Pure culture response of ectomycorrhizal fungi to imposed water stress

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COLEMAN, M. D., BLEDSOE, C. S., and LOPUSHINSKY, W. 1989. Pure culture response of ectomycorrhizal fungi to imposed water stress. Can. J. Bot. 67: 29-39.

The ability of ectomycorrhizal fungal isolates to tolerate imposed water stress in pure culture was examined in 55 isolates of 18 species. Water potential treatments, adjusted with polyethylene glycol, were applied to Petri dish units. These units allowed colony diameter measurements of fungi grown on liquid media. Delayed growth initiation and inhibition of growth rate occurred with increasing water stress. For 87% of the isolates, growth rate was inhibited by the initial water potential treatment applied, leaving only seven isolates where growth increased with initial water potential treatments. No growth was evident under the imposed stress treatments for isolates of *Laccaria bicolor, Laccaria laccata*, and *Lactarius controversus;* growth occurred only in the control. Drought tolerant species, demonstrated by an ability to grow at a water potential of -3 MPa, included *Boletus edulis, Cenococcum geophilum, Rhizopogon vinicolor,* and five out of eight *Suillus* species. Species intolerant of 1 MPa included *Hebeloma crustuliniforme, Laccaria bicolor, Laccaria laccata,* and *Suillus caerulescens.* Fungal drought tolerance was poorly correlated with estimates of annual precipitation for collection locations. Estimates of drought tolerance seems to depend more on fungal classification than on annual precipitation at the site of collection. Reisolation of *Laccaria bicolor* increased growth rate and water stress tolerance when compared with the same fungus prior to reisolation.

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Les auteurs ont examine, en culture pure, 5.5 isolats de champignons ectomycorhiziens appartenant à 18 espèces, la capacité à tolérer un stress hydrique impose. Les traitements faisant appel au polyethylene glycol ont été appliques en Petri. Cette méthode permet de mesurer la croissance en diamètre de colonies de champignon croissant en milieu liquide. Avec une augmentation du stress hydrique, on observe un retard dans le debut de la croissance ainsi qu'une diminution du taux de croissance. Chez 87% des isolats, il y a une inhibition du taux de croissance avec le potentiel hydrique initial, ce qui ne laisse que 7 isolats où ce même traitement conduit à une augmentation de croissance. Avec les isolats de *Laccaria bicolor, Laccaria laccatu* et *Lactarius controversus* aucune croissance ne peut être observée en presence du stress hydrique impose; il n'y a de croissance que chez les témoins. On retrouve une tolerance à la sécheresse, telle que démontrée par la capacité à croitre en presence d'un potentiel hydrique de -3 MPa, chez *Boletus edulis, Cenococcum geophilum, Rhizopogon vinicolor* et chez cinq espèces de *Suillus* sur huit. Les espèces incapables de tolérer − 1 MPa incluent *Hebeloma crustuliniforme, Laccariu bicolor, Laccuria laccata* et *Suillus caerulescens.* La correlation est pauvre, entre la resistance à la sécheresse des isolats fongiques et les precipitations annuelles estimées pour les sites de récolte. Les tolerances à la sécheresse estimées semblent plus fortement corrélées avec l'appartenance systématique du champignon qu'avec la precipitation annuelle moyenne au site de collection. Le réisolement du *Laccaria bicolor* augmente son taux de croissance et sa tolerance à la sécheresse comparativcment au même champignon avant le réisolement.

[Traduit par la revue]

Introduction

Douglas-fir *(Pseudotsuga menziesii* (Mirb.) Franco) may have as many as 1000 different ectomycorrhizal fungal associates (Trappe 1977). Such a large number of fungal species is suspected to represent a broad range of physiological diversity. Although many fungi are known to fruit in association with Douglas fir, very little is known about which fungus is appropriate for any one set of environmental conditions. In this paper one functional aspect of ectomycorrhizal fungi is investigated: the ability of fungi to tolerate water stress. Since relatively few ectomycorrhizal fungi have been examined for water-stress tolerance in pure culture (Cline 1980; Mexal and Reid 1973), the range of physiological response to water stress by these fungi remains unknown. In this study 55 isolates of ectomycorrhizal fungi have been examined for their ability to grow in pure culture with imposed water stress. Such information may be useful in selecting specific fungi for inoculation of seedlings destined for further physiological tests and ultimately for outplanting to dry sites.

Polyethylene glycol (PEG) was used as a solute to adjust media water potential, avoiding problems associated with metabolic utilization of sugars or toxicity and altered tissue water balance due to use of salts (Mexal and Reid 1973). The components of water potential adjusted by PEG include not only osmotic potential, but also, as in soils, matrix potential (Steuter et al. 1981). Therefore, the behavior of PEG is similar to that of soils, in comparison to other methods of adjusting water potential such as sugar or salt. According to Mexal et al. (1975) PEG can create anoxic conditions. In this system, however, fungi were grown on the surface of a liquid medium where sufficient oxygen was presumably available.

Materials and methods

Culture collection

Fungal isolates were obtained from fruit bodies or ectomycorrhizai roots using the methods of Molina and Palmer (1982). Additional cultures were procured from other collections. The site characteristics of collection locations, including annual precipitation, elevation, and ectomycorrhizal host species, for each isolate are listed in Table 1. The location of voucher specimens and cultures is also listed. Mean annual precipitation for these collection locations were obtained from

Printed in Canada, Imprimé au Canada

TABLE 1. Fungal isolates examined for water-stress tolerance and their collection site characteristics

