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NOTE

Seasonal availability of inoculum of the Heterobasidion root disease pathogen in central Wisconsin

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Abstract: After deposition of airborne basidiospores, the root disease pathogen *Heterobasidion irregulare* Garbelotto and Otrosina infects fresh conifer stumps and spreads through root grafts or by root contact to adjacent trees. Infection can be prevented, however, by borate application. Because the need for stump protection depends on inoculum availability, spore trapping was conducted (usually biweekly) from September 2009 through December 2011 in three infested plantations of predominantly red pine (*Pinus resinosa* Aiton) in central Wisconsin. A semiselective medium in Petri plates was exposed for 1 h in daylight at each of four locations in each plantation. After 7–10 days incubation at 20 °C, plates were examined and presence and abundance of colonies of the *Spiniger* asexual stage were recorded. *Heterobasidion irregulare* was detected on most dates during the two growing seasons, but colonies were most abundant during late summer, fall, and early winter. Relatively fewer colonies developed on medium exposed during periods of coldest winter temperatures, but colonies of the pathogen did develop frequently on medium exposed at ≤ 5 °C and occasionally on medium exposed at ≤ 0 °C. Biologically based guidelines for stump treatment require additional studies of seasonal factors influencing inoculum availability, in situ spore germination, infection, and establishment of the pathogen.

Key words: Heterobasidion irregulare, inoculum, *Pinus resinosa*.

Résumé : Après le dépôt des basidiospores disséminées par le vent, le champignon pathogène *Heterobasidion irregulare* Garbelotto & Otrosina, responsable d'une maladie de racines, infecte les souches de conifère fraîchement coupées et se propage vers les arbres adjacents par les greffes de racines et les contacts entre les racines. Il est cependant possible de prévenir l'infection par l'application de borate. Parce que la nécessité de protéger les souches dépend de la disponibilité de l'inoculum, les spores ont été piégées (habituellement sur une base bihebdomadaire) du mois de septembre 2009 jusqu'en décembre 2011 dans trois plantations infectées composées surtout de pin rouge (*Pinus resinosa* Aiton) dans le centre du Wisconsin. Des plats de Petri contenant un milieu de culture semisélectif ont été exposés pendant une heure durant le jour à quatre endroits dans chaque plantation. Après 7 à 10 j d'incubation à 20 °C, les plats ont été examinés et la présence ainsi que l'abondance des colonies de l'anamorphe *Spiniger* ont été notées. *Heterobasidion* a été détecté à la plupart des dates durant les deux saisons de croissance mais les colonies étaient plus abondantes à la fin de l'été, en automne et au début de l'hiver. Relativement moins de colonies se sont développées dans les plats exposés lors des périodes hivernales les plus froides mais des colonies du pathogène se sont fréquemment développées dans les plats exposés à ≤ 5 °C et occasionnellement à ≤ 0 °C. D'autres études des facteurs saisonniers qui influencent la disponibilité de l'inoculum, la germination in situ des spores, l'infection et l'établissement du pathogène seront nécessaires pour élaborer des directives fondées sur la biologie concernant le traitement des souches. [Traduit par la Rédaction]

Mots-clés : Heterobasidion irregulare, inoculum, *Pinus resinosa*.

Introduction

The fungal pathogen *Heterobasidion irregulare* Garbelotto and Otrosina is a recently described species in the former *H. annosum* sensu lato species complex (Otrosina and Garbelotto 2010). Although reported hosts or substrates of *H. irregulare* include a variety of conifer species in both eastern and western North America (Sinclair 1964), this species was previously referred to as the intersterility group P (for pine, *Pinus* L.) of *H. annosum* (Otrosina and Garbelotto 2010). After infection of fresh conifer stump surfaces following deposition of airborne basidiospores, *H. irregulare* spreads through root grafts or by root contact to adjacent trees, which results in expanding root disease centers. Thus, high incidence of *H. irregulare* and severe damage that can include growth loss and mortality of pines has frequently occurred following thinning of commercial pine plantations in the southern United States (Mason 1969; Powers and Hodges 1970; Bradford et al. 1978*a*, 1978*b*). As is known for other Heterobasidion root disease pathogens, application of protective chemicals including urea and borates or the biocontrol fungus *Phlebiopsis gigantea* (Fr.) Julich can prevent infection that occurs after deposition of *H. irregulare* spores on freshly cut stumps (Berry and Bretz 1964; Ross and Hodges 1981; Dumas and Laflamme 2013).

