of 700 g l^{-1} and product uniformly mixed throughout.

Table 2. Characteristics of 57 *Cladobotryum* isolates from the UK, Republic of Ireland and France.

	Origin	Conidial cell number	Camphor odour	Radial growth rate (mm d^{-1})	ED_{50} (ppm)	
					Thiabendazole	Carbendazim
Weakly resistant to thiabendazole[1]						
C. mycophilum						
192B3	W. Sussex	1, 2 (3)	Yes	11.0	1–2	< 1
202A[2]	Kent	1, 2 (3, 4)	Yes	12.4	1–2	< 1
202B	Kent	1, 2	Yes	13.1	1–2	< 1
220D	France	1, 2	Slight	11.0	2–10	< 1
222	Wiltshire	1, 2	Yes	12.8	2–10	< 1
241C[2]	E. Lothian	1, 2, (3, 4)	Yes	19.3	1–2	< 1
257	Kent	1, 2 (3)	Yes	13.1	1–2	< 1
C. dendroides Type I						
187	W. Sussex	(1, 2) 3, 4	No	13.0	2–10	< 1
195A	Devon	2, 3, 4	No	14.8	2–10	< 1
220B	France	(1, 2) 3, 4	No	12.9	1–2	< 1
238A	N. Humberside	2, 3, 4 (5, 6)	No	13.3	2–10	< 1
289	Devon	2, 3, 4 (5, 6)	No	16.0	2–10	< 1
C. dendroides Type II						
193A	Suffolk	1, 2, 3, 4	No	20.0	2–10	< 1
193B	Suffolk	1, 2, 3, 4	No	23.1	1–2	< 1
193C	Suffolk	1, 2, 3, 4	No	20.2	2–10	< 1
220C	France	1, 2, 3, 4	No	21.5	1–2	< 1
Strongly resistant to thiabendazole						
C. dendroides Type II						
5A[3]	Lancashire	1, 2, 3, 4	No	21.0	> 50	2–10
164	Worcestershire	1, 2, 3, 4	No	22.0	> 50	2–10
165	Worcestershire	1, 2, 3, 4	No	22.3	> 50	2–10
166	Hampshire	1, 2, 3, 4	No	19.9	> 50	2–10
167	Hampshire	1, 2, 3, 4	No	18.2	> 50	2–10
169	Republic of Ireland	1, 2, 3, 4	No	21.3	> 50	2–10
174	Dorset	1, 2, 3, 4	No	21.2	> 50	2–10
176	Dorset	1, 2, 3, 4	No	19.0	> 50	2–10
180	Gloucestershire	1, 2, 3, 4	No	19.2	> 50	2–10
192A	W. Sussex	1, 2, 3, 4	No	20.3	> 50	2–10
192A(6a)	W. Sussex	1, 2, 3, 4	No	20.3	> 50	2–10
192A(D6a)	W. Sussex	1, 2, 3, 4	No	21.8	> 50	2–10
192B1[3]	W. Sussex	1, 2, 3, 4	No	20.8	> 50	2–10
192C	W. Sussex	1, 2, 3, 4	No	19.6	> 50	2–10
192C(6a)	W. Sussex	1, 2, 3, 4	No	21.2	> 50	2–10
196A	Devon	1, 2, 3, 4	No	22.0	> 50	2–10
209	Hampshire	1, 2, 3, 4	No	21.5	> 50	2–10
213	N. Yorkshire	1, 2, 3, 4	No	20.0	> 50	2–10
214	Devon	1, 2, 3, 4	No	19.8	> 50	2–10
215A	Fife	1, 2, 3, 4	No	19.4	> 50	2–10
215B	Fife	1, 2, 3, 4	No	20.6	> 50	2–10
217A	E. Lothian	1, 2, 3, 4	No	20.8	> 50	2–10
217B	E. Lothian	1, 2, 3, 4	No	20.8	> 50	2–10
217C	E. Lothian	1, 2, 3, 4	No	22.2	> 50	2–10
229B	W. Sussex	1, 2, 3, 4	No	20.7	> 50	2–10
229C	W. Sussex	1, 2, 3, 4	No	19.0	> 50	2–10
231	Derbyshire	1, 2, 3, 4	No	20.3	> 50	2–10
232B	Shropshire	1, 2, 3, 4	No	21.3	> 50	2–10
235	Norfolk	1, 2, 3, 4	No	21.8	> 50	2–10
239	Surrey	1, 2, 3, 4	No	19.5	> 50	2–10
240	Fife	1, 2, 3, 4	No	19.5	> 50	2–10
243B	Strathclyde	1, 2, 3, 4	No	20.0	> 50	2–10
245	Northern Ireland	1, 2, 3, 4	No	20.0	> 50	2–10
247B	Northumberland	1, 2, 3, 4	No	20.2	> 50	2–10
249C	Lancashire	1, 2, 3, 4	No	19.7	> 50	2–10
260A	W. Sussex	1, 2, 3, 4	No	21.7	> 50	2–10
273	Northern Ireland	1, 2, 3, 4	No	20.5	> 50	2–10
281	Buckinghamshire	1, 2, 3, 4	No	20.5	> 50	2–10
288	Oxfordshire	1, 2, 3, 4	No	20.0	> 50	2–10
297	M. Glamorgan	1, 2, 3, 4	No	19.2	> 50	2–10
298	S. Humberside	1, 2, 3, 4	No	19.7	> 50	2–10

