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# INFECTION THREAT OF HORNBEAM (*CARPINUS BETULUS*) TRANSPLANTS BY PATHOGENIC FUNGI

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**Abstract**: Hornbeam (*Carpinus betulus* L.) transplants examined in an infection experiment with root pathogens *Cylindrocarpon destructans* (Zinss.) Scholt., *C. magnusianum* (Sacc.) Wollenw., *Heterobasidion annosum* (Fr.) Bref. and *Sclerotinia sclerotiorum* (Lib.) De Bary were not infected by either of the fungi. Moreover, neither of the pathogenic species was supported in its growth by the soil fungal community from the forest nursery that provided the transplants. Hornbeam's expansion in Polish forests may be attributable in part to its resistance to infection and to biotic relations in soil that are unfavorable to root infection.

**Key words**: *Carpinus betulus*, hornbeam, *Heterobasidion*, *Cylindrocarpon*, *Sclerotinia*, infection

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## INTRODUCTION

In previous works (Tyszkiewicz & Mańka 1999; Tyszkiewicz 2001) the senior author investigated the biotic relations among saprotrophic soil fungi and root pathogens in two broadleaf stands located in Białystok (NE Poland) – a natural stand (*Tilio-Carpinetum typicum*) and a stand degenerating by pinetization (*Pinus-Lamiastrum*), modified by man (Olaczek 1974; Jakubowska-Gabara 1992; Czerwiński 1995). The former stand is part of the Las Zwierzyniecki Reserve (Sokołowski 1988), with *Carpinus betulus* L. dominating. Less frequent species are *Quercus robur* L., *Fraxinus excelsior* L. and *Populus tremula* L. Sporadically occurring are *Acer platanoides* L., *Ulmus carpinifolia* Gled., *Tilia cordata* Mill. and *Betula pendula* Ehrh. The majority of hornbeams are *ca* 90 years old, and there are single hornbeams, oaks and spruces 150 years old. The latter stand was established about 1940 by introducing *Pinus sylvestris* L. at an oak-hornbeam site after removing the natural stand. Numerous self-seeded hornbeam trees appeared in the stand and are now somewhat younger than the Scotch pines. Maple, ash, lime and oak are also present in the understory and undergrowth (Sokołowski 1988), but natural regeneration of pine is not taking place.

Tyszkiewicz and Mańka (1999) found that the soil fungi communities of the two stands (isolated and analyzed in spring and fall (1993–1996) reflected the difference between their phytocenoses. The saprotrophic soil fungi communities were also examined for their effect on the growth of four pathogenic root fungi isolated from both stand soils: *Cylindrocarpon destructans* (Zinss.) Scholt., *C. magnusianum* (Sacc.) Wollenw., *Heterobasidion annosum* (Fr.) Bref. and *Sclerotinia sclerotiorum* (Lib.) De Bary. The fungal communities from *Ti1io-Carpinetum typicum* always suppressed the growth of all the pathogens, while those from *Pinus-Lamiastrum* suppressed only the growth of *H. annosum* and *S. sclerotiorum*, at the same time supporting the growth of both *Cylindrocarpon* species. The results suggested that the lack of natural regeneration of pine in *Pinus-Lamiastrum* was connected with *C. destructans* activity in the soil but not with the presence of *H. annosum* there, as the latter species was strongly suppressed.

Young self-seeded hornbeams are present and developing very well in both stands. *Carpinus betulus* is an expansive species in Poland (Ilmurzyński 1969), spreading freely in broadleaf forests. The pathogens mentioned, occurring in the soil of the investigated stands, are known to be able to affect tree roots, so it is of interest whether they could harm hornbeam seedlings and play any role in limiting it. The study completes earlier results (Tyszkiewicz & Mańka 1999; Tyszkiewicz 2001) by examining the pathogenicity of the four pathogens to young hornbeam plants and the effect of soil fungi communities from the hornbeam nursery on their growth.

## MATERIALS AND METHODS

Isolates of pathogenic soil fungi *Cylindrocarpon destructans, C. magnusianum, Heterobasidion annosum* and *Sclerotinia sclerotiorum* originated from forest soils. *Heterobasidion annosum* was isolated in spring 1994 from *Pinus-Lamniastrum* community soil, *S. sclerotiorum* and *C. magnusianum* in spring 1994 from *Tilio-Carpinetum typicum*, and *C. destructans* in spring 1995 also from *Tilio-Carpinetum typicum* soil (Tyszkiewicz 2001).

The hornbeams for the infection experiment were taken from a tree nursery in Wronczyn (Czerwonak Forest District near Poznań, central Poland) on May 7, 1998, which was a hot, sunny day. The transplants were just over a year old at the time, produced by sowing local seed material. They were transplanted on the spot into plastic pots (diameter 17 cm, height 14 cm) filled with soil from their bed. The plants were 20–35 cm high. The smaller ones were planted two per pot.