Detection or quantification of basidiospore inoculum of *Heterobasidion* species in forests is commonly accomplished by exposure of conifer stem disks or culture media. After incubation, confirmation of the presence of these fungi is possible due to production of conidiophores with conidia of their asexual *Spiniger* Stalpers stage (Brefeld 1889; Stalpers 1974). These methods have been employed by many previous researchers for pathogen detec-

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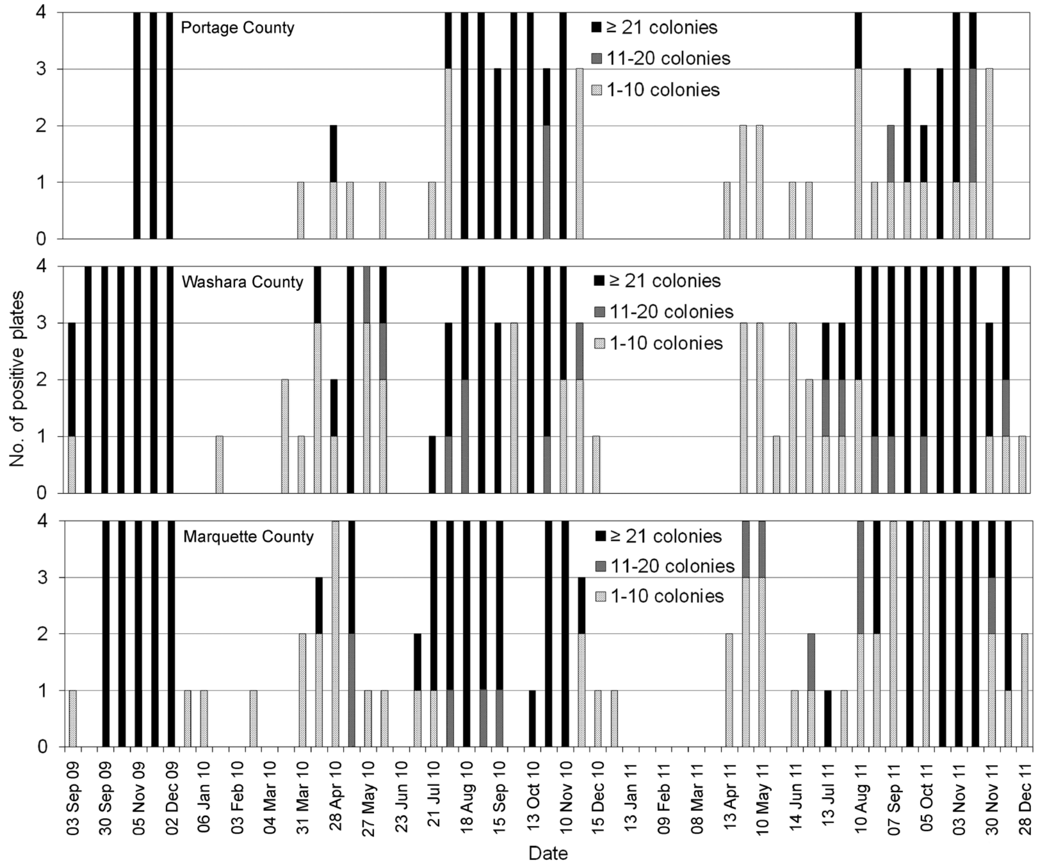
Fig. 1. Numbers of Petri plates of semiselective agar medium exposed for 1 h in daylight at each of three infested sites on which the *Spiniger* asexual stage of *Heterobasidion irregulare* was detected. Spore trapping did not begin until 5 November 2009 in Portage County and did not occur in Waushara County on 23 June and 8 July 2010 and Portage County on 13 July and 26 July 2011 due to harvest activity. Shading of bars indicates the numbers of *H. irregulare* colonies on each plate.



tion and investigations of seasonal and spatial variation in presence of inoculum of *Heterobasidion* species in both Europe and North America (e.g., Rishbeth 1951; Kallio 1970; Stambaugh et al. 1962; Boyce 1963; Sinclair 1964; Möykkynen et al. 1997; Ross 1973; Edmonds et al. 1984; Gonthier et al. 2005).

