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FDC: 172.8 *Armillaria sp.*: 176.1 *Quercus sp.*: 443.3 (436)

DETERMINATION OF THE ARMILLARIA SAMPLES FROM OAK FORESTS IN AUSTRIA

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Abstract

The *Armillaria* samples from several oak stands in lower Austria and in Burgenland were identified in mating with test-samples, after the method of Korhonen. Two species of the genus *Armillaria* were determined: *Armillaria gallica* Marx. et Romagnesi and *Armillaria ostoyae* (Romagnesi) Herink.

Key words: Armillaria gallica, A. ostoyae, oak decline

DETERMINACIJA VZORCEV ŠTOROVK IZ HRASTOVIH SESTOJEV V AVSTRIJI

Izvleček

Vzorce štorovk iz hrastovih sestojev z različnih lokacij v Spodnji Avstriji in Burgenlandu smo identificirali s križanjem s testerji po Korhonenovi metodi. Določili smo dve vrsti štorovk: *Armillaria gallica* Marx. et Romagnesi in *Armillaria ostoyae* (Romagnesi) Herink.

Ključne besede: Armillaria gallica, A. ostoyae, propadanje hrasta

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1 INTRODUCTION

Among the plenty species of fungi, which are involved in oak decline, *Armillaria* can always be found. The two species of genus *Armillaria*, that most often live on roots of the oak trees, are *Armillaria mellea* (Vahl ex Fr.) Kummer and *Armillaria gallica* Marx. et Romagnesi (sin. *A. bulbosa* (Barla) Velen.). *A. mellea* is an aggressive parasitic fungus, which mainly attacks broad-leaved trees. *A. gallica* is a saprophytic fungus, that lives on tree residues and as a facultative parasite attacks the already weakened trees. In oak forests, the latter species is very frequent.

For evaluation of the influence of *Armillarias* and the root rot upon oak decline, it is in the first place necessary to find out, which *Armillaria* species actually infected the roots. The morphology of fruitbodies, which develop on infected roots in autumn, is not a reliable sign for determination of the *Armillarias*. For this purpose, a laboratory method of pairing of *Armillaria* isolates with standard test-samples is used - the so called mating test, which was introduced by the Finnish scientist K. KORHONEN (1978). With this method, which has already for some years been a routine for determination of Armillarias in Slovenia, the Austrian samples were also identified.

2 THE RESEARCH MATERIAL AND THE METHODS OF WORK

In the end of 1990, Dr. Thomas Cech from Federal Forest Research Station (Institute of Forest Protection) in Vienna sent us some *Armillaria* samples, that were gathered in autumn 1990 on several locations in lower Austria and in Burgenland. We got the samples in a form of frozen spore suspensions. There were 13 samples, each in two alternatives (A and B) and several parallels. Unfortunately we did not get any data about the origin and the ecology of the samples.

2.1 Isolation of haploid mycelium from spore suspensions

For determination of species, haploid isolates, isolated from spore suspensions were needed. The appropriately diluted spore suspension was inoculated with a sterile inoculating needle on the nutrient medium in Petri dishes (1.5 % malt agar). The spores were thinly dispersed all over the nutrient medium. They were incubated in a dark room by the temperature of 25 degrees C for five to eight days. Afterwards, the germinating spores were isolated under microscope - according to the method of KORHONEN (1980). For the isolation procedure, the Pasteur pipettes were used, which had earlier been curved above a flame, thinned to a calibre of 0.5 mm and adapted for microscopical work. Previous to every isolation, the pipettes were washed in distilled water, disinfected in alcohol and acetone and burned above a flame. Under the microscope, individual germinating spores were cut out of the nutrient medium with a sterile pipette and inoculated each in its own test tube.

As the germinative faculty of spores of some samples was rather low, it was - in spite

of plenty repeated experiments with different nutrient media - not possible to isolate a haploid mycelium from samples no. 7, 10, 12 and 13.

The haploid, that is single-spore isolates of Armillarias, were kept in a dark room, by a temperature of 4 degrees C, and were every month transplanted to fresh nutrient media.

2.2 Identification of the *Armillarias* with test-samples

Single-spore isolates of *Armillarias* were identified in mating tests with test-samples. The test-samples are haploid isolates of all the European species of genus Armillaria, which are as reference isolates used for identification of *Armillarias*. Two years ago, they were sent to us by Dr. K. Korhonen. As it is not possible for the haploid isolates of *Armillaria* to be kept for a longer time, because they are inclined to degeneration and are afterwards no longer suitable for mating experiments, the Finnish test-samples were partially replaced by the test-samples from our own collection (in TABLE 1, these test-samples are marked by an S).