Species and code	Collection location	Voucher location	Culture location	Annual precipitation (cm)	Elevation (m)	Host tree species
Boletus edulis Bull.: Fr.						
BOED 1	Peavine Cr., Chelan Co.. WA		UW	101	750	PSME, TSHE
Cenococcum geophilum Fr.						
CEGE1 (M347)	Georgia, via C.P.P. Reid		UW			
CEGE2 (A 177)	Wickersham Dome, AL		UW	33		BENA
CEGE3 (A181)	Big Lake, Santiam Pass, OR		UW	242		TSME, ABLA
CEGE4 (A 175)	Chugach, AL	OSC	FSL	980		TSME
CEGE5 (A 145)	H. J. Andrews Forest. Blue River, OR	OSC	FSL	161		PSME
CEGE6	Oklahoma Gulch Pass, Chelan Co., WA		u w	28	900	PIPO, PSME
CEGE7	Mosquito Ridge, Chelan Co., WA		u w	62	1250	PSME. PIPO, ABGR
CEGE8 (A 149)	Tieton Rv., Yakima Co., WA	OSC	FSL	20		PSME
CEGE9 (349)	Rim Drive, Crater Lake, OR	OSC	FSL	198	2710	PSME
Hebelornu aff. *crustuliniforme* (Bull. ex St. Amans) Quel.						
HECR2	Bald Mt., OR		u w	6.5	1890	ABCO, PICO
HECR4	Upper Devil's Club Cr., Chelan Co., WA		u w	101	1250	TSME, PSME
HECR5 (9035)	Lk. Stuart, Chelan Co., WA	WTU	u w	62	1700	Mixed Conifer
HECR6 (MC1 1884-l)	University of Washington, Seattle, WA	WTU	u w	91	30	FASY
HECR7 (7650)	Odell Lk., Klamath Co., OR	o s c	FSL	52	1440	PIMO, TSHE
HECR8 (S 166)	Woods Cr. Rd., Benton Co., OR	o s c	FSL	101	490	PSME
HECR9 (S260)	Starkey Exp. Forest, Union Co., OR	o s c	FSL	46	1440	PICO. PSME
Laccaria bicoior (Maire) Orton						
LAB11 (LIT813)	Sylvan Spawn laboratory, Inc.,					
LAB12	Upper Devil's Club Cr., Chelan Co., WA		u w	101	1250	TSME, PSME
LABI2R (MC 102486- 1)	Reisolate of LAB12	WTU	u w			
L. laccata (Scop.: Fr.) Berk. & Br.						
LALA 1	Bald Mt.. OR		u w	65	1890	ABCO, PICO
LALA4	Upper Devil's Club Cr., Chelan Co., WA		u w	101	1250	TSME, PSME
LALA5	Upper Rainy Cr., Chelan Co., WA		u w	101	1150	TSME, ABAM
LALA6 (238)	Crater Lake. OR	o s c	FSL	198		TSME
Lactarius controversus (Pers.: Fr.) Fr.						
LCCO1	Peavine Cr., Chelan Co., WA		u w	101	750	PSME, TSHE
L. rufus (Scop.: Fr.) Fr.						
LCRU 1	Upper Devil's Club Cr., Chelan Co., WA		u w	101	1250	TSME, PSME
Leccinum aff. *aurantiacum* (Bull. ex St.-Amans) S. F. Gray						
LEAU 1	Upper Devil's Club Cr., Chelan Co., WA		u w	101	1250	TSME, PSME
Rhizopogon vinicolor Smith						
RHVI1 (7412)	Lost Prairie campground, Linn Co., OR	o s c	FSL	242		TSME. PIEN
RHV12 (7889)	Lower Quartz Cr., Merlin, OR	o s c	FSL	82		QUKE, PSME
RHV13 (6861)	Woods Cr., Benton Co., OR	o s c	FSL	101		PSME
RHVI5 (7892)	Quartz Cr., Josephine Co., OR	o s c	FSL	82		PSME
Suillus albidipes (Pk.) Singer						
SUAB1 (MC102384-3)	Winesap, WA	WTU	u w	22	400	PIP0

TABLE 1 (concluded)

Species and code	Collection location	Voucher location	Culture location	Annual precipitation (cm)	Elevation (m)	Host tree species
S. brevipes (Pk.) Kuntze						
SUBR3 (7598)	Ode11 Lake, Klamath Co., OR	OSC	FSL	52	1520	PICO
SUBR4 (767 1)	Elk Lake, Deschutes Co., OR	OSC	FSL	52	1520	PICO
S. caerulescens Smith and Thiers						
SUCR2 (MC62084-1)	Edmonds, WA	WTU	u w	90	90	PSME
S. granulatus (L.: Fr.) Kuntze						
SUGR1	Stevens Pass, WA		u w	197	1070	PICO
SUGR2 (7589)	Union Cr. For. Camp, Jackson Co., OR	o s c	FSL	107	1010	PIP0
SUGR3 (MC102384-2)	Hay Canyon, Cashmere, WA	WTU	u w	22	350	PIP0
SUGR4 (MC102384-1)	Hay Canyon, Cashmere, WA	WTU	u w	22	350	PIP0
S. lakei (Murr.) Smith and Thiers						
SULA I	Peavine Cr., Chelan Co., WA		u w	101	750	PSME. TSHE
SULA2	Upper Rainy Cr., Chelan Co.. WA		u w	101	1150	TSME, ABAM
SULA3	Upper Devil's Club Cr., Chelan Co., WA		u w	101	1250	TSME. PSME
SULA4	University of Washington, Seattle, WA		u w	91	30	
SULA5 (MC62 184-5)	Oklahoma Gulch Pass, Chelan Co., WA	WTU	u w	28	900	PSME, PIPO
S. luteus (L.: Fr.) S. F. Gray						
SULU1	Bald Mt., OR		u w	65	1890	ABCO, PICO
SULU2	Upper Rainy Cr., Chelan Co., WA		u w	101	1150	TSME, ABAM
SULU3	Bald Mt., OR		u w	65	1890	ABCO, PICO
SULU4	Upper Devil's Club Cr., Chelan Co.. WA		u w	101	1250	TSME, PSME
SULU5	Upper Rainy Cr., Chelan Co., WA		u w	101	1150	TSME, ABAM
SULU6 (MC22984-1)	University of Washington, Seattle, WA	WTU	u w	91	30	PIMO
S. ponderosus Smith & Thiers						
SUP02 (MC10185-1)	Swak Pass campground, Kittitas Co., WA	WTU	u w	56	1000	PSME, PIPO
S. sibiricus (Sing.) Sing.						
SUSI1	Balt Mt., OR		u w	65	1890	ABCO, PICO
SUSI2	Balt Mt., OR		u w	65	1890	ABCO, PICO
Tricholoma focale (Fr.) Ricken						
TRFO I	Upper Rainy Cr., Chelan Co., WA		u w	101	1150	TSME, ABAM
TRF02	Upper Devil's Club Cr., Chelan Co., WA		u w	101	1250	TSME, PSME
Root Isolates						
No. 2, 5, 10, 12, 14, 15, 18	Buck Mt.. Douglas Co., OR		HCRL	131	480	PSME
No. 20, 21, 25	O'Shea Cr., Douglas Co., OR		HCRL	93	480	PSME. ABGR
No. 31, 35	Upper Grassy Cr., Douglas Co., OR		HCRL	171	380	PSME

NOTE: Isolates are grouped by species. Numbers in parentheses following the isolate code are the original isolate number. The *Index Herbariorum* abbreviation is listed under voucher location as OSC, Oregon State University; WTU, Univenity of Washington. Cultures are maintained at UW, College of Forest Resources, University of Washington, Seattle. WA: FSL. Forestry Sciences Laboratory, Corvallis, OR: or HCRL, Horticultural Crops Research Laboratory, Corvallis, OR. Abbreviations for ectomycorrhizal host tree species are made up of the first two letters of the genus and species names; ABAM, *Abies amabilis* (Dougl.) Forbes; ABCO, *Abies concolor* (Gord. and Glend.) Lindl.; ABGR, *Abies Grandis* (Dougl.) Lindl.; ABLA, *Abies lasiocarpa* (Hook.) Nutt.: ARME. *Arbutus menziesii* Pursh: BENA, *Betula nana* L.; COCO, *Corylus cornuta* var. *californica* (A. DC.) Sharp; FASY. *Fagus sylvatica* L.; PIEN, *Picea engelmannii* Parry; PICO, *Pinus contorta* Dougl.; PILA. *Pinus lambertiana* Dougl.: PIMO, *Pinus monticola* Dougl.; PIPO, *Pinus ponderosa* Laws.; PSME, *Pseudotsuga menziesii* (Mirb.) Franco; QUCH. *Quercus chrysolepis* Liebm.: QUKE, *Quercus kelloggii* Newb.; TSHE, *Tsuga heterophyla* (Raf.) Sarg.; TSME, *Tsuga mertensiana* (Bong.) Carr.

the U.S. National Climatic Center's Annual Summary of Climatological Data. The precipitation for the nearest weather station was used as an estimate of precipitation for each collection location.