Since initial detection of *H. irregulare* at a single site in central Wisconsin in 1993 (Stanosz et al. 1995), this pathogen has been found much more widely in that state. Heterobasidion root disease is now known to occur in at least 24 of 72 Wisconsin counties, most frequently damaging highly productive plantations of red pine (*Pinus resinosa* Aiton) (Wisconsin Department of Natural Resources 2015). Red pine is the most commonly planted conifer species in the Great Lakes region of the United States, where it is most commonly managed in single species, even-aged plantations (Gilmore and Palik 2006). Pulp, lumber, and utility poles are produced in economic rotations of 55–90 years with thinning at regular intervals, but the red pine is long lived and can reach ages of 200 years or more. At the end of a rotation, stands are typically replanted with red pine seedlings following either complete overstory removal or with retention of small numbers of overstory trees. Among other common economically and ecologically important conifer species in the Great Lakes region, the following are some that are proven or potentially significant hosts of *H. irregulare*: eastern white pine (*Pinus strobus* L.), jack pine (*Pinus banksiana* Lamb.), balsam fir (*Abies balsamea* (L.) P. Mill.), eastern hemlock (*Tsuga canadensis* (L.) Carrière), northern white-cedar (*Thuja occidentalis* L.), eastern red-cedar (*Juniperus virginiana* Sarg.), white spruce (*Picea glauca* (Moench) Voss), black spruce (*Picea mariana* (Miller) B.S.P.), and tamarack (*Larix laricina* (Du Roi) K. Koch).

Almost 20 years after discovery in the state, the Wisconsin Department of Natural Resources provided a “risk-based guide for the fungicide treatment” to prevent Heterobasidion root disease (Wisconsin Department of Natural Resources 2013). A stump protectant that is currently available in the United States is a borate material applied in water either manually or through the heads of harvesting equipment (Cellutreat®, Nisus Corporation, Rockford, Tennessee). Current, local information regarding seasonal variation in presence and abundance of inoculum of *H. irregulare* in North America is limited, however, and these have not been investigated in the Great Lakes region of the United States. The following study was undertaken to obtain data that might assist managers to both justify and increase efficiency of efforts to prevent Heterobasidion root disease in that region. Spores were trapped on a semiselective medium in Petri plates that were exposed in infested red pine plantations at regular intervals for 28 months, including during periods of subfreezing winter temperatures.

Materials and methods

Spore trapping was attempted (usually biweekly except as noted in Fig. 1) during September 2009 – December 2011 in mature, commercial red pine plantations in central Wisconsin counties of Waushara (44.20°N, −89.24°W), and Marquette (43.67°N, −89.35°W) and November 2009 – December 2011 in Portage County (44.58°N, −89.53°W). Sites were flat or gently sloping at elevations of 240–340 m above sea level with well-drained sand, loamy sand, or sandy loam soils (USDA Natural Resources Conservation Service 2013). These soils are relatively infertile and prone to drought in the summer and were historically often occupied by xeric pine forests and scrub oak communities (Curtis 1959). The climate is continental with low to moderate levels of precipitation, cool to warm summers, and cold winters (Moran and Hopkins 2002). Except for temperature during each brief period of spore trapping (as described below), weather conditions at these plantations were not monitored. However, available weather data and snow depths recorded at nearby weather recording stations were obtained (Wisconsin State Climatology Office 2016) and are summarized in Supplementary Table S1[1].

