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BREEDING AND TOXICANT TOLERANCE OF THE DRY ROT FUNGUS SERPULA LACRYMANS

KREUZUNGS- UND HEMMSTOFFTOLERANZVERSUCHE BEI DEM HAUSSCHWAMM SERPULA LACRYMANS

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SUMMARY: The dry rot fungus *Serpula lacrymans* is the most important indoor decay fungus in North and Central Europe. Comparatively little information exists on its genetics. The fungus is tetrapolar. Dikaryons have clamp connections, monokaryons produce oidia. Differences in the tolerance of its dikaryons and monokaryons to wood preservatives have not been investigated.

Twelve out of 20 isolates from different geographical locations and different year of isolation fruited under laboratory conditions. Monokaryotic cultures were grown from basidiospores of two isolates. Their mating types were determined by pairing them on agar. Each of four tester strains were selected from the two parental strains, and inter-stock matings were made to produce dikaryons.

The various strains were investigated for their sensitivity to toxicants. The mycelia of most of the monokaryons showed a greater tolerance to borax and a chromium-copper-boron salt than the parental strains.

ZUSAMMENFASSUNG: Der Hausschwamm *Serpula lacrymans* ist in Nord- und Zentraleuropa der wichtigste holzzerstörende Pilz in Gebäuden. Vergleichsweise wenige Befunde liegen zu seiner Genetik vor, wie sein tetrapolar heterothallisches Verhalten, das Vorkommen von Schnallen bei Dikaryonten und von Oidien bei Monokaryonten. Untersuchungen über unterschiedliche Reaktionen von Di- und Monokaryonten

auf Holzschutzmittel sind nicht bekannt geworden.

Von 20 untersuchten Stämmen des Pilzes von verschiedener geographischer Herkunft und verschiedenem Isolierungsalter fruktifizierten zwölf unter Laborbedingungen. Von zwei Stämmen wurden über Basidiosporen Monokaryonten gezogen, ihr Kreuzungstyp durch Paaren auf Agar bestimmt und die je vier Testerstämme im 'inter-stock mating' zu Dikaryonten gekreuzt.

Das physiologische Verhalten der verschiedenen Stämme wurde anhand ihrer Reaktion auf Hemmstoffe überprüft. Die meisten Monokaryonten zeigten eine größere Toleranz gegenüber Borax und einem Chrom-Kupfer-Bor-Salz als die Ausgangsstämme.

I. INTRODUCTION

The dry rot fungus *Serpula lacrymans* (Wulf.: Fr.) S.F. Gray is the most important destroyer of structural wood indoors in northern and central Europe. A great amount of information on this fungus has been published in monographs (FALCK, 1912; SEGMÜLLER & WÄLCHLI, 1981) and in a recent comprehensive literature review (SEEHANN & HEGARTY, 1988).

The fructification of various strains in the laboratory has been reported recently (CYMOREK & HEGARTY, 1986a; HEGARTY & SEEHANN, 1987; HEGARTY & al., 1987). Comparatively little is known about strain variation in this species (CYMOREK & HEGARTY, 1986b; SEEHANN & V.RIEBESELL, 1988) or its formal genetics. SCHMIDT & KEBERNIK (1989) demonstrated a pronounced homogeneity between 20 isolates regarding the gel-electrophoretic patterns of their mycelial proteins. This uniformity may be used for identification of the fungus and for its separation from other species of the genus *Serpula*. THEDEN & SCHULZE (1942), GERSONDE (1958), ABOU HEILAH & HUTCHINSON (1977) and CYMOREK & HEGARTY (1986b) found considerable differences between various isolates of *S. lacrymans* in the ability to decay wood. ELLIOTT & al. (1979) compared the wood decay capacity of the monokaryotic and dikaryotic progeny of three parental strains, but only performed intra-stock matings.