All the transplants had well-developed leaves. During transplanting half of the leaves were removed and 1/3 to 1/2 of the roots were cut. Two hours later the pots were put in a garden of the Department of Plant Pathology, August Cieszkowski Agricultural University Poznań and the plants were watered. They survived the whole procedure without wilting. They were left there and watered as needed, and inoculated on July 8, 1998. The experiment ended in September 1998.

Inoculation was performed on plants in perfect condition. They were inoculated with *H. annosum* by inserting a piece of beech wood (*ca* $3 \times 5 \times 30$ mm) overgrown with 6-week-old mycelia under cut bark at the stem base; the wound was then covered with parafilm (Stenlid & Swedjemark 1988). Control plants were grafted with sterile wood pieces. The other three pathogens were inoculated by inserting four pieces of mycelium-overgrown beech wood into the soil of each pot, and adding PDA medium overgrown with mycelia from one Petri dish to the soil also. Control plants were inoculated with sterile pieces of wood and sterile PDA placed in the soil. The absolute control consisted of untreated plants. There were 5 plants in every treatment. After two months, on September 2, 1998, the experiment was completed. All the plants were extracted; their roots were washed and carefully examined for disease symptoms. For isolation of fungi on potato-dextrose-agar, 1 inoculum was taken from the stem base and 2 inocula from the main root.

At the time of hornbeam planting (May 7, 1998) the soil from the nursery bed was also sampled from 5–20 cm depth according to Mańka (1974) for mycological analysis. The soil was sandy soil, pH 7.20 in $H_20$ (6.57 in KCl).

Isolation of fungi was performed with Warcup's (1950) soil plate method as modified by Mańka (1964, 1974) and Mańka and Salmanowicz (1987). Two variants were applied: with and without soil extract added to the Martin-Johnson medium (Mańka 1974).

The effect of soil fungi communities on the growth of *C. destructans*, *C. magnusianum*, *H. annosum* and *S. sclerotiorum* was investigated with the biotic series method by Mańka (1974) and Mańka and Mańka (1995). In the biotic test the individual biotic effect (IBE) is evaluated, which is the *in vitro* effect of one isolate of a soil fungus species on pathogen growth. To obtain the general biotic effect (GBE), the effect of all the isolates of the species on pathogen growth, the IBE is multiplied by the species frequency in the community. All the general biotic effects are summarized to give the summary biotic effect (SBE), that is, the effect of the entire soil fungi community on the pathogen. The SBE describes the phytopathological function of the community. Any of the biotic effects mentioned can be positive (suppressing pathogen growth), negative (supporting pathogen growth) or neutral (0). The intensity of the suppressive or supportive effect is described by the absolute value of the effect.

The isolates of all the pathogens were those examined earlier by Tyszkiewicz and Mańka (1999).

## RESULTS AND DISCUSSION

At the end of the infection experiment there were no disease symptoms on the plants. No necrosis were seen at the inoculation sites. Isolation of

fungi yielded only some *Fusarium* spp. and Mucorales (common nursery soil fungi), but failed to recover the pathogens in question. The hornbeam transplants proved resistant to the studied pathogens under the experimental conditions.

The saprotrophic fungi communities obtained after isolation with and without the soil extract were similar – the share of the 3 most frequent species (*Coniothyrium fuckelii, Penicillium decumbens* and *P. daleae*) was over 50% in both cases; there were 9 species occurring in both communities (Tables 1, 2).

The results of the biotic series tests are presented in Tables 1 and 2. Both soil fungal communities suppressed pathogen growth. Suppression was greatest in the case of *H. annosum* and least in the case of *C. destructans*. This means that *H. annosum* cannot pose a threat to hornbeam roots in the soil investigated, while *C. destructans* (a well-known nursery root pathogen) is only slightly suppressed. These biotic test results may explain why the infection experiment, performed in the very soil from which the communities derived, was unsuccessful; it could have been due at least in part to the biotic relations in the soil.

In this study we extended earlier investigations of biotic relations among soil fungi in broadleaf forest (Tyszkiewicz & Mańka 1999; Tyszkiewicz 2001) where hornbeam (*Carpinus betulus* L.) spreads very actively by self-seeding. Here we examined the effect of soil fungi and root pathogens on the health status of the species.

Transplants of hornbeam (just over a year old) were not infected by *Cylindrocarpon destructans*, *C. magnusianum*, *Heterobasidion annosum* or *Sclerotinia sclerotiorum* in an infection experiment. The communities of saprotrophic fungi isolated from the hornbeam nursery soil suppressed the growth of all the pathogens *in vitro*.