[1] Each number represents an individual farm except for 192A, B and C and 193A, B and C which represent three individual farm units within two larger farm complexes, and also 220B, C and D which are from different French farms.

[2] Isolates identified by CABI Bioscience as *C. mycophilum*.

[3] Isolates identified by CABI Bioscience as *C. dendroides*.

radius after 3 d incubation was calculated by linear interpolation (Method I). ED_{50} values were also calculated for each isolate using radial growth rate data collected over a 2 wk period (Method II).

RESULTS

Isolate characterization

Selected isolates which were sent to IMI for identification were confirmed as either *C. mycophilum* (Oudem.) W. Gams & Hooz.: isolates 202A (IMI 372796) and 241C (IMI 368694) or *C. dendroides* (Bull. Fr.) W. Gams & Hooz.: isolates 5A (IMI 359310) and 192B1 (IMI 372795). The conida and conidiogenous cells of *C. mycophilum* isolates were similar to the descriptions given by Gams & Hoozemans (1970) and Cole & Kendrick (1971) for this species. *C. dendroides* isolates were divided into two groups according to the nature of the conidiogenous cells. In Type I isolates the conidiogenous cells extended irregularly as a result of successive sympodial spore production and were similar to the description given for this species by Gams & Hoozemans (1970). The conidiogenous cells of Type II isolates, which included two isolates identified by IMI as *C. dendroides*, did not produce an irregularly shaped extending tip as described by Gams & Hoozemans (1970); they ranged from being relatively long with narrow apices to relatively short with wide apices and were similar to the description and diagrams by Cole & Kendrick (1971) for *C. mycophilum*, except that a greater number of three- and four-celled conidia were produced. The two types of isolates also differed in their radial growth rate with Type I isolates growing more slowly than Type II isolates.

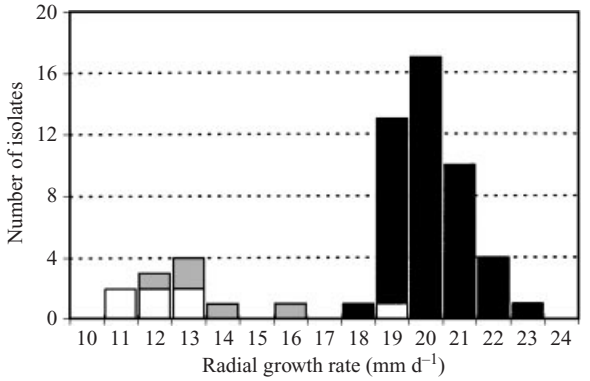

Fig. 1. Frequency distribution of radial growth rate for 57 *Cladobotryum* isolates; □, *C. mycophilum*; ▨, *C. dendroides* Type I and ■, *C. dendroides* Type II.