In the following account, mating between two strains, chosen from 20 isolates of *S. lacrymans*, is reported. Wood infection can occur with dikaryotic mycelium and by spores. In the tetrapolar species *S. lacrymans* (SEGMÜLLER & WÄLCHLI, 1981) compatible monokaryons of four mating types per fungal strain may combine to form dikaryons. Monokaryons and dikaryons may differ in their sensitivity to

toxicants. DA COSTA & KERRUISH (1965) recorded the monokaryons of *Lenzites (Gloeophyllum) trabea* (Pers.) Fr. and of *Poria vaillantii* (DC. ex Fr.) Cke. in general to be more tolerant towards a chromium-copper-arsenic preservative than the parental dikaryotic strains. In contrast, a comprehensive investigation by OSBORNE & DA COSTA (1970) with *Polyporus palustris* Berk. et Curt., including its monokaryons and the resulting dikaryons up to the third generation, showed a greater tolerance for copper in the dikaryons.

In the present work, two parental strains, both their monokaryotic tester strains and the resulting inter-stock dikaryons were screened regarding their sensitivity towards borax and a chromium-copper-boron salt.

II. MATERIALS AND METHODS

1. Strains: The 20 strains of *Serpula lacrymans* (Wulf.: Fr.) S. F. Gray investigated are listed in table 1. They were kept as stock cultures on malt agar slants at 4°C.

2. Fruiting on agar: For fruitbody development, fungi were inoculated on the partly modified 'fruiting medium' of CYMOREK & HEGARTY (1986a): 7.5 g wheat flour, 7.5 g malt extract, 5 g K_3PO_4, 3 g glutamic acid, 50 µg thiamine HCl, 20 g agar per litre demineralized water (pH 5.8). To stimulate fruitbody formation, petri dishes were placed in a glass tank closed by Parafilm and were kept for 3 months (March to May) under natural temperature variation (2 to 20°C) in a wooden shed, followed by 4 weeks of culture at 20°C.

3. Isolation of spores and germination: From the fruitbodies of *S. lacrymans* strain nos. 5 (BAM 133) and 11 (BF 025) basidiospores were collected under sterile conditions with a moistened inoculation needle and were put into sterile tap water. Sets of 0.1 ml spore suspension were plated on malt agar with 0.1% citric acid sterilized separately (pH 4) and incubated at 20°C. To separate dikaryons on the germination plates (somatogamy of neighbouring germlings), the mycelia chosen for subculturing were previously examined microscopically to confirm the monosporous origin.

4. Monokaryons: Fifteen monokaryons were labelled as M 1, M 2 etc. in chronological order of isolation. They were microscopically examined (phase contrast, 788x) to confirm the presence of one nucleus per cell, occurrence of oidia and absence of clamp connections.

5. Mating type and breeding: Determination of mating type and

breeding followed the procedures of RAPER & MILES (1958) and EGER (1978). Monokaryons were paired on malt agar in all 105 possible combinations. To corroborate the results, reciprocal crosses were made with essentially the same result. After 6 weeks of incubation, subcultures from the contact zone of the mycelia were made. The remaining plates were used for microscopical detection of dikaryotization (true clamp connections). Misleading results may be obtained because of false clamp connections (no fusion of the hook cell with the subterminal cell) in the contact zone by mating of monokaryons with common B factor and by heterokaryons of the common A type (no clamp connection, nuclear content per cell ranges from none to many). From the dikaryons obtained, the mating type of the monokaryon from strain no. 5 with the smallest number (M 1) was labelled as A_1B_1 and the compatible monokaryon M 7 to be A_2B_2. A_1B_2 and A_2B_1 monokaryons were detected according to EGER (1978) by the development of heterokaryons with the A_1B_1 and A_2B_2 monokaryons yielding - in a further pairing - dikaryons from the heterokaryons and the A_2B_1 and A_1B_2 monokaryons. The mating types of the monokaryons from the strain no. 11 were determined from crosses with strain no. 5. Each monokaryon with the smallest number was used as tester strain, and tester strains were inter-stock mated to produce dikaryons.