Isolates of common origin were compared for their response to imposed water stress. Two *Suillus granulatus* isolates (SUGR3 and SUGR4) were collected at the same location from separate sporocarps 3 m apart. In a reisolation experiment, an isolate from a *Laccaria bicolor* sporocarp growing with a containerized seedling inoculated with LABI2 was obtained and assigned number LABI2R.

Culture maintenance

Isolates were maintained in culture plates on modified Melin–Norkran's agar media, pH 6.3 (MMN; Marx 1969). These cultures were grown at 20–22°C and used for screening tests when in an actively growing condition. Uniform inoculum plugs were taken from the colony edge.

Preparation of polyethylene glycol solutions

Polyethylene glycol (PEG-3350, Carbowax, Union Carbide Corp.) was used to adjust water potential in MMN solutions. A range of water potentials was prepared using increasing amounts of PEG in MMN media. These solutions were measured psychrometrically using a Wescor, Inc. (Logan, Utah) C-51 sample chamber and a S-B Systems (Manhattan, Kansas) model 500-B volt meter. Water potential as a function of PEG concentration was used to determine the proper concentration. To prepare solutions, PEG was added to double strength MMN and brought to single strength volume with distilled water. Thus nutrient concentrations were constant between treatments.

Two water potential treatment series were used, mesic and xeric. Water potential levels in the mesic series included -0.16 (water potential of MMN without PEG), -0.36, -0.56, -0.76 and -0.96 MPa and those in the xeric series included -0.16, -0.86, -1.56, -2.26, and -2.96 MPa. Fungi found incapable of tolerating stress levels in the xeric series were then tested in the mesic series. The media pH averaged 6.5 ± 0.2. For convenience, water potential values were rounded to the nearest 0.1 MPa (e.g., untreated MMN: -0.2). The -0.2 MPa treatment level is referred to below as the control treatment level.

Experimental system

Polyethylene glycol cannot be incorporated into agar because the components separate, reducing solidification of the agar with increasing PEG concentrations. Therefore, PEG can only be used in liquid culture. Colonies were suspended on the surface of the liquid for two reasons: (i) Submerged cultures must be destructively sampled for dry weight determination, demanding many replicates to define the growth curve. Diameter growth of surface cultures, on the other hand, can be measured repeatedly from a single colony. (ii) Oxygen can be limiting in PEG solutions due to its viscous nature (Mexal et al. 1975). Growing colonies at the surface avoids problems of oxygen diffusion in PEG solutions.

A glass bead Petri dish (60 mm) unit, originally described by Cline (1980), was used to grow fungi under different levels of water stress. The unit consisted of a 7-mm diameter agar inoculum plug placed on a nylon mesh disk that was supported at the liquid medium surface by glass beads. To remove impurities that inhibit fungal growth the nylon mesh was washed in detergent, soaked in 20% acetic acid solution, rinsed thoroughly in distilled water, and oven dried. Each nylon disk was preweighed and fungal dry weight was obtained by difference. After inoculation, the Petri dish unit was sealed with Parafilm and maintained in the dark at 22°C for 6–10 weeks. Each isolate in each treatment was replicated four times.

Growth determination

Colony diameters were measured weekly to the nearest millimetre. The average of two diameter measurements along perpendicular axes was used to estimate colony size at weekly intervals. Diameter measurements were collected until growth in the control ceased; then fungal dry weight was measured. Maximum growth rates, i.e., the average maximum change in diameter between any two successive measurements, divided by the number of days between measurements, were used for comparison between treatments and fungi. Further examination of the growth curve also allowed evaluation of the effect of water stess of the initiation of growth.

Results

Polyethylene glycol und water potential

Solution water potential increased with increasing PEG concentrations in a logarithmic manner. The regression equation generated from our data was:

$$Y = 10.29 \ln X + 19.05 \quad (r^2 = 0.997)$$

where Y is molar concentration of PEG and X is water potential (MPa). This equation was used to calculate PEG concentrations for each of the desired water potentials. These psychrometrically measured water potential values were very similar to published data (Lawlor 1970; Mexal and Reid 1973).

Growth as a function of time

Growth curves at different water-stress treatments for *Suillus luteus* (SULU1) and *Cenococcum geophilum* (CEGE4) are plotted in Fig. 1. Figure 1A shows a pattern typical of most fungal isolates tested under xeric conditions and Fig. 1B a less common pattern. The typical growth pattern, illustrated by SULU1 (Fig. 1A), exhibits an initial lag phase followed by an exponential growth phase, then a maximum rate phase; finally growth slows and the colony becomes inactive. The growth curve for the control level illustrates only the phases beyond exponential growth; the lag phase is not evident. At increasing water potentials, however, water stress reduces growth and lengthens the lag phase. Thus, under water stress, only the early phases of growth may be evident. For example, the growth curve for the -3 MPa treatment has only lag and exponential phases, since the other phases did not occur during the time period studied.

For most isolates that had an extended lag phase due to water stress (including many *Cenococcum geophilum, Rhizopogon vinicolor,* and *Suillus* isolates), colony diameter in the control treatment was always greater than colony diameter in stress treatments (Fig. 1A). In contrast to the pattern of SULU1 , a less typical growth response is illustrated by CEGE4 (Fig. 1B), an isolate of a species believed to be drought tolerant (Mexal and Reid 1973). Initially colony diameter in the control treatment exceeded that of the stress treatments, but later colony diameter in the -0.9, -1.6, and -2.3 MPa treatments was greater than the control. Only six isolates (CEGE4, CEGE6, CEGE7, CEGE8, SUGR3, and SUGR4) demonstrated this less typical growth response to stress.

The examples in Fig. 1 illustrate the difficulties in selection of a consistent time period for comparison of growth rates at different water potentials. It is important, therefore, to compare a similar phase of growth regardless of the time in which it occurs. Thus, maximum growth rates were chosen for comparison.

Growth rates

Fungal diameter growth rates for the mesic series are presented in Table 2 and for the xeric series in Tables 3 and 4. Several isolates of *Cenococcum, Hebeloma,* and *Laccaria* grew slowly in the control treatment level (< 100 μm day-') while certain isolates of *Suillus* and *Rhizopogon* grew more rapidly (1000 -2000 μm day-'). Some fungi that grew slowly at the control level were not very stress tolerant. Table 2 shows