Following the initial processing of the 57 isolates there appeared to be two distinct groups in terms of growth on MEA at 25° (Fig. 1). All *C. mycophilum* isolates (apart from 241C) and all *C. dendroides* Type I isolates had a radial growth rate of between 11 and 16 mm d^{-1} with an average of 13.2, and 14.0 mm d^{-1} (S.E.M. = 1.07, and 0.61), respectively. One *C. mycophilum* isolate (241C) had a faster, more variable radial growth rate of 16.5–20 mm d^{-1}. All *C. dendroides* Type II isolates had a radial growth rate of 18.2–23.1 mm d^{-1} with an average for the group of 20.5 mm d^{-1} (S.E.M. = 0.15). *C. dendroides* Type II isolates accounted for 45 out of 57 isolates examined of which 41 were from Britain, three were from Ireland and one was from France. Isolates of *C. mycophilum*

Table 3. Radial growth rate at 25° and ED_{50} values of fungicides for selected *Cladobotryum* isolates.

	Radial growth rate (mm d^{-1})	ED_{50} values (ppm)			
		Thiabendazole Method I*	Carbendazim Method I	Prochloraz Method I	Prochloraz Method II†
C. mycophilum					
220D	13.8	1.9	(−1.13)‡ 0.32	0.33	0.19
222	11.3	3.4	(−0.87) 0.42	0.34	0.73
241C	18.5	2.5	(−0.89) 0.41	0.18	0.14
C. dendroides Type I					
195A	12.1	1.9	(−1.49) 0.22	0.46	4.2
220B	13.6	1.5	(−1.43) 0.24	0.85	7.8
C. dendroides Type II					
169	19.8	> 500	(1.25) 3.8	0.56	3.7
192B1	20.8	> 500	(1.43) 4.2	0.62	3.5
213	20.7	> 500	(1.25) 3.5	0.63	2.4
214	21.2	> 500	(1.33) 3.8	0.36	1.5
215	19.7	207	(1.26) 3.5	0.74	2.2
239	20.8	221	(1.48) 4.4	0.52	1.1
245	21.2	> 500	(1.47) 4.3	0.56	1.8
193A	20.5	1.2	(−1.37) 0.25	0.38	0.54
S.E.M. (26 d.f.)	0.67	23.3§	(0.082)	0.118	0.545
L.S.D. ($P = 0.05$)	1.37	47	(0.16)	0.24	1.12

* Method I: ED_{50} calculated after 3 d growth.
† Method II: ED_{50} calculated using radial growth rate data.
‡ Log_n-transformed data.
§ ANOVA carried out on data for eight isolates with ED_{50} values < 500, S.E.M., d.f. = 16.

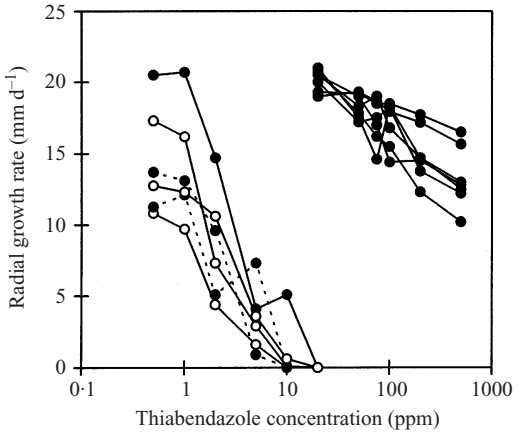

Fig. 2. Growth response curves of *Cladobotryum* isolates to thiabendazole: —○—, *C. mycophilum*; – –●– –, *C. dendroides* Type I; —●—, *C. dendroides* Type II.