6. Toxicants: To investigate the effect of toxicants on mycelial growth of di- and monokaryons of *S. lacrymans*, borax ($Na_2B_4O_7$ 10 H_2O) and a commercial CCB (chromium-copper-boron) wood preservative (Impralit Weyl) were used in 2% malt agar. Agar and boron compounds were separately autoclaved. The concentration increments were 0.06, 0.08, 0.10, 0.12, 0.14, 0.16, 0.18% borax and 0.05, 0.07, 0.09, 0.11, 0.13, 0.15% CCB. Three replicate plates per concentration were inoculated with the 19 strains (see table 2) by placing 5 mm diameter mycelial plugs at the edge of the dish. Plates were incubated at 20°C for 8 weeks, and growth was recorded every 2 to 3 days as mm radial mycelial increment (see table 2).

III. RESULTS AND DISCUSSION

1. Fruiting. The 20 strains of *Serpula lacrymans* investigated are listed in table 1 together with their coding and, when known, with their origin and year of isolation. Twelve strains fruited in the laboratory on agar after they had received a cold shock in a wooden shed under natural temperature variation (see HEGARTY & SEEHANN,

1987). With few exceptions, these strains correspond to the strains of CYMOREK & HEGARTY (1986a) fruiting under similar conditions. Unlike HEGARTY & SEEHANN (1987), an artificial ventilation of the cultures was apparently not necessary for the development of fruitbodies, although most sporophores occurred between lid and basal part at the edge of the petri dishes. It appears remarkable that very old isolates fruited, such as strain 4 from 1935, and, furthermore, that culture nos. 7 and 8 from the same strain BAM 238 differed regarding fruiting ability.

2. Spore germination, monokaryons, mating type and breeding. From two strains of *S. lacrymans*, which differed in geographical origin and year of isolation (no. 5: Berlin 1937, no. 11: Krefeld 1984), basidiospores were plated on agar. Germination - yielding between 1 and 10 germlings per plate - and subsequent growth to macroscopically visible mycelia took up to 3.5 weeks. HEGARTY & al. (1987) reported seven to ten days for germination using two other strains of the fungus.

S. lacrymans is tetrapolar (SEGMÜLLER & WÄLCHLI, 1981). Seven monokaryons (M 1 - M 7) from strain no. 5 and eight monokaryons (M 8 - M 15) from no. 11 were mated. Fifty dikaryons were obtained. From matings and by additional pairings for detection of A_1B_2 and A_2B_1 types via heterokaryons (EGER, 1978), the monokaryons were grouped into seven mating types. One type (A_2B_1) was represented by two monokaryons (M 3 and M 11) originating from different parental strains. False clamps were hardly seen, and barrage formation was not distinct.

To increase outbreeding, crosses were only performed as interstock mating between tester strains deriving from the different parental stocks. The strains are listed in table 2.

All dikaryons showed true clamp connections and never produced oidia. In contrast, all monokaryons of *S. lacrymans* produced abundant oidia. This was already reported by FALCK (1912) and NOBLES (1948). Conidia were not produced (see SEGMÜLLER & WÄLCHLI, 1981). When pairing compatible monokaryons, the outgrowing dikaryotic mycelia often looked like bow-ties as seen in *Stereum hirsutum* (Willd.: Fr.) Pers. (RAYNER & BODDY, 1988). Generally, the subcultured dikaryons grew faster and with thinner mycelium than the monokaryons, similarly to *Coprinus cinereus* (Schaeff.: Fr.) S.F. Gray (CASSELTON, 1978) and to *Lentinus (Lentinula) edodes* (Berk.) Sing. (SCHMIDT & KEBERNIK, 1987).

Because dikaryons of *S. lacrymans* may revert to monokaryons at

high temperature (LOMBARD, pers. comm.), the temperature during cultivation never exceeded 20°C.