The good development of self-seeded hornbeam in Poland may be connected with factors including its resistance to infection and the biotic

**Table 1**. Effect of soil fungi community (isolated with soil extract) on growth of *Cylindrocarpon* spp. and *Heterobasidion annosum*. Freq – frequency, IBE – individual biotic effect, GBE – general biotic effect.

| Soil fungi species | Freq | Biotic effect towards | | | | | |
|---|---|---|---|---|---|---|---|
| | | *C. destructans* | | *C. magnusianum* | | *H. annosum* | |
| | | IBE | GBE | IBE | GBE | IBE | GBE |
| *Coniothyrium fuckelii* Sacc. | 37 | –2 | –74 | +1 | +37 | +3 | +111 |
| *Penicillium decumbens* Thom | 24 | –1 | –24 | +5 | +120 | +6 | +144 |
| *Penicillium daleae* Zaleski | 11 | +4 | +44 | –3 | –33 | +2 | +22 |
| *Pestalozzia hartigii* Tub. | 10 | +5 | +50 | +4 | +40 | +7 | +70 |
| *Mortierella minutissima* van Tiegh. | 9 | +4 | +36 | –4 | –36 | +4 | +36 |
| *Chloridium virescens* (Pers.: Fr.) W. Gams et Hol.-Jech. | 8 | –2 | –16 | –3 | –24 | +3 | +24 |
| *Gliocladium virens* Giddens et Foster | 7 | +9 | +63 | +7 | +49 | +8 | +56 |
| *Sesquicillium candelabrum* (Bonard.) W. Gams | 6 | +3 | +18 | –1 | –6 | +4 | +24 |
| *Paecilomyces variotii* Bain. | 4 | +1 | +4 | +4 | +16 | +2 | +8 |
| *Mortierella vinacea* Dixon-Stewart | 3 | –3 | –9 | –2 | –6 | +1 | +3 |
| *Acremonium kiliense* Grutz | 3 | –3 | –9 | +2 | +6 | +2 | +6 |
| *Penicillium jensenii* Zaleski | 3 | –2 | –6 | –2 | –6 | +3 | +9 |
| *Penicillium decumbens* Thom | 3 | –2 | –6 | –2 | –6 | +2 | +6 |
| *Fusarium oxysporum* Schlecht. | 1 | +6 | +6 | +5 | +5 | +7 | +7 |
| *Zygorhynchus moelleri* Vuill. | 1 | +6 | +6 | +8 | +8 | +8 | +8 |
| | 130 | | | | | | |
| Summary biotic effect | | | +83 | | +164 | | +534 |

**Table 2**. Effect of soil fungi community (isolated without soil extract) on growth of *Cylindrocarpon* spp. and *Sclerotinia sclerotiorum*. Freq. – frequency, IBE – individual biotic effect, GBE – general biotic effect.

| Soil fungi species | Freq. | Biotic effect towards | | | | | |
|---|---|---|---|---|---|---|---|
| | | *C. destructans* | | *C. magnusianum* | | *S. sclerotiorum* | |
| | | IBE | GBE | IBE | GBE | IBE | GBE |
| *Coniothyrium fuckelii* Sacc. | 33 | –2 | –66 | –2 | –66 | –2 | –66 |
| *Penicillium decumbens* Thom | 20 | –1 | –20 | +3 | +60 | +6 | +120 |
| *Trichoderma viride* Pers. ex Gray | 15 | +8 | +120 | +5 | +75 | +9 | +135 |
| *Penicillium daleae* Zaleski | 7 | +1 | +7 | +2 | +14 | +5 | + 35 |
| *Penicillium daleae* Zaleski | 6 | +3 | +18 | +1 | +6 | +1 | +6 |
| *Mortierella vinacea* Dixon-Stewart | 5 | –2 | –10 | –1 | –5 | –2 | –10 |
| *Trichocladium opatam* (Corda) Hughes | 4 | –1 | –4 | –1 | –4 | –3 | –12 |
| *Penicillium janczewskii* Zaleski | 4 | +2 | +8 | –1 | –4 | +4 | +16 |
| *Penicillium citreonigrum* Dierckx | 4 | –2 | –8 | –1 | –4 | –1 | –4 |
| *Sesquicillium candelabrum* (Bonard.) W. Gams | 3 | –2 | –6 | –2 | –6 | +1 | +3 |
| *Mortierella minutissima* van Tiegh. | 3 | –1 | –3 | +3 | +9 | –3 | –9 |
| *Pestalozzia hartigii* Tub. | 2 | +3 | +6 | +3 | +6 | +1 | +2 |
| *Absidia spinosa* Lendner | 2 | +7 | +14 | +8 | +16 | +8 | +16 |
| *Chrysosporium pannorum* (Link) Hughes | 2 | –2 | –4 | –2 | –4 | –2 | –4 |
| *Fusarium oxysporum* Schlecht. | 2 | +3 | +6 | +4 | +8 | +2 | +4 |
| *Penicillium waksmanii* Zaleski | 2 | –3 | –6 | +1 | +2 | –2 | –4 |
| *Zygorhynchus moelleri* Vuill. | 2 | +7 | +14 | +8 | +16 | +7 | +14 |
| | 116 | | | | | | |
| Summary biotic effect | | | + 66 | | +119 | | +242 |

relations in the soil. If the relations do not favor root pathogens, as was the case in this work, the conditions for hornbeam growth may be greatly improved.

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