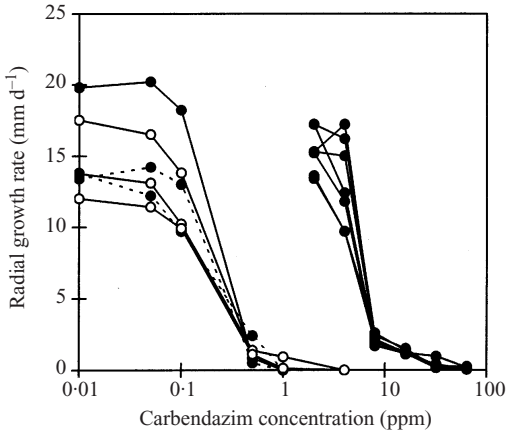

Fig. 3. Growth response curves of *Cladobotryum* isolates to carbendazim: —○—, *C. mycophilum*; – –●– –, *C. dendroides* Type I; —●—, *C. dendroides* Type II.

and *C. dendroides* Type I accounted for the remaining 12 isolates examined, of which 10 were from Britain and two were from France. With one exception (isolates 192B1 and 192B3), different isolates obtained from the same farm were all similar to each other.

Fungicide resistance profiles in vitro

Thiabendazole. All *C. mycophilum*, *C. dendroides* Type I and four *C. dendroides* Type II isolates (193A, 193B, 193C and 220C) demonstrated an ability to tolerate thiabendazole at low concentrations. They were capable of good growth at 1 and 2 ppm but most isolates were severely inhibited at concentrations of 10 ppm or above. ED_{50} values were between 1–2 or 2–10 ppm (Table 2). Repeat tests on selected isolates indicated that ED_{50} values ranged from 1.2 to 3.4 ppm (Table 3) and growth response curves were similar (Fig. 2), but they reflected differences in the radial growth rates of each isolate in the absence of any fungicide. These isolates, which were severely inhibited at 10 ppm thiabendazole, are thus considered to be weakly resistant to thiabendazole. The remaining 41 isolates, all *C. dendroides* Type II, were strongly resistant to thiabendazole with ED_{50} values of > 50 ppm. Repeat testing indicated that most had an $ED_{50} > 500$ ppm (Table 3) and growth response curves were similar (Fig. 2). ED_{50} determination using either radial growth after 3 d (Table 3) or radial growth rate (data not shown) gave similar results.

Carbendazim. The 41 strongly-thiabendazole-resistant *C. dendroides* Type II isolates all demonstrated some ability to tolerate carbendazim. They were capable of good growth at 1 and 2 ppm but were severely inhibited at 10 ppm and above. ED_{50} values were between 2 and 10 ppm (Table 2). Repeat tests indicated that ED_{50} values ranged from 3.5 to 4.4 ppm (Table 3) and growth response curves were similar (Fig. 3). These isolates were considered to be weakly resistant to carbendazim. All *C. mycophilum*, *C. dendroides* Type I, and the four Type II isolates which were weakly resistant to thiabendazole, were very sensitive to carbendazim with ED_{50} values of < 1 ppm. Repeat tests indicated that ED_{50} values ranged from 0.22 to 0.42 ppm. Growth response curves were similar (Fig. 3) but reflected differences in control growth rates. ED_{50} determination using either radial growth after 3 d (Table 3) or radial growth rate (data not shown) gave similar results.

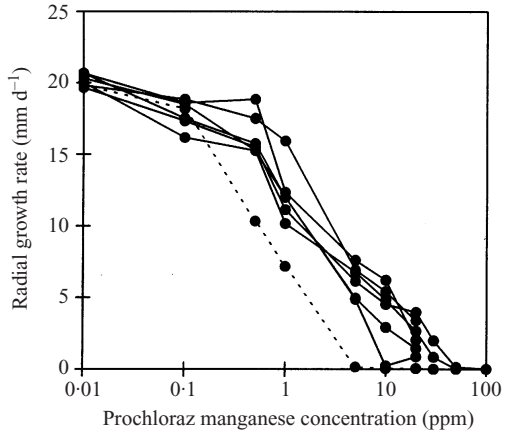

Fig. 4. Growth response curves of *C. dendroides* Type II isolates to prochloraz manganese. —●—, strongly resistant to thiabendazole; – –●– – weakly resistant to thiabendazole.

