Heterobasidion Infection in *Abies nordmanniana* ssp. *bornmülleriana* Stands in Kastamonu Province

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Abstract

In this study, the proportion of *Abies bornmülleriana* trees infected by *Heterobasidion* spp. was investigated in Kastamonu Province in different types of stands. One 1 cm-thick disc was taken from each of 100 freshly cut trees, washed under a running tap, placed into plastic bags and incubated in growth chamber at 24 C° for 7 days. The area occupied by the conidial stage of *Heterobasidion annosum* s.l. was determined under a stereomicroscope using transparent film placed onto the upper surface of each disc. During this investigation conidia of *Heterobasidion annosum* s.l. were taken with a needle and placed onto agar plates. The obtained isolates were identified with pairing tests and DNA-based methods.

All 36 isolates responded as *Heterobasidion abietinum* to the tester isolates. Identification based on PCR amplification with MJF – MJR and KJF-KFR primers gave the same result. 34% (34 out of 100) of the discs taken from the fir forests were found to be infected with *H. abietinum*. The characteristics of the colonized patches on the discs indicated that the *H. abietinum* colonies originated from stem infections of the trees. The proportion of the disc area covered by the conidial stage of the fungus was 80% in one of the discs, 6-10% in 6 discs, 1-5% in 18 discs, and 0-1 % in 11 discs. The conidiophores were observed in heartwood in only five samples.

Key words: *Abies bornmülleriana*, Fir Stands, Heterobasidion

Introduction

Root rot caused by *Heterobasidion annosum* s.l. leads to severe economical losses to forestry in the northern boreal and temperate regions of the world. Economic losses due to *Heterobasidion* infection in Europe are estimated at 800 million euros annually (Woodward et al., 1998).

Heterobasidion is capable of spreading over long distances by means of airborne spores. Basidiospores are produced in basidiocarps that often occur in the cavities of old stumps, on logs containing advanced decay, and on the roots of wind thrown trees. Basidiospores are able to infect wounded roots in the soil. Also the roots of suppressed trees are considered to be susceptible to spore infections (Rishbeth, 1951b; Schönhar, 1995). However, in managed forests, the spore infections occurring through wounds in the roots are considered to be of minor importance as compared with infections occurring through fresh stump surfaces or deep wounds above ground level (Redfern and Stenlid, 1998). Therefore, in general, *Heterobasidion* infection occurs via fresh wounds and freshly cut stump surfaces, and spreads to neighbouring trees via root-to-root contacts (Redfern and Stenlid, 1998; Stenlid and Redfern, 1998). Once the fungus has entered a stand, control of the disease is very difficult and the forest management practices aiming to reduce losses caused by *Heterobasidion* are of great value. In Europe and U.S.A, forest managers are aware of the presence and threats of *Heterobasidion* on coniferous forests and therefore, chemical, biological or silvicultural measurements are routinely applied to control the disease. In Turkey, in contrast, both the forest managers and the forest pest specialists are usually unaware of its presence or its danger. Nevertheless, the presence of *Heterobasidion* in Turkish coniferous forest has already been proved (Doğmuş-Lehtijärvi et al., 2006, 2007, 2008, 2010).

In Europe, the fungus has been devided into three species, *H. annosum* s.s., *H. parviporum* and *H. abietinum* (former P, S and F intersterility groups) based on their main host preferences, pine, spruce, and fir, respectively. In Turkey, *Heterobasidion abietinum* (Fr.) Niemelä & Korhonen is the dominant species in the genus on stumps of

native *Abies* taxa: *Abies cilicica* (Ant. & Kotsch.) Carr., *A. nordmanniana* ssp. *bornmülleriana* (Mattf.) Coode & Cullen, *A. nordmanniana* ssp. *equi-trojani* (Aschers & Sint. ex Boiss.) Coode & Cullen and *A. nordmanniana* (Steven) Spach ssp. *nordmanniana* (Doğmuş-Lehtijärvi et al. 2006, 2007). The disease incidence caused by *Heterobasidion* has received little attention on fir in Turkey until now. Recently, Doğmuş-Lehtijärvi et al. (2008) demonstrated that the disease incidence on freshly-cut stumps of approximately 90 year-old *A. cilicica* and 75- and 120 year-old *A. nordmanniana* ssp. *bornmülleriana* was 11.5, 18.8 and 28.2%, respectively.

The aim of this study was to investigate the incidence of butt rot caused by *H. annosum* s.l. in *A. nordmanniana* ssp. *bornmülleriana* stands in Western Black Sea Region of Turkey.