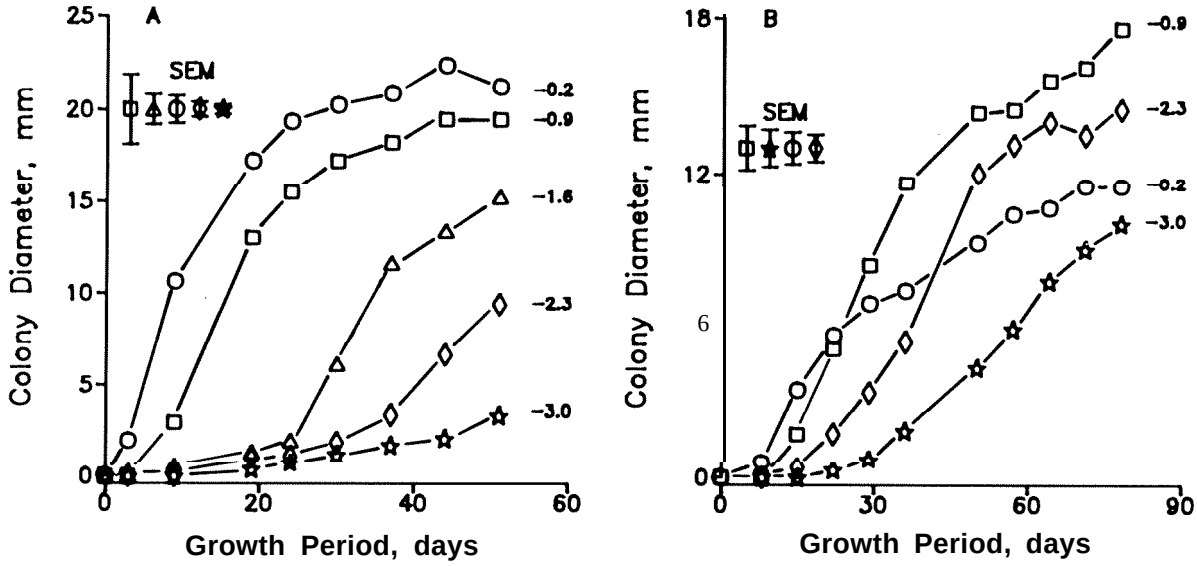

FIG. 1. Growth curves at five different water potentials at 22°C. Water potentials in PEG-amended liquid MMN media were -0.2, -0.9, *-1.6, -2.3,* and *-3.0* MPa. (A) SULU1, *Suillus luteus. (B)* CEGE4, *Cenococcum geophilum* (- 1.6 MPa treatment deleted for clarity). Values are the mean of 4 replicates. Bars indicate standard error of the mean.

TABLE 2. The mesic series showing maximum growth rates and growth response types (see Fig. 2) of 20 different ectomycorrhizal fungi grown on PEG-amended liquid MMN media at 22°C in the mesic series (-0.2 to -1.0 MPa)

Species and code	Type	Growth rate (μm day-') at water potential of: -0.2 MPa	-0.4 MPa	-0.6 MPa	-0.8 MPa	-1.0 MPa
Hebeloma crustuliniforme						
HECR2	II	219±31	141±119	214±29	63±63	71±41
HECR4	III	41±24	31±31	63±40	146±53	63±21
HECRS	III	47±47	161±45	188±36	125±18	107±21
HECR6	II	94±60	54±34	0	36±36	21121
HECR7	II	156±60	161±45	71±29	63±36	63±26
HECR8	II	333±39	222±23	232±34	143±51	161±34
HECR9	II	911±356	89±90	0	0	71±72
Luccaria bicolor						
LAB11	I	143±144	*0*	*0*	*0*	0
LAB12	I	125±61	*0*	*0*	*0*	0
LABI2R	II	464±133	107±36	36±36	*0*	0
L. laccata						
LALA1	II	482±298	72±72	357±66	179±69	0
LALA4	I	71±29	0	0	0	0
LALA5	II	1210±41	89±54	36±36	0	36±36
LALA6	II	375±179	125±85	71±72	0	0
Lactarius controversus						
LCCO1	I	375±80	*0*	*0*	*0*	0
Lactarius rufus						
LCRU 1	II	1160±129	1040±246	607±216	179±179	143±144
Leccinum auruntiacum						
LEAU1	II	554±45	357±101	393136	232±34	232±54
Suillus brevipes						
SUBR3	II	1100±261	438±188	565±185	188±162	188±110
SUBR4	II	1810±252	896±373	604±398	161±74	141±141
S. caerulescens						
SUCR2	II	1170±234	536±285	268±172	54±54	0

NOTE: Standard error of the mean is calculated from four replicate determinations. See Table 1 for isolate descriptions.

TABLE 3. The xeric series showing maximum growth rates and growth response types (see Fig. 2) of 36 different ectomycorrhizal fungi grown on PEG-amended liquid MMN media at 22°C in the xeric series (-0.2 to -3.0 MPa)

Species and code	Type	Growth rate (μm day^{-1}) at water potential of: -0.2 MPa	-0.9 MPa	-1.6 MPa	-2.3 MPa	-3.0 MPa
Boletus edulis						
BOED 1	II	1210±51	714±29	571±88	571±106	375±26
Cenococcum geophilum	II	875±45	232,233	0	0	0
CEGE 1	II	875±45	232±233	0	0	0
CEGE2		232±34	0	0	0	0
CEGE3	II	339±129	232±115	125±126	107±108	18±18
CEGE4	III	411±18	482±34	518±111	473±96	268±90
CEGE5	II	643±276	411174	0	0	0
CEGE6	III	208±100	250±62	482±107	179±36	214±51
CEGE7	III	250±29	375±139	393±69	339±34	286±78
CEGE8	III	475±48	661±126	554±532	0	54±34
CEGE9	II	275±25	36±36	107±108	36±36	0
Rhizopogon vinicolor						
RHVI 1	II	1090±659	271±272	*0*	*0*	0
RHVI2	II	1580±184	854±310	536±85	281±41	47±30
RHV13	II	1960±153	778±94	422±47	250±21	125±42
RHVI5	II	1390±40	569±70	250±145	125±42	78±30
Suillus albidipes						
SUAB 1	II	766±278	313±105	188±71	109±16	125±61
S. granulatus						
SUGR1	II	1790±106	1460±62	1060±87	1040±42	228±35
SUGR2	II	1390±212	768±34	232±54	232±34	125±34
SUGR3	III	500±51	604±40	357±41	393±46	18±18
SUGR4	II	6431128	589±135	375±85	250±21	54±34
S. lakei						
SULA 1	II	1170130	333±76	179±36	125±24	71±29
SULA2	II	1380±300	281±137	234±78	109±16	146±21
SULA3	II	2230±291	396±63	196±54	89±18	146±21
SULA4	II	656±116	438±120	172±30	143±0	125±0
SULA5	II	2340±79	458±121	357±29	146±21	146±40
S. luteus						
SULU1	II	1440±105	1000±224	786±163	464±182	179±62
SULU2	II	488±63	232±54	161±45	100±0	107±36
SULU3	II	1330±488	429±101	250±21	161±54	143±29
SULU4	II	813±120	536±112	125±63	89±34	125±48
SULU5	II	750±85	625±73	607±151	143±29	125±18
SULU6	II	1310±31	550±132	357±0	411±99	196±18
S. ponderosus						
SUP02	II	938±68	604±40	268±18	125±74	0
S. sibiricus						
SUSI1	II	1380±63	446±156	393±85	286±88	179±208
SUSI2	II	1360±53	1280±267	679±272	286±59	54±34
Tricholoma focale						
TRFO 1	II	643±93	5361242	286±155	*0*	0
TRF02	II	339±179	143±144	125±85	18±18	0

NOTE: Standard error of the mean is calculated from four replicate determinations. See Table I for isolate descriptions.

several isolates of *Laccaria* and *Lactarius* that grew slowly and were not capable of growth beyond -0.8 MPa. Tables 3 and 4 show that growth was rapid for many fungi at the control level. This often was associated with an ability to grow at the maximum stress applied (-3 MPa).