Prochloraz manganese. The response of all isolates to prochloraz manganese in the initial tests, carried out over a period of time, was variable with no clear patterns emerging (data not shown) unlike the responses to the two benzimidazole fungicides. A selected number of isolates from each species group were re-tested together to minimise variation. This entailed using a single batch of media, a single preparation of fungicide, and inoculum plugs taken from fresh cultures with a uniform growing edge. On this occasion, the strongly-thiabendazole-resistant *C. dendroides* Type II isolates all gave very similar response curves (Fig. 4) while the weakly resistant isolates were more variable (Figs 4, 5). A repeat experiment confirmed these results. The ED_{50} values for prochloraz manganese differed depending on how it was calculated (Table 3). When radial growth after 3 d was used, all ED_{50} values were < 1 ppm, ranging from 0.18 to 0.85, whereas when radial growth rate data were used (collected over a 2 wk period), ED_{50} values ranged from 0.14 to 7.8. There was a

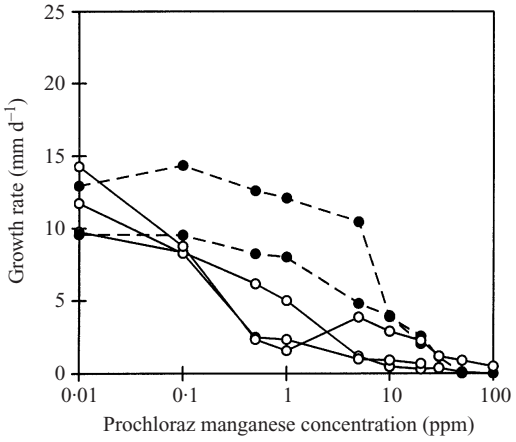

Fig. 5. Growth response curves of *Cladobotryum* isolates to prochloraz manganese: —○—, *C. mycophilum*; −−●−−, *C. dendroides* Type I (all weakly resistant to thiabendazole).

significant correlation ($P = 0.01$) between the two sets of values with $r = 0.77$ (95% confidence limits = 0.37, 0.93) indicating that 41% ($1 - r^2 \times 100$) of the variation between the two methods was unexplained. This is likely to reflect the fact that zero growth was recorded after 3 d at higher prochloraz manganese concentrations whereas radial growth rate, calculated after 2 wk growth, gave significantly fewer zero values.

DISCUSSION

Cladobotryum dendroides Types I and II and *C. mycophilum* were isolated from mushroom farms around Britain during the height of a cobweb epidemic in 1995. Isolates of *C. dendroides* Types I and II produced many three- and four-celled conidia but differences in conidiophore morphology warranted separating them into two groups. McKay *et al.* (1999) have recently reported on genetic characterization of *Cladobotryum* species which included *C. dendroides* Type II isolates 192B1 and 213 and *C. mycophilum* isolates 202A, 222, and 241C (Table 2), and which are listed by them as isolates numbers 87, 89, 88, 90 and 91, respectively. They demonstrated that isolates from Britain and Ireland associated with the 1993–5 epidemic, and initially identified as *C. dendroides*, were genetically more similar to *C. mycophilum* than to a *C. dendroides* isolate from the U.S.A. which was identified by W. Gams (CBS, The Netherlands) as *C. dendroides*. The conidiogenous cells of this U.S.A. isolate produced a secondary extension (similar to the Type I isolates reported here) while their other '*C. dendroides*' isolates did not. Based on the genetic data, they have concluded that the '*C. dendroides*' isolates associated with the epidemic form a distinct subgroup (subgroup 2) within the *C. mycophilum* species group. McKay *et al.* (1999) recorded no morphological differences between their *C. mycophilum* subgroups 1, 2 and 3 but we have separated subgroup 2 (isolates 192B1 and 213) from subgroups 1 and 3 based on conidia cell number, presence or absence of a camphor odour and radial growth rate at 25° (Table 2). We have not found morphological data to separate *C. mycophilum* subgroup 1 (isolates 202A and 241C) from subgroup 3 (isolate 222). The genetic data of McKay *et al.* (1999) clearly indicate a closer relationship between these taxa. Rogerson & Samuels (1994) record that the teleomorphs of *C. mycophilum* (*H. odoratus*) and *C. dendroides* (*H. rosellus*) are identical and that they differ only in their anamorphic forms. Attempted crosses between single ascospore isolates taken from perithecia of *H. odoratus* or *H. rosellus* failed to produce any perithecia while crosses between single ascospore isolates within each species were successful. Mating interactions between selected isolates in Table 2 are currently being carried out to investigate the relationship between *C. mycophilum* and *C. dendroides* Types I and II in the light of the genetic information provided by McKay *et al.* (1999). Descriptions of *C. mycophilum* by Gams & Hoozemans (1970), De Hoog (1978) and Rogerson & Samuels (1994), do not indicate that three- and four-celled conidia are a feature of this species although the diagram of Cole & Kendrick (1971) includes them. Three- and four-celled conidia have been observed in many of the *C. mycophilum* isolates examined in this study but they were not the most common, and for some isolates were very rare. Further investigation is necessary to determine the critical morphological characteristics of these two species in relation to their genetic characterization.

