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Diversity of Ectomycorrhizal Morphotypes and Oak Decline

By

G. KOVACS¹⁾, M. PAUSCH²⁾ & A. URBAN³⁾

K e y w o r d s : Ectomycorrhizae, diversity, oak decline, ectomycorrhizal microcoenosis.

Summary

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In two oak stands in the north-east of Austria, the root systems of declining oak trees (Quercus petraea and Q. robur) were compared to those of vigorous trees. Ectomycorrhizal roots were morphotyped and counted. Fortysix ectomycorrhizae could be differentiated. While all morphotypes occurred in Patzmannsdorf, only 38 morphotypes were found in Niederweiden. The presence of some morphotypes was highly correlated with the crown-status of the trees. In both stands, the Shannon index of diversity calculated for the ectomycorrhizal microcoenosis was higher for the root systems of vital trees. It was possible to construct a discriminant function which discerned the ectomycorrhizal microcoenoses of vital and of declining trees. The possibility of reducing the number of morphotypes selected for the construction of the discriminant function without significant loss of information about the tree status was tested. Possible implications of the correlation of morphotypes with the crown-status of oak trees are discussed.

Introduction

The investigation of below ground factors may yield an improved understanding of the processes involved in oak decline, which has been observed in Western Europe, Central Europe (DONAUBAUER 1987), Eastern Europe (VARGA 1987) and in the Mediterranean (RAGAZZI & al. 1989). Oak die-back is widely regarded as a complex disease which cannot be reduced to a single abiotic or biotic

¹⁾ Federal Office and Research Centre of Agriculture, Spargelfeldstraße 191, A-1226 Vienna.

²⁾ Universität f. Bodenkultur, Inst. f. Forstentomologie, Forstpathologie u. Forstschutz, Hasenauerstr. 38, A-1190 Wien.

³⁾ Universität Wien, Institut f. Botanik, Rennweg 14, A-1030 Wien.

stress factor (KOWALSKI 1991, HAGER 1993, DONAUBAUER 1998). The fungal communites on the roots and in the rhizosphere; mutualistic symbionts (CAUSIN & al. 1996, VAN DRIESSCHE & PIÉRART 1995), pathogenic fungi (BLASCHKE 1994, JUNG & al. 1996) and endophytes (HALMSCHLAGER & KOWALSKI 1998), are all subjected to certain shifts during the process of oak decline. In this study, the ectomycorrhizal communities of vigorous and declining trees were compared.

Material and Methods

Two oak stands in the north-eastern part of Austria were selected. Both are situated in the Pannonian Basin and are exposed to dry-warm, subcontinental climate. The description of the sites follows SCHUME 1992. "Patzmannsdorf", 310 m above sea level, 16°18'80'' east, 48°36'25'' north, is located in a hilly region about 40 km north of Vienna. The investigated stand grows on pseudogleyic loamy brown earth. The middle forest is of about 110 years old. "Niederweiden", 140 m above sea level, 16°54'70'' east, 48°12'30'' north, is located about 30 km east of Vienna. The soils were classified as wet black earth. Locally, the soil is very sandy and thus has a reduced water retention capacity. The high forest is about 80 years old.

In both stands, three trees with a low defoliation index ("vigorous trees") and three trees with a high defoliation index ("declining trees") were selected. In Niederweiden one of the selected declining trees died during the investigation period. The trees were sampled successively from 1993 to 1995, twice a year in July and October. Below each tree soil cores were taken with a ground auger in a distance of 1 m, 2 m and 3 m from the centre of the stem. Each soil core was divided into four samples: 0-5, 5-10, 10-15, and 15-20 cm depth, each sample containing about 200 cm³ soil.

Complete mycorrhizal systems (aggregates of mycorrhizal tips of the same morphology, following AGERER 1987-1998) of each morphotype were classified and counted. Vitality of roots and mycorrhizae was discerned by their appearance (turgor, colour).

The statistical analysis was based on non-parametric tests (binomial, Man-Whitney-U) and discriminant analysis and executed with SPSS for Windows 8.0.0 (SPSS, Chicago, IL). The Shannon index of diversity (logarithm base e) was calculated for the morphotypes per sample and for the aggregated samples from both stands (BEGON & al. 1990). Discriminant functions for the tree status were calculated on the basis of random selections of samples (60 of 72 cases for Patzmannsdorf) and applied to the remaining samples as a test group. This process was repeated with 18 random selections of samples, the mean classification scores of the test groups were recorded.