Every Austrian sample was paired with 24 testers - four for each *Armillaria* species. Beside the species with a veil (*A. borealis, A. cepistipes, A. gallica, A. mellea* and *A. ostoyae*), the ringless species A. tabescens, which often lives parasitically on oak trees in warmer climates, was used in mating tests as well.

Table 1: The list of test-samples, used for identification of the haploid *Armillaria* isolates

A. borealis:	1 - S900930.1.2	2 - S890930.1.1	3 - 880800.2.2/5	4 - 901002.1.2/1
A. cepistipes	5 - S891019.3.1	6 - S901028.3.1	7 - 870912.1.2/7	8 - 880902.2.1/2
A. gallica	9 - S891107.1.2	10 - S901006.1.2	11 - S891014.1.2	12 - 861024.1.1/1
A. mellea	13 - S891019.2.2	14 - S881025.1.2	15 - S891112.1.1	16 - S901013.4.1
A. ostoyae	17 - 870919.2.2/3	18 - S901006.2.1	19 - 870923.4.2/2	20 - S891021.2.2
A. tabescens	21 - 871104.8.1/2	22 - 871104.8.1/5	23 - 871111.1.1/3	24 - 871111.1.1/5

Both of the partners (the test-sample and the isolate, which was to be identified), were inoculated in Petri dishes with a diameter of 9 cm, on a nutrient medium with 1.5 % of malt agar. The isolates were inoculated near to each other (at a distance of 3 - 5mm), so that the results of mating would be as clear as possible. There were four pairings in every Petri dish.

The Petri dishes were incubated in dark, by room temperature. The results of mating tests were first evaluated after three weeks, and after six weeks for the second time.

When the studied sample was of the same species as the tester, diploid mycelium developed after mating, which was easily recognized by its morphological characteristics. The differences betwen haploid and diploid mycelium by genus *Armillaria* are very evident: the haploid mycelium is white and airy, while the diploid mycelium is dark and crusty.

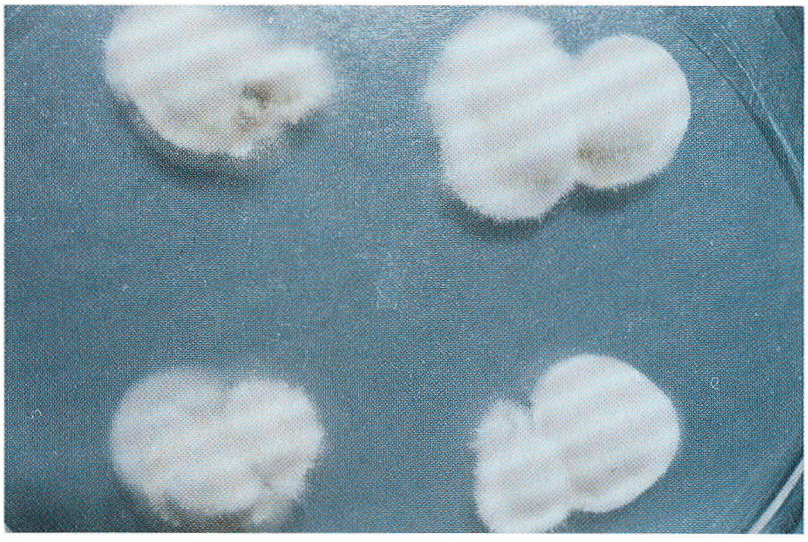

Figure 1: Pairing of haploid *Armillaria* isolates with test-samples (10 days after inoculation)

Figure 2: The haploid (left) and the diploid (right) mycelium of *Armillaria*

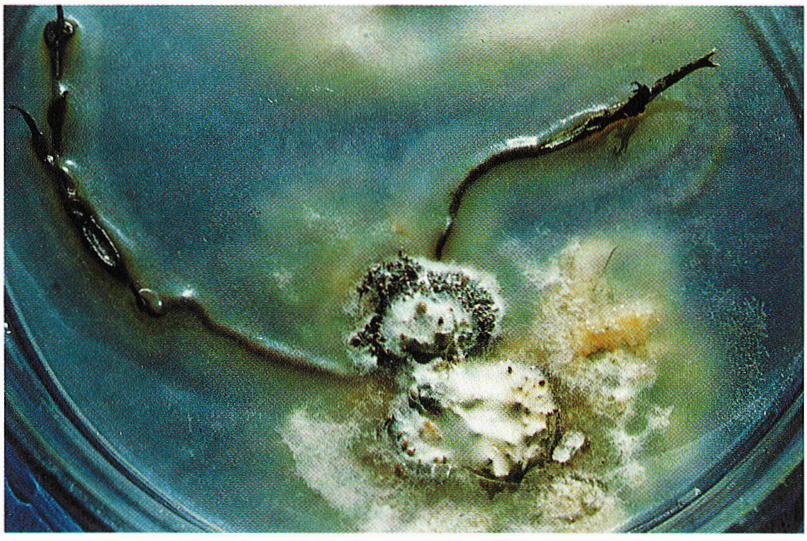

Figure 3: Compatible mating (20 days after inoculation)

Figure 4: Incompatible mating (20 days after inoculation). All the photos: documentation of IGLG. The photographer: Dušan Jurc.

