ABSTRACT OF HABILITATION THESIS

The August Cieszkowski Agricultural University, Poznań, Poland

ENVIRONMENTAL CONDITIONS OF DECIDUOUS TREE STUMPS COLONIZATION BY CHOSEN SAPROTROPHIC FUNGI SPECIES AND *ARMILLARIA* SPP.

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Key words: *Armillaria*, saprotrophic fungi, biological control, stumps

The objectives of this study was to determine relationships among isolates of *Armillaria* species and isolates of saprotrophic fungi which colonize and decompose wood of deciduous trees, under different environment conditions. The following saprotrophic species were taken under consideration: *Bjerkandera adusta, Hypholoma fasciculare, H. sublateritium, Kuehneromyces mutabilis, Lentinus lepideus, Pluteus atricapillus, Pleurotus ostreatus, Pholiota squarrosa* and *Trametes versicolor*.

The study was focused on conditions of wood colonization and decomposition by fungi of forest site and relationships among pathogen, saprotrophs, environment and time in forest ecosystem. The investigation of variable effect of the pathogens on saprotrophic fungi and the effect of the saprotrophs on the pathogens resulted in selecting isolates able to decompose and colonize wood most effectively. Those isolates were the most suppressive to the pathogens' growth in wood. The studies were performed under laboratory and forest conditions.

In the study 36 isolates of nine saprotrophic fungi species and 10 isolates of five *Armillaria* species (*A. borealis, A. cepistipes, A. gallica, A. mellea* and *A. ostoyae* – two isolates each) were used.

In laboratory mycelium growth rate of saprotrophic fungi was determined to find out their competitive ability against pathogens. Colonization rate of beech, birch and oak wood by saprotrophs was also studied to determine their ability to decrease the growth of *Armillaria* spp. in the wood species. Deciduous wood decomposition rate by saprotrophic fungi was determined with the view of their potential ability of decomposing stumps in forest. The mutual effect of metabolites produced by *Armillaria* and saprotrophs on each other was tested to describe possible reactions between the organisms in wood:

– the effect of saprotrophs' mycelia on the growth of *Armillaria* isolates and of *Armillaria* isolates on the growth of saprotrophs' mycelia *in vitro* to determine the ability of restricting or stimulating mycelium development by competitive fungi,

– the wood colonization rate by *Armillaria* and by the saprotrophs' isolates in competitive conditions of food base and ecological niche *in vivo*.

Forest study. Some isolates of nine saprotrophic fungi species were used to colonize stumps of beech, birch and oak trees. The inoculum (sawdust overgrown by mycelium) was put on stump surface and covered with soil. The stump (thinned or clear-cut) inoculations were performed in oak, oak-beech and pine with oak understorey stands. The experimental plots were established in Babki, Podanin, Strzelce Krajeńskie Forest Districts and Murowana Goślina (Experimental Forest of The August Cieszkowski Agricultural University in Poznań). Monitoring of *Armillaria* species in experimental stands and the development of *Armillaria* disease in Scots pine plantations established one year after clear-cutting (where oak stumps were inoculated) was also conducted.

Several isolates of *B. adusta, H. fasciculare, H. sublateritium, P. atricapillus, P. ostreatus* and *T. versicolor* initiated growth one day after inoculation. Mycelium of *H. fasciculare* HF16 isolate and all isolates of *P. squarrosa* started to grow three days after inoculations (other isolates – two days). The *in vitro* growth rate of *B. adusta* and *T. versicolor* isolates was the biggest among all isolates investigated.

Isolates of *B. adusta, P. ostreatus* and *T. versicolor* were the most effective (41–83%) in beech and birch colonization, while in oak wood – the isolates of *B. adusta* and *T. versicolor* (60–93.7%).

Armillaria isolates colonized all wood species in a similar way, but the biggest rate of colonization was found for *A. borealis, A. cepistipes* and *A. gallica*. The entire wood samples were colonized 10 to 15 days after inoculation.

Beech wood was decayed most and oak wood least intensively by most isolates of the saprotrophic fungi. The isolates of *B. adusta* and *T. versicolor* caused the most effective decay and *L. lepideus* and *P. squarrosa* isolates decomposed wood the least intensively after three and six months of incubation. *Bjerkandera adusta* and *T. versicolor* caused the most intensive decay of wood after six months of incubation (beech – respectively 56.35% and 69.42%, birch – 56.13% and 72.72%, oak – 64.76% and 70.49%).