Results and Discussion

Fortysix morphotypes were morphologically differentiated (data not shown) and 25 were tentatively identified (Table 1). All morphotypes were present in Patzmannsdorf, while only 38 morphotypes were found in Niederweiden. The lower age of the latter stand, the specific site history and pedology and the more intense influence of drought in this stand may be responsible for the lower number of morphotypes in Niederweiden.

CAUSIN & al. 1996 discerned 43 ectomycorrhizal morphotypes in an oak stand in northern Italy. In Belgium, 31 morphotypes of oak ectomycorrhizae were found in one stand (VAN DRIESSCHE & PIÉRART 1995). Mycosociological records of the investigation sites of this study (KOVACS 1997) and of other oak stands near Vienna (KRISAI 1992) have shown that at least 74 ectomycorrhizal macromycetes are associated with oak species in this region. The total number of ectomycorrhizal species associated with oak is probably higher, as it is difficult to detect e.g. hypogeous species like *Genea verrucosa*, corticoid fungi like *Byssocorticium atrovirens* and imperfect species, like the omnipresent *Cenococcum geophilum*.

It can be concluded that the number of morphotypes underestimates the number of species involved, due to the irregular distribution of ectomycorrhizal fungi and because of limits concerning sample size and taxonomic resolution.



♦ Patzmannsdorf, vigorous

Patzmannsdorf, declining

△ Niederweiden, vigorous

Niederweiden, declining

Fig. 1. Relative abundance of ectimycorhhizal morphotypes in vigorous and declining oaks in two stands.

Fig. 1 shows very moderate differences between the relative abundances of morphotypes in the two stands and under vigorous and declining trees. A log-linear rank-abundance curve is indicative of mature communities, where a relatively large number of species is involved in complex interactions (SMITH & READ 1997). Table 2 presents details of the parameters which describe the diversity of the ectomycorrhizal communities. In Niederweiden, the mean number of morphotypes per sample was lower than in Patzmannsdorf, for vital and for declining trees, while the Shannon diversity index was higher in Niederweiden for both groups. In this stand a lesser number of morphotypes was more evenly distributed than in Patzmannsdorf. In both stands, the mean number of morphotypes per sample was slightly higher under vigorous trees, although not significantly (p = 0.05). The average Shannon diversity index was also higher for samples taken under vigorous trees. When all samples from both stands were compared, this difference was significant (p = 0.05). When the Shannon diversity index was calculated for the aggregated samples in both stands, differences were slighter or even inverse. A moderate decrease of ectomycorrhizal diversity during oak decline could be observed on the level of soil samples, while overall diversity did not seem to be affected. We hypothesise that ectomycorrhizal diversity in declining oak stands is temporarily maintained by a spread of "weedy" species at the expense of K-selected fungi in a patchwork of declining and regenerating roots.

Suspected species	Туре	Reference
Lactarius cf. quietus	A	VAN DRIESSCHE & PIÉRART 1995
cf. Russula sp.	A1	
Cenococcum geophilum (single tips)	A2	
cf. Genea sp.	В	Brand 1991, Jakucs & Bratek 1998
Byssocorticium atrovirens	B1	Brand 1991
Lactarius sp.	B2	
Lactarius sp.	B3	
Lactarius sp.	С	
cf. Fagirhiza granulosa	D	Brand 1991
cf. Boletus	F	Agerer & Gronbach 1990
cf. Fagirhiza cystidiophora	G	Brand 1991
Russula sp.	G1	
Amanita rubescens	H	
Xerocomus sp.	J	PALFNER 1995
cf. Tricholoma sp.	K	
cf. Cortinarius sp.	K1	
cf. Tricholoma sulphureum	K3	
Laccaria amethystina.	L1	Brand 1991
Cenococcum geophilum (aggregates)	M	
Quercirhiza setifera	0	PALFNER 1994
cf. Quercirhiza squamosa	R	PALFNER 1994
cf. Paxillus involutus	Т	Agerer & Gronbach 1989
cf. Russula sp.	v	
cf. Russula sp.	V1	
Lactarius sp.	X1	

Table 1. List of tentatively classified morphotypes.