Patterns of growth response to water stress

Growth response to imposed water stress showed three different patterns (Fig. 2), referred to as types I, II, and III. In the type I pattern growth occurred only in the control treatment level and no growth occurred with increasing stress. The only isolates with a type I response were CEGE2, LABI1, LABI2, LALA4, and LCCO1. The majority of the isolates (78%) exhibited a type II pattern. In the type II pattern growth rates decreased with increasing stress and the maximum growth rate always occurred in the control. In the type III pattern, maximum growth rate

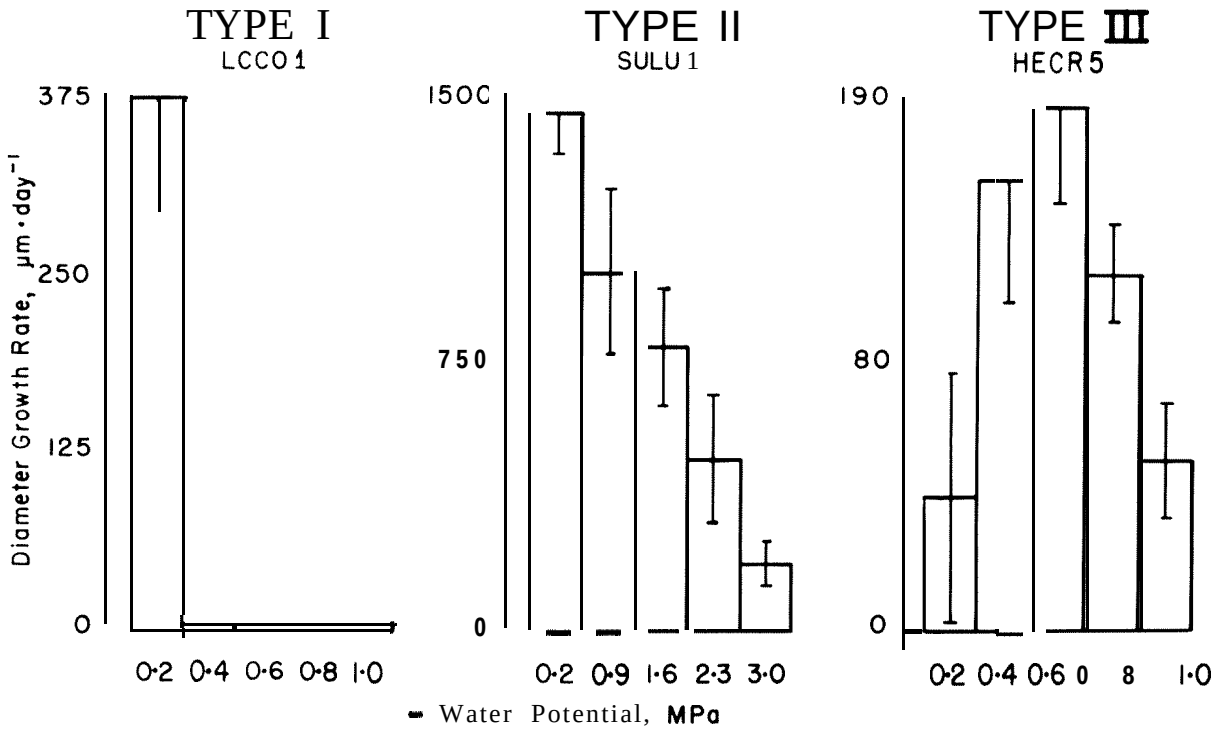

FIG. 2. Typical maximum diameter growth response patterns (22°C) of ectomycorrhizal fungi on liquid MMN media to increasing water stress created with PEG. Type I response is exemplified by LCCO1, Type II by SULU1 and Type III by HECR5. Growth rates are the mean maximum rates observed for four replicates ± standard error of the mean.

TABLE 4. Maximum growth rates and growth response types (see Fig. 2) of 12 different ectomycorrhizal root isolates grown on PEG-amended liquid MMN media at 22°C in the xeric series (-0.2 to -3.0 MPa)

		Growth rate (µm day-') at water potential of:				
Root isolate No.	Type	-0.2 MPa	-0.9 MPa	-1.6 MPa	-2.3 MPa	-3.0 MPa
RHVI-like						
5	II	1580±104	1390±70	656±65	304±61	214±78
10	II	875±92	411±135	393 i-62	268±34	107±62
1.5	III	1050±40	1230±94	547±65	321±208	143±59
18	III	1000±99	1130±256	688±73	339±111	0
20	II	1130±74	1110±70	484± 150	482±222	12.5±74
21	II	1040±46	953± 187	609±116	344±146	161±94
25	II	1210±29	1050± 175	734 ± 107	609±54	482±68
31	II	969±91	891±40	422±65	304±34	0
35	II	1610±150	828 ±277	734±83	469±98	0
Non-RHVI-like						
2	III	750±89	766±83	1070±264	1410±448	719±83
12	III	922± 104	1050±90	922 ± 101	891±144	1200± 157
14	III	547± 104	484±65	500±112	719±179	828±269

NOTE: **Isolates which resemble *Rhizopogon vinicolor* are indicated under RHVI-like; other isolates are under non-RHVI-like. Standard error the mean is calculated from four replicate dctenninations. See** Table 1 for isolate descriptions.

did not occur in the control but at some greater stress level, followed by decreasing growth with further increases in stress. The seven isolates that exhibited this pattern were CEGE4, CEGE6, CEGE7, CEGE8, HECR4, HECR5, and SUGR3. Growth response patterns are listed for each isolate tested in Tables 2, 3, and 4.

Comparisons among isolates of the same species

Isolates of the same species were compared for their abilities to grow under water stress. For some species, isolates within a species were extremely variable in their response to water stress while isolates of other species demonstrated surprising homogeneity. These contrasting responses are illustrated in Fig. 3 for two fungi *(Cenococcum geophilum* and *Rhizopogon vinicolor).* For ease of comparison, growth rates of isolates were expressed as a percentage of the control.

Large variation occurred among selected isolates of *Cenococcum geophilum.* CEGE4 and CEGE1, as discussed previously, had a type III growth pattern in response to increasing water