Each plantation had been thinned at least twice without treatment to prevent stump infection and was known to be infested with *H. irregulare*. The identity of the pathogen at these sites was confirmed by comparing ITS sequences from each of 14 isolates (4–6 per site) obtained from host material to the ITS sequence for the well-characterized *H. irregulare* strain TC32-1 (GenBank accession No. FJ627586) (Olson et al. 2012). Sequence data from each of these isolates was greater than 99% similar to sequence data of strain TC32-1 (data not shown). At each site, four red pine stumps that bore basidiocarps of *H. irregulare* at the start of the study were used throughout as locations for placement of spore traps. Spore traps consisted of 9 cm diameter Petri plates with semiselective medium (20 g agar, 5 g Bacto-peptone, 0.5 g $KHPO_4$, 0.51 g $MgSO_4$, 1000 mL H_2O, plus the following added after autoclaving: 2 mL 50% lactic acid, and both 0.1 g streptomycin sulfate and 0.19 g pentachloronitrobenzene suspended in 10 mL 95% EtOH) (modified from Kuhlman and Hendrix (1962)). On each date of sampling, one plate was placed on each of the four selected stumps in each plantation and opened to expose the medium for 1 h, during which there was no measurable precipitation. All three sites were sampled in succession during daylight on the same day. A digital thermometer was placed next to one stump at each site to monitor temperature during the 1 hr exposure period. Plates were closed, brought to the laboratory, and after 7–10 days incubation at approximately 20 °C, were examined using a dissecting microscope at approximately 30× magnification. If necessary, slides were prepared and examined using a compound microscope at up to 400× magnification.

The number of plates deployed at each site on each date on which *H. irregulare* was identified after incubation was recorded. These data were plotted by date, and correlations of these data among sites were tested using Minitab Release 14.1 (Minitab Inc., State College, Pennsylvania). Numbers of colonies of this fungus per plate were recorded as 0, 1–10, 11–20, or ≥ 21, and these data also were plotted by date.

Results

Although a variety of fungi (not identified) did develop on the exposed medium, radial growth was somewhat restricted to allow distinct colonies to be examined. After 7–10 days, colonies of hyaline *H. irregulare* hyphae were ≤ 5 mm in diameter and thin, appearing transparent to faintly white. Characteristics of the *Spiniger* asexual conidiophores and conidia borne at their swollen apices allowed differentiation of *H. irregulare* colonies from others with similar morphology.

The number of plates for each date on which *H. irregulare* was identified were correlated among the three sites (values of r for pairwise comparisons ≥ 0.65). Further, due to similarity among sites in the number of colonies per plate on respective dates, a pattern is evident (Fig. 1). Spores were frequently trapped and were often most numerous in late summer and fall. For example, if each discrete *H. irregulare* colony detected originated from a single spore, deposition resulting in one or 20 colonies on medium exposed for 1 h in a 9 cm diameter Petri plate (area of approximately 64 cm^2) is equivalent to approximately 160 or 3200, respectively, viable spores deposited per m^2 per hour). Colonies of *H. irregulare* also developed, though less abundantly than on plates exposed in autumn, on plates exposed in spring and summer months beginning as early as April in 2010 and 2011. Trapping of spores, sometimes in very large numbers, continued into December of each year. At least 21 colonies of *H. irregulare* were recorded for multiple plates exposed at two of the three sites in mid-December 2011, but numbers of positive plates and colonies per plate diminished at all three sites in mid-December 2009 and 2010 after persistent snow cover (Fig. 1; Supplementary Table S1[1]). Spores were infrequently trapped during months of January–March 2010 and were not trapped during those months in 2011. However, spores were trapped on numerous dates in the winter when temperatures recorded during exposure remained ≤ 5 °C or even ≤ 0 °C (Table 1; Supplementary Table S1[1]).

Discussion

Knowledge of the availability of *H. irregulare* inoculum gained during this study should contribute to recognition of the increasing risk posed by Heterobasidion root disease to forests of the Great Lakes region. Not only were viable spores trapped during much of the growing season (and some winter periods), but they were also sometimes very abundant. Thus, there is great potential for inoculation of additional unprotected, fresh stump surfaces in already infested stands and also in adjacent areas during much of the year. Further investigation of the possibility of inoculum dissemination during winter, especially during periods of mild weather, and implications regarding infection are justified.