Table 2. Ectomycorrhizal diversity. St...status, no...number of trees, Ms...mycorrhizal systems per sample (mean), mn...total number of morphotypes, ms...morphotypes per sample (mean), hs...Shannon diversity index (per sample, mean), ha...Shannon diversity index (aggregated samples), v...vigorous, d...declining.

stand	tree status	no.	Ms	mn	ms	hs	ha
Patzmannsdorf	v	3	92.93	43	9.08	1.35	2.74
	d	3	114.81	44	8.33	1.18	2.64
Niederweiden	v	3	87.63	38	8.97	1.48	2.57
	d	2	98.35	33	7.5	1.24	2.67

Table 3. Mean number of morphotypes (*) and Shannon diversity index (*).

	Patzma	annsdorf	Niederweiden		
tree status	vigorous	declining	vigorous	declining	
soil depth 0-5 cm	15.0+/1.89*	14.7+/1.53*	14.7*/1.70*	10.8+/1.57*	
soil depth 5-10 cm	8.8+/1.17*	7.4+/0.97*	10.3+/1.43*	9.7*/1.42*	
soil depth 10-15 cm	5.8+/1.00*	5.7+/1.13*	4.0+/1.22*	5.5+/1.01*	
soil depth 15-20 cm	6.8+/1.35*	5.6+/1.09*	7.2/*1.50*	4.0+/0.91*	

The vertical distribution of the mean number of morphotypes per sample is shown in Table 3. The number of ectomycorrhizal morphotypes was highest in the uppermost 5 centimetres in both stands, and under declining trees in Niederweiden it was markedly reduced, probably as a consequence of drought. This pattern of vertical variation is similar for the Shannon diversity index. Ectomycorrhizal diversity as expressed by the Shannon diversity index had a second maximum in the deepest investigated soil layer (15-20 cm) in both stands only under vigorous trees (Table 3). In this soil layer ectomycorrhizal diversity was rather due to high equitability than due to a high number of morphotypes.

A stable ectomycorrhizal community in deeper soil layers may reduce the susceptibility of the tree to environmental stress (drought, nitrogen input, irregular carbon supply due to crises in the crown) and pathogen attack.

It can be concluded that ectomycorrhizal diversity decreases, at least on a small scale, slowly but significantly during oak decline. In the investigated stands, vigorous and declining trees were growing close to each other. In these old forest stands, the abundant resources of ectomycorrhizal inoculum may explain the relative inertia of the ectomycorrhizal diversity in response to disturbance. If oak decline and the responsible environmental changes continue, the decrease of the specific symbiotic mycoflore will be more marked. In spruce forests a reduction of ectomycorrhizal diversity due to pollution was shown by KRAIGHER 1999.

Under vigorous and declining trees in each stand, different morphotypes were dominant (Table 4). Some morphotypes are significantly associated with either vigorous or declining trees (Table 5).

Niederweiden vigorous Patzmannsdorf vigorous declining vigorous declining cf. Russula sp. unidentified (M) cf. Cortinarius sp. (K1) unidentified				
vigorous declining		vigorous	declining	
cf. Russula sp. (V1)	unidentified (M)	cf. Cortinarius sp. (K1)	unidentified (S)	
cf. <i>Genea</i> sp. (B) basidiomycet (P) unidentified (U1) basidiomycet (N) cf. <i>Fagirhiza cys-</i> <i>tidiophora</i> (G) cf. <i>Quercirhiza</i> <i>squamosa</i> (R)		Lactarius cf. quietus (A) unidentified (S) unidentified (J1)	cf. <i>Boletus</i> (F) unidentified (V2) basidiomycet (P)	
basidiomycet (K2) cf. <i>Genea</i> sp. (B)		Cenococcum geophilum (A2)	Lactarius cf. quietus (A)	
cf. Fagirhiza unidentified (S)	unidentified (U1) cf. <i>Boletus</i> (F)	cf. Genea sp. (B) unidentified (H2)	Quercirhiza granulosa (D) Byssocorticium atrovirens (B1)	
	cf. Russula sp.(V1)	cf. Boletus (F)	cf. Fagirhiza cystidio- phora (G)	
	Lactarius sp. (X1)	cf. Russula sp. (V)	cf. Quercirhiza squamosa (R)	
		Lactarius sp. (C) cf. Russula sp.(V1) cf. Triaboloma sp. (K)		

Table 4. Morphotypes forming more than 10 mycorrhizal systems per 100 $\rm cm^3$ soil, ranked by abundance.