Of the 51 British *Cladobotryum* isolates collected during the height of the cobweb epidemic in 1995, 25% were weakly resistant to thiabendazole (ED_{50} 1–10 ppm), sensitive to carbendazim ($ED_{50} < 1$ ppm) and showed a variable range of responses to prochloraz manganese (ED_{50} 0.19–7.8 ppm). These isolates included all *C. mycophilum* and *C. dendroides* Type I isolates and three *C. dendroides* Type II isolates. The remaining 75% of British isolates were all strongly resistant to thiabendazole ($ED_{50} > 200$ ppm), weakly resistant to carbendazim (ED_{50} 3.5–4.4 ppm) and weakly resistant to prochloraz manganese (ED_{50} 1.1–3.7 ppm), and all were identified as *C. dendroides* Type II. These results suggest that the 'thiabendazole-resistant' isolate of Lockely & Gay (1983) was not the same as the thiabendazole-resistant *C. dendroides* Type II isolates reported here, as it was incapable of growth at 100 ppm thiabendazole, whereas the thiabendazole-resistant *C. dendroides* Type II isolates associated with the cobweb epidemic grew very well at this concentration. The 'thiabendazole-resistant' isolate from one farm described by Fletcher & Jaffe (1993) may have been a *C. dendroides* Type II isolate as it was capable of growth at 20 ppm thiabendazole and 2 ppm benomyl. No data were presented for growth at higher concentrations of thiabendazole, so it is difficult to comment with certainty on the nature of the observed resistance. Most of the thiabendazole-weakly-resistant isolates in this survey failed to grow at 20 ppm thiabendazole or 1 ppm carbendazim (= 1.5 ppm benomyl approx.), so there is a good chance the isolate described by Fletcher & Jaffe (1993) was similar to a thiabendazole-resistant *C. dendroides* Type II. Three thiabendazole-weakly-resistant isolates in this survey had growth rates of 25% or less at 20 ppm thiabendazole and this suggests that there may be some wild isolates which are more tolerant to thiabendazole or that some mutations do not give rise to high levels of resistance. It is important, therefore, to test a pathogen's response to a wide range of fungicide concentrations in order to determine more precisely the degree of resistance present. Thiabendazole, carbendazim and