The pattern of seasonal variation in availability of *H. irregulare* inoculum observed at all three sites of this study is generally similar to that often described for other Heterobasidion root disease pathogens. Gonthier et al. (2005) investigated the spore deposition of *Heterobasidion* species in four forests of the western Alps and found that "inoculum concentration consistently peaked in late summer or early fall". The very large numbers of spores deposited in our study were sometimes much greater that those reported in two studies of deposition of *H. irregulare* inoculum in the northeastern United States (Sinclair 1964; Stambaugh et al. 1962). Well-documented influences of local, temporal environmental factors on both inoculum production and dispersal (Sinclair 1964; Kallio 1970) that were not within the scope of this study and a steep, decreasing gradient in deposition of *Heterobasidion* inoculum with increasing distance from sources (e.g., Kallio 1970; Möykkynen et al. 1997) also are likely sources of such differences. Results could also be affected by differences in trapping efficiency and ease of pathogen recognition between pine stem disks or stumps (used in those studies) and Petri plates of a semiselective medium. However, our results might still underestimate *Heterobasidion* inoculum availability that can occur under the most conducive conditions. For example, Kallio (1970) reported deposition equivalent to > 45 × 10^6 spores·m^{-2}·$hour^{-1}$ on agar exposed for just 5 s under *H. annosum* sensu lato basidiocarps.

The observed availability of *Heterobasidion* inoculum early in the growing season in central Wisconsin is consistent with reports from numerous, widely distributed European studies (e.g., East Anglia, England, Rishbeth 1951; southern Finland, Kallio 1970; western Italian Alps, Gonthier et al. 2005). Although knowledge from research in northern temperate forests of eastern North America is much more limited, early spring fertility of basidiocarps was reported by Sinclair (1964). Of those collected at various sites in the state of New York from mid-April to mid-June, 23% bore mature basidia and basidiospores. Additionally, red pine stem disks were exposed as spore traps in an infested plantation of Scots

[1]Supplementary data are available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/cjfr-2016-0136.

Table 1. Detection of *H. irregulare* (×) on semiselective agar medium in at least one of the four Petri plates exposed for approximately 1 h during which temperatures did not exceed 5 °C or 0 °C on various dates over 28 months at three sites in central Wisconsin.

Site	Date													
	05 Nov 09	02 Dec 09	16 Dec 09	06 Jan 10	20 Jan 10	16 Feb 10	01 Dec 10	15 Dec 10	28 Dec 10	03 Nov 11	16 Nov 11	30 Nov 11	15 Dec 11	28 Dec 11
Temperature: ≤ 5 °C														
Portage County	×	×					×			×	×	×	×	
Waushara County		×			×		×	×			×	×		×
Marquette County		×	×	×		×	×	×	×		×	×	×	×
Temperature: ≤ 0 °C														
Portage County							×						×	
Waushara County					×		×	×						
Marquette County			×	×		×	×	×	×					

Note: Dates are given as day month year.

pines (*Pinus sylvestris* L.) and red pines near Varna, New York (42.46°N, –76.44°W) from late June to mid-October 1960 and early June to mid-November 1961. Although the highest deposition rates occurred in late summer and fall, deposition of over 1000 viable spores·m^{-2}·$hour^{-1}$ occurred on two different dates in June in one of the two years of that study. Thus, *H. irregulare* inoculum from basidiocarps produced the previous fall and that have survived relatively harsh winter conditions is likely to occur and occasionally be abundant, even in very early spring.

Development of colonies from spores deposited on plates exposed at low and subfreezing temperatures also indicates survival of mature basidia with basidiospores with at least potential for periodic dissemination during winter. Abundant discharge of *H. annosum* sensu lato basidiospores after a period of cold weather during which minimum air temperatures ranged from –3 °C to –7 °C was reported by Rishbeth (1951). Kallio (1970) noted an occasion on which basidiospores were trapped at –1.0 °C. Basidiospores trapped at subfreezing temperatures in our study may have matured during an earlier, warmer period and been simply dislodged by air currents during the hour of subfreezing exposure of spore traps. Alternatively, incident solar radiation may warm a basidiocarp sufficiently to allow discharge.