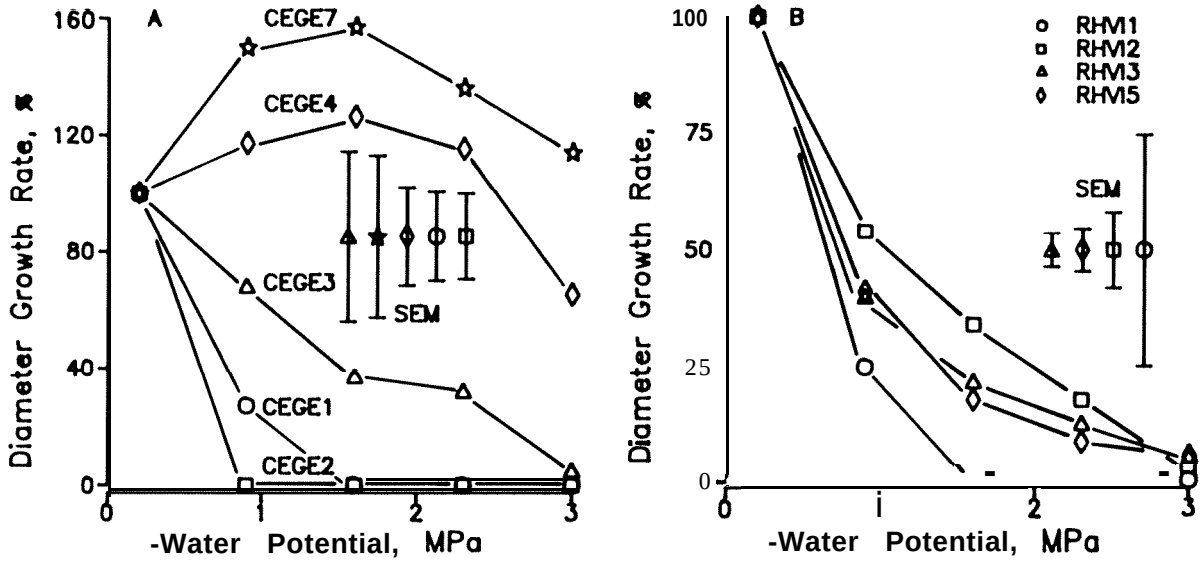

FIG. 3. Comparison of isolates of a single species showing the effect of water stress of maximum diameter growth rate of (A) five select isolates of *Cenococcum geophilum* and (B) four isolates of *Rhizopogon vinicolor*. Growth was at 22°C on liquid MMN media with PEG amendments to impose water stress. Values are expressed as a percent of the control and are the mean of four replicates for each isolate. Bars indicate standard error of the mean.

stress; maximum growth rate occurred at intermediate stress levels. A type II pattern was exhibited by CEGE1 and CEGE3, while CEGE2 showed the type I pattern, where growth occurred only in the control treatment level. In addition to the *Cenococcum geophilum* isolates, growth responses of *Hebeloma crustuliniforme* isolates were extremely variable.

There was less variation among the isolates of *R. vinicolor* (Fig. 3B). All isolates had the predominant type II pattern. *Suillus* isolates also had low variability and a consistent response to stress.

Comparisons among different species

Of all the genera studied, *Suillus* was represented by the greatest number of species. Growth rates for isolates within each species were combined, enabling comparison among species of a single genus (Fig. 4). Three of the species were represented by only one isolate; however, the remaining five species were represented by at least two or as many as six isolates (Table 1).

Although each *Suillus* species demonstrated maximum growth in the control treatment level, the amount of growth inhibition resulting from imposed stress differed among species. Selected *Suillus* species representing the range of stress tolerance are illustrated in Fig. 4. *Suillus caerulescens* (and *S. brevipes,* not shown) did not grow in the xeric series and growth of *S. lakei* was severely inhibited under the stress levels of the xeric series. The moderately tolerant species were *S. albidipes* and *S. ponderosus* (and *S. luteus* and *S. sibericus,* not shown). The most tolerant *Suillus* species was *S. granulatus.*

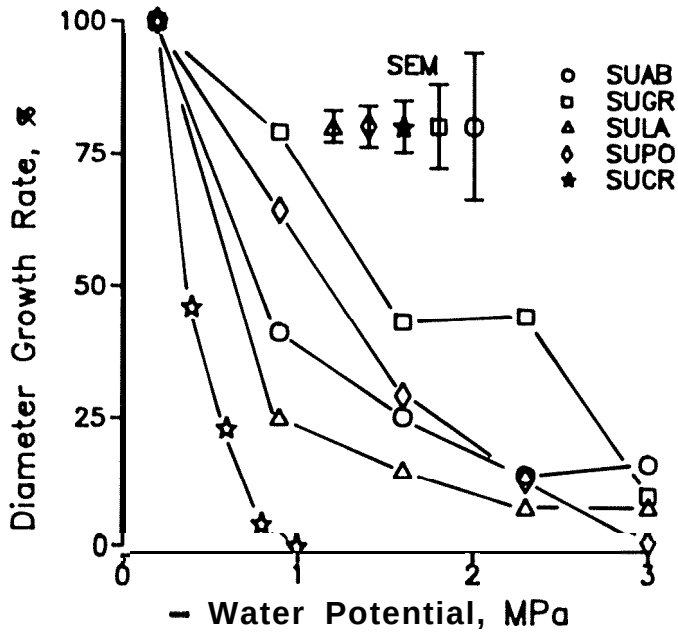

FIG. 4. Effect of water stress on maximum diameter growth of five different *Suillus* species. Growth was at 22°C on liquid MMN media with PEG amendments to impose water stress. Data from several isolates for each species were combined: *S. albidipes* (one); S. *granulatus* (four); S. *lakei (five); S. ponderosus* (one); *S. caerulescens* (one). Values are expressed as a percent of the control and are the mean of isolates replicated four times. Bars indicate standard error of the mean.

Comparisons among different genera

Growth rate data for all species within the nine genera were averaged (Fig. 5). With the wide variation that occurred among isolates of *Cenococcum* and *Hebeloma,* average response does not wholly represent the range of response within the genera. However, average responses of all other genera are representative. The −1.0 MPa value of the abscissa can be used to compare fungi in Fig. 5A and 5B. *Laccaria*, *Lactarius*, and more mesic *Suillus (S. brevipes* and S. *caerulescens*) species were relatively intolerant of water stress and were tested under mesic conditions (Fig. 5A). In contrast, *Cenococcum* and *Boletus* were much more tolerant (Fig. 5B). The rapid decline in growth with increasing water stress is evident for genera in both mesic and xeric series.

Root isolates

Root isolates were also examined for their response to water stress (Table 4). With the exception of isolate numbers 2, 12,

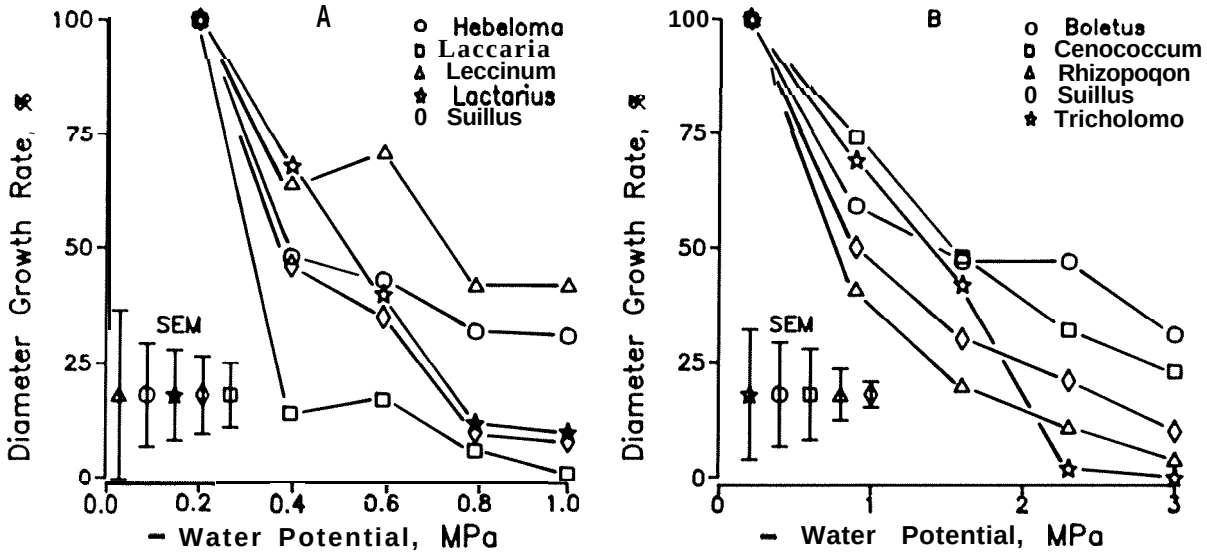

FIG. 5. Effect of water stress on maximum diameter growth rate of 9 different fungal genera. (A) Mesic. (B) Xeric. Note that *Suillus* has both mesic and xeric species (see Tables 2 and 3). Growth was at 22°C on liquid MMN media with PEG amendments to impose water stress. Genera are the average of the following number of isolates and species: *Boletus*, 1 isolate; *Cenococcum*, 8 isolates, 1 species; *Hebeloma*, 6 isolates, 1 species; *laccaria*, 7 isolates, 2 species; *Lactarius*, 2 isolates, 2 species; *Leccinum*, 1 isolate; *Rhizopogon*, 4 isolates, 1 species; *Suillus*-mesic, 3 isolates, 2 species; *Suillus*-xeric, 19 isolates, 6 species; *Tricholoma*, 2 isolates, 1 species. Values arc expressed as a percent of the control and each isolate used for the averaging is replicated four times. Bars indicate standard error of the mean.