Material and Methods

Study sites and ecological characterisation

This study was carried out in two stands of *A. nordmanniana* ssp. *bornmülleriana* in Western Black Sea Region of Turkey in Kastamonu province (Fig. 1). The size of the stands varied between 0.6-10 hectares. The age of the stands was 25-80 years (Table 1).

Sampling area	Coordinates	Altitude (m)	Status	Stand properties
Küre	36 560 367 D 46 27 772 K	1018	Managed forest	Pure stand
Ilgaz (Kırkpınar yaylası)	36 553 286 D 45 399 30 K	1795	Managed forest	Mixed with *Pinus sylvestris*

Figure 1. Location of sampling areas in Kastamonu province

Field survey and sample collection

In each of the two stands, 50 *A. nordmanniana* ssp. *bornmülleriana* trees were randomly selected according to root rot symptoms and cut with a chainsaw. Discs were placed into nylon bags and brought to the laboratory. Height and d.b.h. of sampled trees were recorded.

Fungal isolation and Assessment of *Heterobasidion* species based on DNA techniques

Discs (without washing or removing the bark) were incubated in the dark at room temperature (approx. 23 ºC) for 7 days. The area colonized by *H. annosum* s.l. was determined as described in Korhonen (2003). A transparent film with a sq.cm grid was fixed onto the upper surface of each disc and the area occupied by the conidial stage of the fungus determined under a stereomicroscope.

During this investigation, conidia of *H. annosum* s.l. were taken with a needle and placed onto malt-extract agar (Merck) plates. The isolates were paired with known tester strains. The pairings were made in Petri plates containing malt extract agar medium. The inoculates were approximately 5 mm apart from each other and they were incubated at room temperature for 3 to 5 weeks. After this period, the change of the

mycelial appearance and occurrence of clamp connection were observed.

Prior to DNA extraction, fungal isolates were grown on agar media covered with cellophane membrane at 25°C for two weeks. After incubation the growing mycelia was collected and ground in liquid nitrogen in a mortar. Fungal DNA was extracted using DNeasy Plant Mini Kit (Qiagen). DNA of each isolates was amplified with MJF (GGTCCTGTCTGGCTTTGC) and MJR (CTGAAGCACACCTTGCCA) and KJF (CCATTAACGGAACCGACGTG) and KFR (GTGCGGCTCATTCTACGCTATC**)** primers specific to *H. annosum* s.str and *H. parviporum* respectively. The amplifications were carried out using a 'hot start' protocol where DNA samples and primers (each in a concentration of 0.5 uM) were denaturated at +95 °C for 10 minutes, after which a dNTP-mix (each deoxynucleotide in a concentration of 0.2 mM) and 20 U/ml of DNA-polymerase (Promega) were added. Then 40 cycles of amplification (30 s denaturation at 95 °C, 35 s annealing at 67 °C, 1 min extension at 72 °C) and a final extension of 7 minutes were carried out.

Results and Discussion

A total of 100 trees were examined, of which 33 contained advanced decay and were found by inspection to be caused by *H. annosum*. All fungal isolates were determined to be *H. abietinum,* based on morphological and DNA-based techniques.

Table1. Laboratory and field results

	A. bornmülleriana					
	Ilgaz		**Küre**		**Total**	
		Mean		**Mean**		**Mean**
Disease incidence (%)	**Min-max**	13	**Min-Max**	20	**Min-Max**	
Diameter	27-50	11	25-70	22	26-60	43

Annosum root and butt rot incidence varied between sampled stands, 28-34% on average (Table 1).

There was a positive correlation between stand age and root rot frequency ($r = 0.317$, $p < 0.001$). Larger stumps were more likely to contain decay.

The present study indicates that about one-fifth of *A. bornmülleriana* trees in Kastamonu province in Black Sea region forests are infected by *Heterobasidion abietinum* and that infection frequency among individual stands varies greatly. The results are comparable with 11.5, 18.8 and 28.2% rot frequency (Doğmuş-Lehtijärvi et al., 2007, 2008, 2010) detected during previous inventories in Turkish fir forests.

At high altitudes the incidence of disease is lower than at sea level, probably due to climatic factors and a shorter growing season (Korhonen & Stenlid, 1998). The extension of decay is also affected by genotype to the same extent as other investigated traits, e.g. height growth (Swedjemark, Stenlid & Karlsson, 1997, 2001; Swedjemark & Karlsson, 2002, 2004).