Although winter dissemination and subsequent basidiospore germination and conifer stump infection by *Heterobasidion* pathogens in northern forests may be less frequent when harvesting is accomplished in winter, it does occur. For example, Thor and Stenlid (2005) investigated efficacy of materials and methods used to treat *Picea abies* (L.) Karst stumps from both summer and winter thinning in stands infested by *H. annosum* sensu lato in southern to central Sweden. Several weeks after trees were cut, disks were cut from stump surfaces and incubated in the laboratory. In spite of development of substantially smaller *Heterobasidion* colonies on most disks from the winter-thinned stumps, the probability of infection of these stumps was approximately 0.1. Comparable studies are lacking in northern portions of eastern North America with severe winters. In a more southern site, however, Stambaugh et al. (1962) investigated infection of eastern white pine stumps resulting from winter thinning in the Tuscarora State Forest (40.30°N, 77.59°W) of Pennsylvania. Frequency of stump infection was ≥ 70% for stumps from the December, January, and February thinnings, even when maximum recorded temperatures were usually ≤ 5 °C, sometimes not exceeding 0 °C, and when minimum recorded temperatures were well below 0 °C.

The prolonged availability of *H. irregulare* inoculum is relevant in the context of the response to its detection and current proliferation in Wisconsin and also the very recent report of Heterobasidion root disease in neighboring Minnesota (Blanchette et al. 2015). The Wisconsin Department of Natural Resources "risk-based guide for the fungicide treatment" to prevent Heterobasidion root disease (Wisconsin Department of Natural Resources 2013) has been developed using very limited knowledge of *H. irregulare* biology or host and environmental influences on disease development in the Great Lakes region. Presumably due to the practical difficulty in application of a water-borne chemical in winter, treatment is recommended from April 1 to November 30 but not after November 30 "except under unusual weather patterns". Examples provided include "prolonged unusually warm weather during the winter period (Dec 1 – Mar 31)". Dissemination of viable basidiospores during this period, including at subfreezing temperatures, is the first requirement for infection of fresh stumps when left untreated in winter. The potential for survival of either winter-deposited *H. irregulare* inoculum or incipient colonies of the pathogen in stumps resulting from winter thinning in the Great Lakes region, and contribution to continued proliferation, is a subject of ongoing studies.

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References

Berry, F.H., and Bretz, T.W. 1964. Urea and other chemicals effective against colonization of shortleaf pine stumps by *Fomes annosus* in Missouri. Plant Dis. Rep. **48**(11): 886–887.

Blanchette, R.A., Held, B.W., Mollov, D., Blake, J., and D'Amato, A.W. 2015. First report of *Heterobasidion irregulare* causing root rot and mortality of red pines in Minnesota. Plant Dis. **99**(7): 1038. doi:10.1094/PDIS-11-14-1232-PDN.

Boyce, J.S., Jr. 1963. Colonization of pine stem sections by *Fomes annosus* and other fungi in two slash pine stands. Plant Dis. Rep. **47**: 320–324.

Bradford, B., Alexander, S.A., and Skelly, J.M. 1978*a*. Determination of growth loss of *Pinus taeda* L. caused by *Heterobasidion annosus* (Fr.) Bref. Eur. J. For. Pathol. **8**(3): 129–134. doi:10.1111.j.1439-0329.1978.tb01459.x.

Bradford, B., Alexander, S.A., and Skelly, J.M. 1978*b*. Incidence and severity of annosus root rot in loblolly pine plantations in Virginia. Eur. J. For. Pathol. **8**(3): 135–145. doi:10.1111/j.1439-0329.1978.tb01460.x.

Brefeld, O. 1889. Untersuchungen aus dem Gesammtgebiete der Mykologie, Heft 8. Leipzig, Germany. doi:10.5962/bhl.title.3887. [In German].