benomyl (structurally and functionally very similar to carbendazim) are benzimidazole fungicides whose mode of action is interference with the division of cell nuclei by disrupting the assembly of tubulin into microtubules (Hassalls, 1990). Cross resistance to these chemicals often occurs and has been demonstrated for the mushroom pathogen *V. fungicola* which is highly resistant to thiabendazole and benomyl (Bonnen & Hopkins, 1997). The molecular structure of thiabendazole is, however, sufficiently different from that of benomyl and carbendazim to imply that their abilities to bind to tubulin may differ (Hassalls, 1990). Differences in benzimidazole sensitivity have been shown to reflect differences in the (beta-tubulin gene) mutations which confer resistance (Davidse, 1986; Fujimura *et al*., 1990) so that a mutation which leads to poor binding of, and therefore resistance to, thiabendazole may not necessarily affect the binding of carbendazim to the same extent. This scenario could describe the pattern of resistance recorded for *C. dendroides* Type II isolates. Bonnen & Hopkins (1997) also reported that 12% of *V. fungicola* isolates tested were not cross resistant to both thiabendazole and benomyl indicating that at least two different mutation sites may have been involved. McKay *et al*. (1998) have recently shown that benzimidazole resistance in *C. dendroides* isolates from Ireland is caused by a single base transition mutation at codon 50, causing an amino acid substitution from TAC (tyrosine) to TGC (cysteine). This location had not previously been recorded as being associated with benzimidazole resistance in field isolates of pathogens. The three thiabendazole-resistant Irish isolates in Table 2, 169, 245 and 273, were all classified as *C. dendroides* Type II, so there is a possibility that the mutation conferring benzimidazole resistance to all *C. dendroides* Type II isolates in Table 2 is the same as that described by McKay *et al*. (1998). It would appear, therefore, that this mutation has a greater inhibitory effect on the binding of thiabendazole compared with carbendazim. Of interest, also, is the fact that no *C. mycophilum* or *C. dendroides* Type I isolates were thiabendazole-resistant and this may suggest that the structure of their tubulin is different from that of *C. dendroides* Type II.

C. dendroides Type II appears to represent *Cladobotryum* isolates which have recently developed benzimidazole resistance, since a previous survey found only one farm with a thiabendazole-resistant strain (Fletcher & Jaffe, 1993). This may have occurred as a result of regular use of benzimidazole fungicides to control other mushroom pathogens and this phenomenon is widely recognized following benzimidazole use (Hassall, 1990; Bonnen & Hopkins, 1997). Under more stringent experimental conditions, however, *C. dendroides* Type II responses to prochloraz manganese were very similar to each other, unlike the responses of *C. dendroides* Type I and *C. mycophilum* isolates, and this finding suggests that the *C. dendroides* Type II isolates may be clonal in nature. All *C. dendroides* Type I and *C. mycophilum* isolates, and a few *C. dendroides* Type II isolates, were much more sensitive to both carbendazim and thiabendazole, and had more variable responses to prochloraz manganese so these may represent sensitive wild-type isolates. Such isolates represented 25% of British isolates received during this survey at the height of the 1994–5 epidemic which suggests that, despite their sensitivity to benzimidazoles, they were nonetheless associated with serious incidences of cobweb. This in turn suggests that although resistance to benzimidazole fungicides probably made control of cobweb more difficult, there may have been an underlying increase in the incidence of cobweb (Gaze, 1995, 1997). Work is in progress to identify cultural factors which may influence the epidemiology of *C. dendroides*.

The concentration of thiabendazole and carbendazim in casing following application of a standard commercial dose is in the region of 35 ppm (Table 1) although concentrations are likely to be higher in the surface layers of the casing following a drench application. Most *C. dendroides* Type II isolates have an $ED_{50} > 200$ ppm for thiabendazole so it is unlikely that this fungicide would control outbreaks of cobweb caused by such isolates. Isolates which are only weakly-resistant to thiabendazole, however, with ED_{50} values of < 10 ppm, such as *C. mycophilum* and *C. dendroides* Type I may well be controlled by this fungicide. Carbendazim might be expected to be very effective against *C. mycophilum* and *C. dendroides* Type I isolates ($ED_{50} < 1$ ppm) but probably less effective against most *C. dendroides* Type II isolates ($ED_{50} < 10$ ppm).

The fungicide resistance profile of individual mushroom pathogens gives good information on whether or not a given fungicide will be effective in controlling a disease outbreak. With the evolution of strongly, or even weakly, resistant isolates, the efficacy of fungicides may be significantly compromized. It is important, however, to determine exactly what role fungicide resistance plays in the loss of disease control by a fungicide, as there are complex interactions between fungicide resistance, fungicide persistence in mushroom casing and on-farm disease management, in addition to the effects of growing conditions and environment. Work is ongoing in this area to understand the relative importance of individual factors.

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