H. abietinum occurs generally more frequently in managed pure fir forests than in mixed stands. Additionally, butt rot frequency in a stand is influenced by different factors such as site history, site management, previous tree rotation, and stem damage by game (Isomäki and Kallio, 1974; Swedjemark and Stenlid, 1993; Piri, 2003). In forests lacking previous management, the fungus seldom causes significant damage to the trees (Korhonen et al., 1998; Redfern and Stenlid, 1998). The high infection incidence of *H. abietinum* in pure stand in Küre was most likely due to the spread via root contact and recent cuttings in the forest.

Felling in warmer seasons during the growth period creates a higher risk of infection of the trees by pathogens via both fresh stumps, which are known as the main *Heterobasidion* spp. infection source, and mechanical wounds (Rönnberg, 2000; Vasiliauskas, 2002).

This study showed that *H. abietinum* is the main cause of butt rot in Kastamonu province in *A. bornmülleriana* stands, which indicates that such forest sites are

permanently infested and that the disease will persist over future forest generations.

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References

Bendz- Hellgren, M., Lipponen, K., Solheim, H., Thomsen, I.M., 1998. The Nordic countries. *In*: Woodward, S., Stenlid, J., Karjalainen, R., Hüttermann, A. (Eds), *Heterobasidion annosum*: Biology, Ecology, Impact and Control, CAB Internatinal, Wallingford, UK, pp. 333- 345.

Doğmuş-Lehtıjärvı, H.T., Lehtijärvi, A. and Korhonen, K., 2006. *Heterobasidion abietinum* on Abies species in western Turkey. Forest Pathology, 36 (4), 280-286.

Doğmuş- Lehtıjärvı, H.T., Lehtijärvi, A., Hatat- Karaca, G., Aday, A.G., 2007b. *Heterobasidion annosum* s. l.'un Uludağ göknarında oluşturduğu alt gövde çürüklüğünün arazi ve laboratuar metotları ile tespiti. S.D.Ü. Orman Fakültesi Dergisi. Seri A, Sayı 1: 58–67.

Doğmuş Lehtijärvi, H.T., Lehtijärvi, A., Aday, A.G., Oskay, F. *Annosum* kök Çürüklüğüne Karşı Uygulanan Biyolojik Kontrol Ajanı; Phlebiopsis gigantea. 3. Ulusal Karadeniz Ormancılık Kongresi, 20-22 Mayıs 2010, Artvin. Cilt IV: 1403–1410

Korhonen, K., 1978. Intersterility groups of *Heterobasidion annosum*. Communicationes of Instituti Forestalis Fenniae 94 (6), 25-25.

Korhonen, K., Capretti, P., Karjalainen, R. and Stenlid, J., 1998. Distribution of Heterobasidion annosum intersterility groups in Europe. In: *Heterobasidion annosum*. Biology, Ecology, Impacr and Control. Ed by Woodward, S., Stenlid, J., Huttermann, A., Karjalainen, R. Oxon, New York: CAB International, 93-104.

Pırı, T., Korhonen, K. and Sairanen, A., 1990. Occurence of *Heterobasidion annosum* in pure and mixed spruce stands in Southern Finland. Scandinavian Journal of Forest Research 5, 113-125.

Redfern, D. B. and Stenlid, J., 1998. Spore dispersal and infection. In: *Heterobasidion annosum*. Biology, Ecology, Impact and Control. Ed. by Woodward, S.; Stenlid, J.; Hu¨ ttermann, A.; Karjalainen, R. Oxon, New York: CAB International, pp. 105–124.

Stenlıd, J., 1994b. Regional differentiation in Heterobasidion annosum. In: Johansson, M. and Stenlid, J. (Eds), Proceeding of the Eight IUFRO Conference on Root and Butt Rots. Sweden/Finland August 1993. Swedish University of Agricultural Sciences, Uppsala, Sweden, pp. 243-248.

Stenlid, J. and Wästerlund, I., 1986. Estimating the frequency of stem rot in *Picea abies* using an increment borer. Scandinavian Journal of Forest Research, 1, 303-308.

Swedjemark, G. and Stenlid, J., 1993. Population dynamics of the root rot fungus *Heterobasidion annosum* following thinning of Picea abies. Oikos 66: 247- 254.

Vasiliauskas, R., Juska, E., Vasiliauskas, A., Stenlid, J., 2002. Community of Aphyllophorales and root rot in stumps of *Picea abies* on clear-felled forest sites in Lithuania. Scand. J. Forest Res. 17, 398–407.