Various reactions of *Armillaria* and saprotrophic fungi mycelia in dual cultures 20 days after inoculation were observed. In most cases saprotroph mycelium grew faster and often overgrew *Armillaria* mycelium, but despite the situation *Armillaria* produced rhizomorphs which penetrated the medium under the saprotroph's mycelium. The brownish or yellowish contact zone between mycelia appeared occasionally. The most suppressed *Armillaria* isolates were *A. borealis* AB72, *A. mellea* AM51 and *A. ostoyae* and the weakest effect of saprotrophs' mycelia on *Armillaria* growth was observed for *A. cepistipes* as well as *A. borealis* AB60 and *A. mellea* AM51. On the other hand, in 75% cases *A. mellea* AM51 caused a significant reduction of saprotroph growth in dual cultures, as compared to control. The situation occurred also in dual cultures with *A. mellea* AM48 (53.6%), *A. cepistipes* AC37 (60.7%) and AC68 (41.4%). In many cases in dual cultures, *Armillaria* mycelium was significantly smaller, whereas the size of saprotroph mycelium did not differ from control.

Armillaria borealis, A. cepistipes and *A. ostoyae* were the most resistant to saprotrophs' metabolites effect (isolates of these *Armillaria* species were able to restrict the growth of the saprotroph isolates in dual cultures). Almost all isolates of the saprotrophic fungi produced metabolites, which suppressed the growth and development of *Armillaria* isolates. Only metabolites of *P. squarrosa* Ps34 stimulated the growth of *A. cepistipes* AC68 mycelium (116.94%, $p < 0.05$). Metabolites of *B. adusta* Ba322 and *T. versicolor* Tv31 restricted the growth of *Armillaria* mycelia isolates most effectively (respectively in 93.2% and 91.3%). Both *A. ostoyae* isolates were the most susceptible to the influence of saprotrophs' metabolites and the *A. cepistipes* isolates were the most resistant. The mycelium growth analysis of saprotrophs' mycelia three, five and seven days after inoculation on medium containing metabolites of *Armillaria* isolates showed that in most cases the saprotrophs' mycelia grew as in controls or a little faster. Only a few isolates grew slower than in controls. *Armillaria borealis* AB60, AB72 and *A. cepistipes* AC68 suppressed the saprotrophs' growth most effectively.

Armillaria isolates were able to compete with saprotrophs' isolates in wood colonization but not in Petri dishes, where *Armillaria* isolates grew slowly and were suppressed by fast growing saprotrophs. Almost all saprotroph isolates colonized totally the wood samples. All isolates of *B. adusta*, as well as *T. versicolor* Tv31, *H. fasciculare* HF21 and HF23, colonized wood very fast. Almost in all combinations: saprotroph × *Armillaria*, the pathogens produced shorter rhizomorphs in comparison to control.

For the study 354 specimens of *Armillaria* spp. (basidiomes and rhizomorphs) were collected, which yielded 232 isolates of *Armillaria* spp. In stands with the experimental plots the occurrence of *A. cepistipes, A. gallica, A. mellea* and *A. ostoyae* was confirmed.

Only a few isolates of those used in forest study effectively colonized stumps. After stump inoculation, only *B. adusta, H. fasciculare, H. sublateritium, K. mutabilis, P. ostreatus* and *T. versicolor* have colonized the stump's wood and the root system deeply during four years. Wood colonization and decay by saprotrophs caused a slow exclusion of *Armillaria* from these stumps and the process was faster in oak and birch than in beech stumps. The isolates of *L. lepideus, P. atricapillus* and *P. squarrosa* colonized the stumps least intensively of all saprotrophic fungi used in the experiments.

Biological control of *Armillaria* spp. with saprotrophs colonizing stump wood did not decrease the number of pines killed by *A. ostoyae*. Pathogen's basidiomes appeared on the stumps, soil and dead pines every year of observation. One and two years after inoculation *A. ostoyae* basidiomes occurred also on the stumps treated with saprotrophs, while three years after inoculation pathogen's basidiomes appeared rarely on these stumps and completely disappeared four/five years after inoculation.

Conclusions. Among the isolates of saprotrophic fungi and isolates of *Armillaria* species there was competition for food base and ecological niches, antibiosis and mycoparasitism. *Bjerkandera adusta, H. fasciculare, H. sublateritium, K. mutabilis, P. ostreatus* and *T. versicolor* seem suitable for biological control of *Armillaria* spp. in beech, birch and oak stumps. The saprotrophic fungi species colonized the wood of stumps and root system relatively deeply. The colonization and decay of the stump wood by saprotrophs caused slow exclusion of *Armillaria* species from stumps; the process occurring faster in oak and birch than in beech stumps. The biological control method used in pine plantations, established one year after clear-cutting, did not cause the decrease of the number of pines killed by *Armillaria* in the following years, due to slow process of excluding *Armillaria* from stumps. Saprotrophic fungi, which colonize and decompose stump wood, should be used as an element to work out the long-term strategy of *Armillaria* control in stands. More studies are needed on integrated pest management (IPM) of *Armillaria* root rot in high risk site stands.

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