The compatible reaction, resulting from pairing of fungi of the same species, was recognized by the following characteristical signs (KORHONEN 1978, GUILLAUMIN, BERTHELAY 1981): 15 - 20 days after the inoculation, the isolates grew together in a homogenous diploid colony; the separating line, which in the beginning appeared between the mycelia, disappeared completely; the growth of mycelia was slowed down; the exuberant airy mycelium darkened gradually, became thicker and grew into the agar, finally it was covered by a crusty pseudostroma, the rhizomorphs appeared.

For pairing of isolates, not belonging to the same species it was typical, that the mycelia did not grow together and their morphology did not change significantly - they remained white and airy, and a separating black demarcation line appeared in the fusion area. Often, the signs of antagonism between both isolates were also noticed.

3 THE RESULTS AND CONCLUSIONS

Table 2: Identification of haploid *Armillaria* isolates with test-samples

The isolate	Number of the tester																							
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	Species
1A1	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	*A. gallica*
1B1	-	-	-	-	-	-	?	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	*A. gallica*
2A2	-	-	-	-	-	-	-	-	-	-	-	?	-	-	-	-	+	+	+	+	-	-	-	-	*A. ostoyae*
3A4	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	*A. gallica*
3A5	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	*A. gallica*
3B2	0	0	-	-	-	-	-	-	+	+	+	+	-	-	-	-	0	0	-	-	-	-	-	-	*A. gallica*
3B5	-	-	-	-	-	-	-	-	?	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	*A. gallica*
4A4*	-	-	-	-	-	-	?	-	-	-	-	?	?	+?	-	-	+?	+	+?	?	-	-	-	-	? *A.ostoyae*
4A1*	-	?	-	-	-	-	-	-	?	-	-	-	?	?	?	-	-	+	?	-	-	-	-	?	?
5A3	-	-	-	-	-	-	-	?	-	-	-	-	+	+	+	?	-	-	-	-	-	-	-	-	*A. gallica*
5A5	-	-	-	-	-	-	-	-	+	+	+	+	-	?	-	-	-	-	-	-	-	-	-	-	*A. gallica*
5B1	-	-	-	-	-	-	-	-	+	+	+	?	-	-	-	-	-	-	-	-	-	-	-	-	*A. gallica*
6A4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	*A. ostoyae*
8A2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	?	+	-	-	-	-	*A. ostoyae*
8B1	-	-	-	-	-	-	-	-	-	-	-	?	-	-	-	-	+	+	+?	+	-	-	-	-	*A. ostoyae*
8B2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	?	+	-	-	-	-	*A. ostoyae*
9A4	-	-	-	-	-	-	?	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	*A. gallica*
9B5	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	*A. gallica*
11A1	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	*A. gallica*
11A5	0	0	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	*A. gallica*
11B5	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	*A. gallica*

+ = compatible mating
0 = contamination
* = the sample number 4 is badly contaminated by bacteria
- = incompatible mating
? = the results of mating tests are unclear

Among the studied *Armillaria* samples, the prevailing species was *A. gallica*. *A. gallica* normally lives saprophytically, but in spite of this, it sometimes also causes root rot of weakened trees. It is characteristical for this species, that it first infects the roots superficially and penetrates inwards, when the tree has already been sufficiently weakened by some other causes. It is rather surprising, that among the samples, not even one of *A. mellea* was found, although this very species is a very frequent oak parasite. Surprisingly as well, the *A. ostoyae* was present, although this species is normally found in coniferous forests. *A. ostoyae* also grows in mixed forests, together with *A. cepistipes* and *A. gallica*, yet its frequency there depends upon the history of a stand (LEGRAND, 1990). In the saprophytic phase of this, otherwise very pathogenic fungus, an oak tree is even a better substratum for its growth and development as a conifer (GUILLAUMIN, LUNG 1985). The mentioned species (*A. ostoyae*) has already been found on oaks in Slovenia too, yet never in unmixed oak stands.

On the mere basis of taxonomical data and with such a small number of samples, it is not possible to make conclusions, about the significance of the determined two species for oaks. For this purpose, accurate data about the growing sites, the history of stands, the condition of trees from which the samples were taken, and especially the data about the pathogenicity of individual *Armillaria* samples would be necessary. As it was not possible for us to get these data, we have to leave the estimation to be done by our Austrian colleagues.

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