and 14, all root isolates appeared similar to *Rhizopogon vinicolor* in cultural characteristics. In agar cultures the RHVI-like colonies ranged from white to dark brown with the colony edge contrasting in shade with the center; the agar and advancing mycelium were often dark brown. All isolates that resembled *R. vinicolor* had a water stress response pattern similar to *R. vinicolor*. The three isolates of the non-RHVI-like class did not resemble *R. vinicolor* in cultural character or in stress tolerance. With imposition of stress the non-RHVI-like isolates increased in growth rate and appeared to reach a maximum at -2.3 or -3.0 MPa (Fig 6).

Neighboring sporocarps

The two *Suillus granulatus* isolates (SUGR3 and SUGR4) collected at the same location but from sporocarps 3 m apart were very similar in their response to stress (Fig. 7). Interestingly, for both isolates, growth in the control ceased after only 2 weeks, while growth at -0.9 MPa continued much longer.

Reisolation

Reisolation of LABI2 from a sporocarp in a seedling container, giving LABI2R, resulted in enhanced growth and tolerance of water stress. Growth of LABUR in the control level was nearly four times greater than before reisolation (Table 2). Furthermore, this fungus was able to grow at a lower water potential after reisolation.

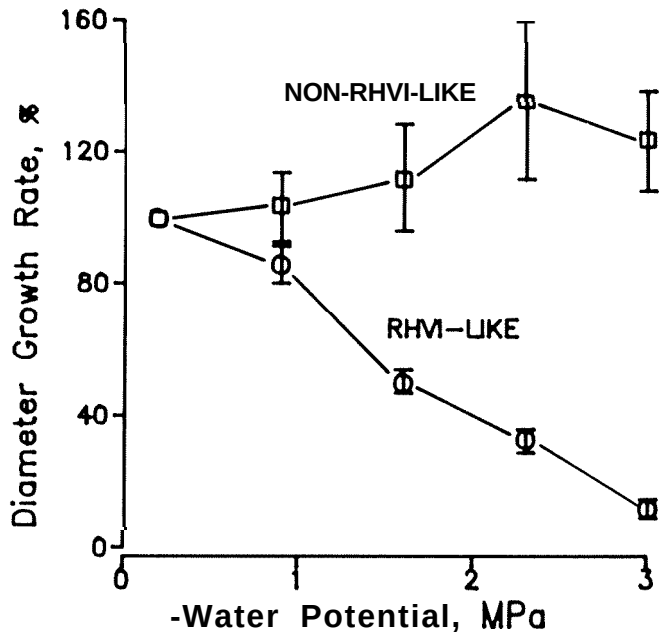

FIG. 6. Effect of water stress on maximum diameter growth rate of root isolates. The RHVI-like group (nine isolates) were similar to *Rhizopogon vinicolor*, while the non-RHVI-like group (three isolates) were not. Growth was at 22°C on liquid MMN media with PEG amendments to impose water stress. Values are expressed as a percent of the control (-0.2 MPa) and are the mean (± standard error of the mean) of isolates replicated four times.

Discussion

Ecophysiologicul characteristics

The low stress tolerance of ectomycorrhizal fungi observed in this study is also characteristic of many other Basidomycetes. Tresner and Hayes (197 1) compared salt tolerance of nearly 1000 species of terrestrial fungi representing the four major fungal classes and found that Basidiomycetes were the least stress tolerant. Other, more specific studies confirmed these results. Deuteromycetes can grow at an osmotic potential of − 10 MPa or below and will generally grow optimally between − 1 and -3 MPa, demonstrating a type III response (Brownell and Schneider 1985; Manandhar and Bruehl 1973; Griffin 1972). Basiodiomycetous wood and litter decay fungi, conversely, have been shown to cease growth near -4 MPa with an almost exclusive type II response pattern (Boddy 1983; Griffin 1977; Wilson and Griffin 1979). However, working with saprophytic Basidiomycetes, Dix (1984) demonstrated

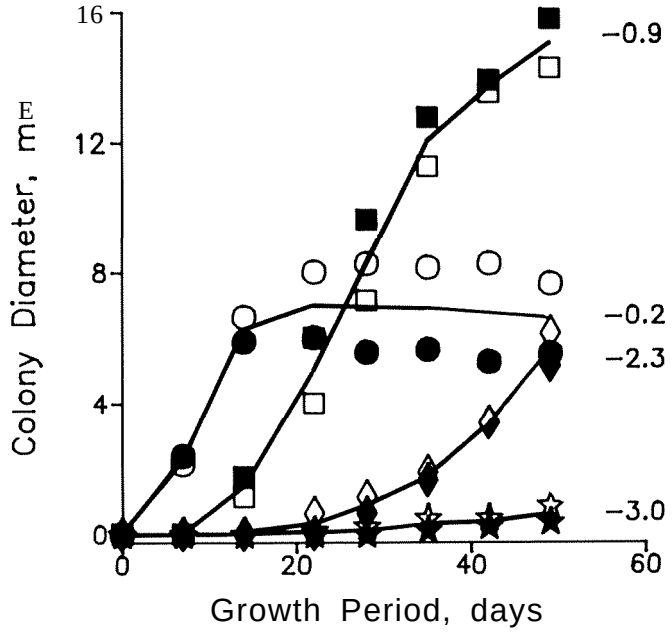

FIG. 7. Comparison of isolates from adjacent sporocarps of the same species, *Suillus granulatus*. Effect of water stress on maximum diameter growth in liquid culture. The different isolates are indicated by closed (SUGR3) and open (SUGR4) symbols. Water potentials in media were -0.2, -0.9, -2.3, and -3.0 MPa. Values are the mean of four replicates. The -1.6 MPa treatment was omitted for clarity.

lower minimum water potentials for growth (to -7.2 MPa) and Koske and Tessier (1986) found some Basidiomycetes to have maximum growth under some stress.

The type II response was most common among the mycorrhizal fungi in this study. This is consistent with the response of many nonmycorrhizal Basidiomycetes (Boddy 1983; Griffin 1977; Wilson and Griffin 1979). Cline (1980), who examined 32 cultures of four mycorrhizal species, also found that the majority of cultures displayed a type II growth response, although a few fungi had a type III response.

The difference between reports of type II and type III response of particular mycorrhizal fungi may be that tested responses of a limited number of isolates are assumed to be representative of an entire species. There are also procedural reasons why, in some experiments, a type III pattern may be displayed, shifting the majority of isolates from type II response. These experimental problems include measurement of growth by dry weight vs. hyphal extension or the length of time for growth determinations.