	Туре	vt	dt	sign	ind
Patzmannsdorf	Type cf. Russula sp. (A1) Cenococcum geophilum (A2) basidiomycet (F1) cf. Fagirhiza cystidiophora (G) cf. Tricholoma sulphureum (K3) Cenococcum geophilum (M) cf. Paxillus involutus (T) basidiomycet (Y) Lactarius sp. (B2)				
	cf. Russula sp. (A1)	0.80	0.32	0.05	vitality
	Cenococcum geophilum (A2)	3.20	0.60	0.01	vitality
	basidiomycet (F1)	0.06	4.96	0.01	decline
	cf. Fagirhiza cystidiophora (G)	0.11	3.00	0.01	decline
	cf. Tricholoma sulphureum (K3)	0.20	0.84	0.01	decline
	Cenococcum geophilum (M)	2.19	0.32	0.01	vitality
	cf. Paxillus involutus (T)	0.05	3.27	0.01	decline
	basidiomycet (Y)	1.36	0.11	0.05	vitality
Niederweiden					
	Lactarius sp. (B2)	1.51	0.0	0.05	vitality
	Amanita rubescens (H)	0.75	0.23	0.05	vitality

Table 5. Morphotypes associated with vigorous or decling trees (Man-Whitney-U-test). vt...vigorous trees, dt...declining trees, sign...significance, ind...indicative for.

In the two stands, different morphotypes were indicative of the tree status. Thus for some morphotypes, their indicative significance is limited to the specific site. It would require further investigation of more sites with an improved identification of ectomycorrhizae to identify more universal indicators. Such a type may be O, which was significantly associated with vigorous trees of both stands combined. Type A2/M (*Cenococcum geophilum*) on the contrary was significantly associated with vigorous trees in Patzmannsdorf while it was very abundant on the roots of declining trees in Niederweiden. In this site, the declining trees were growing on the driest and sandiest soil patches, where the nitrophilic and drought tolerant *C. geophilum* proliferated. The different distribution of this fungus in both sites may either be due to environmental parameters, like anthropogenic nitrogen input and drought stress, or be due to the presence of different ecotypes (ERLAND & al. 1999). HALMSCHLAGER & KOWALSKI 1998 found *C. geophilum* in Patzmannsdorf as an endophytic fungus in living oak roots.

CAUSIN & al. 1996 found in an investigation of a *Q. robur* stand in northern Italy that four morphotypes (of 43 morphotypes) were associated with healthy trees, while most morphotypes where distributed homogeneously. VAN DRIESSCHE & PIÉRART 1995 could show that the ectomycorrhizae of *Scleroderma areolatum*, of *Xerocomus chrysenteron* and of some unidentified types were significantly associated with vigorous trees. They supported the hypothesis that ectomycorrhizae with abundant rhizomorphs are more effective under conditions of water stress. Either stochastic or interactive (quantity of carbohydrate supply) processes may determine the distribution of these ectomycorrhizae.



Fig. 2. Classification of test samples by the discriminant functions.

Discrimant analysis was chosen as a method of testing if substantial information about the tree status can be extracted from the composition of the mycorrhizal community. The discriminant analysis was started with 21 frequent morphotypes as variables, optimal classification (81 % correctly classified) was obtained with a selection of 8 morphotypes. Classification on the basis of only one type (67 % correctly classified) was still significantly (p < 0,1, binomial test) better than random classification (50 %) (fig. 2).

Different physiological and anatomical properties of ectomycorrhizal species, like the maintenance or shedding of absorptive structures under conditions of drought, are the basis of their protective role against abiotic stress and against pathogens. The selection for more opportunistic fungi during oak decline may modify the beneficial effect of the ectomycorrhizal community for the tree. Further work is needed to consolidate the indicative status of ectomycorrhizae for stress factors responsible for oak decline.

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