3. Toxicant tolerance in monokaryotic and dikaryotic strains. Monokaryotic and dikaryotic strains of wood decay fungi were shown to behave differently regarding their tolerance to wood preservatives (DA COSTA & KERRUISH, 1965; OSBORNE & DA COSTA, 1970). To study the sensitivity of monokaryotic and dikaryotic strains of *S. lacrymans*, borax and a chromium-copper-boron preservative were used.

The toxic limit of borax against one strain of *S. lacrymans* is reported by FINDLAY (1953), showing a decay inhibition of Scots pine sapwood samples by treatment with a preparation containing 0.25% of borax. Lower concentrations have never been investigated (see BAVENDAMM, 1958; BECKER, 1959). Regarding the growth inhibition by CCB on agar, SCHMIDT (1977) and LIESE & SCHMIDT (1976) recorded 0.2% CCB as the limiting concentration for the strain no. 4 of *S. lacrymans* (Ebw. 1, table 1).

Table 2 shows the mycelial increment of the 19 *S. lacrymans* strains after eight weeks of cultivation on agar containing increasing borax levels. The means of three replicate cultures are given. At the lowest concentration of 0.08% of borax most strains reached the possible maximum of 80 mm of radial growth. Minimum and maximum readings are included, since variation among replicate cultures at sub-inhibitive concentrations of a toxicant usually increases (WILLEITNER, 1975; WILLEITNER & al., 1977). For example, two cultures of the monokaryon M 9 did not grow at 0.14% of borax and the third one yielded 29 mm growth.

The column in table 2 with the growth inhibiting concentrations of borax shows the two parental strains of *S. lacrymans* to be more sensitive against borax than seven out of eight derived monokaryons. Both parental strains reacted similarly with total growth inhibition by 0.10 to 0.12% of borax. In the monokaryons, growth was inhibited by 0.12 to 0.16% of borax. The inter-stock mating of the monokaryons from the two parental strains to dikaryons yielded for one strain (7 x 9) a greater borax tolerance (0.14% inhibiting concentration) than for the parental strains. Nevertheless, the exceptional values of the monokaryons M 5 and M 9 with 0.16% were not reached.

Single readings with respect to growth at increasing CCB levels are omitted, since growth again continuously decreased. The last column in table 2 shows that the parental strains of *S. lacrymans* were inhibited by 0.11% CCB and the monokaryons by 0.13 to 0.15% CCB.

Seven of the mated strains showed a greater tolerance to CCB than the parental strains. But a repetition of the experiment after six months gave similar results for both collectives. The reason for this is not clear.

Among 138 dikaryons which were mated from 40 monokaryons of three *S. lacrymans* strains, 79 dikaryons were less effective in wood decay than either of the component monokaryons, 46 strains behaved intermediately, and 13 dikaryons produced more weight loss than either of the component monokaryons (ELLIOTT & al., 1979).

All dikaryons of *S. lacrymans* were microscopically examined after cultivation to ascertain the occurrence of clamp connections because dikaryons may revert to the monokaryotic condition by chemical agents. This effect was shown with arsenate and *Lenzites trabea* (KERRUISH & DA COSTA, 1963; AMBURGEY, 1967) and *Trametes lilacinogilva* (Berk.) Lloyd (OSBORNE, 1970). The investigation revealed that the cultures of *S. lacrymans* on the sub-inhibitive concentrations of borax and CCB were still dikaryotic.

In the case of a greater tolerance of dikaryons, OSBORNE & DA COSTA (1970) discuss that the presence of more nuclei may give a fungus a greater potential output of RNA and enzymes and hence more toxicant is required to inactivate them. The reason for the greater tolerance of monokaryons, as found by DA COSTA & KERRUISH (1965) and in the above experiments, is not clear. This tolerance may enable monokaryotic mycelium to leave a poisoned substratum and, after mating with a compatible mycelium, to continue the life cycle by fruitbody development and spore-dispersal. It may therefore be significant in understanding the interaction between wood preservatives and *S. lacrymans*. Consequently, decay tests with chemically treated wood samples in Kolle flasks with the 19 strains are in progress (SCHMIDT & MORETH-KEBERNIK, 1989).