Fungal dry weight and hyphal extension (diameter growth) are correlated; however, this relationship may not be consistent for each species, or each isolate of a species, and can vary depending upon growth conditions. A description of the relationship between dry weight and hyphal extension is needed in order to compare results of water stress studies using these different methods. Mexal and Reid (1973) measured dry weight, while data of Cline (1980), Boddy (1983), and this report are based on hyphal extension. The former showed a type III response to stress, while the latter usually observed a type II response. As suggested by the dry weight data collected, density (mass per unit colony surface area) increases as stress increases (data not shown). Type III patterns, more commonly found in dry weight experiments, may be due to an increased colony density with increased stress. Alternatively, with the difficulty of removing PEG from the fungal tissue, inadequate rinsing could create an artificially high dry weight value under stress. Tests showed that soaking followed by rinsing with over 2 L of water was required to eliminate PEG. Mexal and Reid (I 973) also found that PEG was not easily leached from the tissue. Therefore, increased dry weight from PEG-imposed stress may result from inadequate separation of media and mycelium (Griffin 1978).

The time at which growth is measured may influence the estimate of the response to stress. For mycorrhizal fungi, if growth rates are measured after 1 3 weeks, control rates often exceed rates in stress treatments, resulting in type II response. However, acclimation periods exceeding 6 or 7 weeks may be necessary for stress treatments to initiate growth. Fungi grown under stress pass through an extended lag phase before entering the exponential growth phase (Fig. 1). Estimating growth rates over a shorter period favors the control, which rapidly enters exponential growth. Estimating growth rates after longer periods favors the stress treatments because fungi under stress have time to acclimate while growth in the control has ceased. Clearly, in comparison of water-stress treatments, it is desirable to examine the growth curve and use the maximum growth rates rather than estimates of growth based on weight or diameter increases during a specified period.

Drought-tolerant fungi

Based on their ability to grow under stress, several fungal species tested in this study are not drought tolerant (e.g., isolates of *Laccaria* and *Lactarius*, Table 2 and Fig. 5). Interestingly, the slow-growing genera were generally intolerant (e.g., Laccaria and *Hebeloma*), while those capable of rapid growth were more stress tolerant (e.g., *Suillus* and *Rhizopogon)*. The major exceptions were *Cenococcum* isolates (CEGE3, CEGE6, CEGE7, and CEGE9), which were stress tolerant but grew relatively slowly (Table 3), and the mesic *Suillus* isolates, which grew rapidly but were not stress tolerant (Table 2).

Selection of drought tolerant fungi was based on growth at -2.3 MPa. If the growth rate as a percent of the control exceeded 30%, the isolate was designated drought tolerant. Using these criteria, nine isolates were drought tolerant: BOED1, **CEGE3**, CEGE4, CEGE6, **CEGE7**, **SUGR3**, **SUGR4**, SULU1, and SULU6. Many of these isolates originated from relatively moist locations (rainfall > 105 cm). Of those isolates found in more arid locations (precipitation < 40 cm annually), CEGE6, **SUGR3**, and SUGR4 were the only isolates that were also drought tolerant. Other isolates tested from xeric locations were much less tolerant of drought.

In general, drought tolerance of fungi was poorly correlated with average annual moisture conditions at the collection site. However, even the wettest sites can experience a summer drought, the severity of which may not be reflected in annual precipitation depending more on seasonal fluctuation. Therefore, annual precipitation values may not reflect the extreme seasonally dry conditions that may occur on moist sites and to which a fungus from that location may be adapted.

Poor correlation also may suggest that water-stress tolerance is not a highly adaptable trait. Growth under water stess may be more characteristic of individual species rather than the site of origin. The species response to water stress was, with some exceptions, consistent among isolates of a species regardless of collection site conditions. This suggests that if there is adaptation to water stress by isolates of a species, it was not evident under these test conditions.

Tolerance of water stress may result from the ability of the

fungus to adjust osmotically during stress. The extension of the lag phase with increasing water stress may be a period of osmotic adjustment. Mycorrhizal fungi such as *Boletus edulis*, *Cenococcum geophilum,* Rhizopogon vinocolor, and *Suillus* spp. need to be tested in a more rigorous manner and compared with less tolerant fungi such as *Laccaria* and *Hebeloma* to determine how fungi physiologically acclimate to stress and if acclimation occurs during the lag phase.

Root isolates

Based on cultural characteristics (color, growth form, and growth rate), *Rhizopogon vinicolor* isolates and RHVI-like root isolates were very similar. The general pattern of response to stress was also similar (Figs. 3B and 6).

Root isolates 2, 12, and 14 (non-RHVI-like) demonstrated a unique type of growth. They rapidly colonized the plate with many small colonies separate from the main colony of the inoculum plug. These isolates appeared extremely drought tolerant, since growth increased with increasing stress (Fig. 6). The unique form of growth and high stress tolerance suggest that either *(i)* these fungi are exceptionally different from most mycorrhizal fungi, or *(ii)* they are not mycorrhizal fungi. The fact that an imperfect contaminant, inadvertently tested, had similar unique growth form and high stress tolerance to the non-RHVI-like root isolates (data not shown), indicates that perhaps these root isolates are not mycorrhizal. Attempts have been made to synthesize mycorrhizae with these root isolates under nonaxenic conditions (N. Mosier, unpublished data). Eight out of nine RHVI-like isolates formed ectomycorrhizae with Douglas-fir while none of the non-RHVI-like isolates had this capacity (No. 12 was not tested); this supported the contention that the ability of the non-RHVI-like isolates to tolerate high water stress is not characteristic of mycorrhizal fungi.

Selection of fungi for inoculation

A large number of ectomycorrhizal fungi must be examined to select those that confer drought resistant to host seedlings. A rapid, simple test is obviously desirable. Either the pure culture technique or examination of the seedling associations will likely be the basis for the selection process; the simpler of the two tests is the pure culture technique. Using seedlings inoculated with various fungi, Parke et al. (1983) determined that seedlings inoculated with *Rhizopogon vinicolor* were more drought resistant than all other seedlings, including those inoculated with *Laccaria laccata.* In the current pure culture experiments, *R. vinicolor* was more drought tolerant than *L. laccata,* which suggests a correlation between the two experiments. However, Parke et al. (1983) found no relation between their pure culture experiments and seedling experiments. As a result, it is not clear if the drought tolerance of fungi in pure culture is transmitted to associated host plants. If this transmission can be shown, use of this simpler pure culture method to screen fungi will be possible. Otherwise, the selection of drought tolerant fungi will likely involve the more difficult procedure of screening ectomycorrhizal host-fungus associations.

Acknowledgments

Our appreciation is extended to J. F. Ammirati, P. S. Homann, R. G. Lindermann, and R. Molina for manuscript review, M. A. Castellano for providing sporocarp isolate cultures, N. Mosier for providing ectomycorrhizal root isolates cultures and related mycorrhizal formation data, and K. Do for technical assistance. Funding was provided by USDA Forest Service Cooperative aid grant No. PNW-83-222 and National Science Foundation grant No. BSR-8510858. This work was done in partial fulfillment for the Ph.D. degree, College of Forest Resources, University of Washington, Seattle, WA, by M.D.C.

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