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Table 1: Strains of *Serpula lacrymans* investigated.
The original coding, year of isolation, source from culture collection and fruiting ability on agar are given.

Strain	Original coding	Isolation	Source	Fruiting
1	FPRL 12c	1949	Princess Risborough Laboratory, England	+
2	L.U.5(A-169)		Liverpool University	-
3	L.U.6	1971	Liverpool University	-
4	Ebw.1	1935	BAM, Berlin	+
5	BAM 133	1937	BAM, Berlin	+
6	570C (BAM 133)	1937	CTBA, Paris	+
7	BAM 238	1939	BAM, Berlin	+
8	BAM 238	1939	BFH, Hamburg	-
9	BAM 261	1940	BAM, Berlin	+
10	BF 022	1984	Dr. Hegarty	+
11	BF 025	1984	Dr. Hegarty	+
12	BF 026	1984	Dr. Hegarty	+
13	BF 028	1984	Dr. Hegarty	-
14	570E (BAM 239)		CTBA, Paris	+
15	HFP 780 I	1978	For. Prod. Res. Inst., Hokkaido	-
16	HFP 780 II	1978	For. Prod. Res. Inst., Hokkaido	-
17	A 186	1975	Princess Risborough Laboratory, England	-
18	FP 90876-R	1946	For. Prod. Lab., Madison	-
19	Warsaw III		CSIRO, Melbourne	+
20	DFP 16521	1981	CSIRO, Melbourne	+

Table 2: Strains of *Serpula lacrymans* involved in the breeding and growth inhibition of the mycelia by borax and a chromium-copper-boron (CCB) salt on agar.
The 2 parental strains used, their monokaryons with the mating type, the dikaryons, the mycelial increment within 8 weeks of cultivation and the growth inhibiting concentrations by borax and CCB are shown. The tester strains used for mating and toxicant studies are underlined. Mycelial plug radii which had only grown by 5 mm or less were regarded as inhibited. No growth occurred at 0.16% of borax.

Strain	Mating type	Mycelial increment (mm) at % borax								Growth inhibit. conc. (%)	
		0.08		0.10		0.12		0.14			
		mean	min-max	mean	min-max	mean	min-max	mean	min-max	Borax	CCB
Parental strain 5 (BAM 133)		80		0		0		0		0.10	0.11
Monokaryon <u>M1</u>, M2, M4	A_1B_1	80		65	60-70	16	0-30	0		0.14	0.13
<u>M5</u>, M6	A_1B_2	80		54	50-56	23	20-25	3	0-10	0.16	0.15
<u>M3</u>	A_2B_1	80		57	55-60	9	0-28	0		0.14	0.15
<u>M7</u>	A_2B_2	80		56	53-65	2	0- 5	0		0.12	0.15
Parental strain 11 (BF 025)		80		4	0- 6	0		0		0.12	0.11
Monokaryon <u>M8</u>, M12, M13, M14	A_2B_3	80		62	55-65	6	0-15	0		0.14	0.15
<u>M9</u>, M15	A_3B_3	80		57	52-66	17	12-23	10	0-29	0.16	0.13
<u>M10</u>	A_3B_1	80		49	46-52	14	0-25	0		0.14	0.15
<u>M11</u>	A_2B_1	80		13	0-40	0		0		0.12	0.15
Dikaryon 1 x 8		80		36	7-80	0		0		0.12	0.15
1 x 9		73	60-80	3	0- 7	0		0		0.12	0.11
3 x 9		80		2	0- 3	0		0		0.10	0.13
5 x 8		80		5	3-10	0		0		0.12	0.15
5 x 9		80		0	0- 1	0		0		0.10	0.13
5 x 10		80		5	0- 8	0		0		0.12	0.11
5 x 11		80		15	5-35	0		0		0.12	0.15
7 x 9		77	70-80	9	3-20	5	0-11	0		0.14	0.13
7 x 10		80		12	3-30	0		0		0.12	0.13