Curtis, J.T. 1959. The vegetation of Wisconsin: an ordination of plant communities. University of Wisconsin Press, Madison, Wisconsin.

Dumas, M.T., and Laflamme, G. 2013. Efficacy of two *Phlebiopsis gigantea* formulations in preventing *Heterobasidion irregulare* colonization of red pine stumps in eastern Canada. Phytoprotection, **93**(1): 25–31. doi:10.7202/1018887ar.

Edmonds, R.L., Hinshaw, R.W., and Leslie, K.B. 1984. A 24-hour deposition sampler for spores of *Heterobasidion annosum*. Phytopathology, **74**: 1032–1034. doi:10.1094/Phyto-74-1032.

Gilmore, D.W., and Palik, B.J. 2006. A revised managers handbook for red pine in the North Central Region. USDA Forest Service, North Central Research Station, St. Paul, Minnesota, General Technical Report NC-264.

Gonthier, P., Garbelotto, M.M., and Nicolotti, G. 2005. Seasonal patterns of spore deposition of *Heterobasidion* species in four forests of the Western Alps. Phytopathology, **95**: 759–767. doi:10.1094/PHYTO-95-0759. PMID:18943007.

Kallio, T. 1970. Aerial distribution of the root-rot fungus *Fomes annosus* (Fr.) Cooke in Finland. Acta For. Fenn. **107**: 55.

Kuhlman, E.G., and Hendrix, F.F., Jr. 1962. A selective medium for the isolation of *Fomes annosus*. Phytopathology, **52**: 1310–1312.

Mason, G.N. 1969. An evaluation of the incidence of *Fomes annosus* in East Texas. Plant Dis. Rep. **53**(12): 936–939.

Moran, J.M., and Hopkins, E.J. 2002. Wisconsin's weather and climate. University of Wisconsin Press, Madison, Wisconsin. p. 376.

Möykkynen, T., von Weissenberg, K., and Pappinen, A. 1997. Estimation of dispersal gradients of S- and P-type basidiospores of *Heterobasidion annosum*. Eur. J. For. Pathol. **27**: 291–300. doi:10.1111/j.1439-0329.1997.tb01083.x.

Olson, Å., Aerts, A., Asiegbu, F., Belbahri, L., Bouzid, O., Broberg, A., Canbäck, B., Coutinho, P.M., Cullen, D., Dalman, K., Deflorio, G., van Diepen, L.T.A., Dunand, C., Duplessis, S., Durling, M., Gonthier, P., Grimwood, J., Fossdal, C.G., Hannson, D., Henrissat, B., Hietala, A., Himmelstrand, K., Hoffmeister, D., Högberg, N., James, T.Y., Karlsson, M., Kohler, A., Kües, U., Lee, Y.-H., Lin, Y.-C., Lind, M., Lindquist, E., Lombard, V., Lucas, S., Lundén, K., Morin, E., Murat, C., Park, J., Raffaello, T., Rouzé, P., Salamov, A., Schmutz, J., Solheim, H., Ståhlberg, J., Vélëz1, H., de Vries, R.P., Wiebenga, A., Woodward, S., Yakovlev, I., Garbelotto, M., Martin, M., Grigoriev, I.V., and Stenlid, J. 2012. Insight into trade-off between wood decay and parasitism from the genome of a fungal forest pathogen. New Phytol. **194**(4): 1001–1013. doi:10.1111/j.1469-8137.2012.04128.x. PMID:22463738.

Otrosina, W.J., and Garbelotto, M. 2010. *Heterobasidion occidentale* sp. nov. and *Heterobasidion irregulare* nom. nov.: a disposition of North American *Heterobasidion* biological species. Fungal Biol. **114**(1): 16–25. doi:10.1016/j.mycres.2009.09.001.

Powers, H.R., Jr., and Hodges, C.S., Jr. 1970. Annosus root rot in eastern pines. USDA Forest Service, For. Pest Leaflet 76.

Rishbeth, J. 1951. Observations on the biology of *Fomes annosus* with particular reference to East Anglian pine plantations. II. Spore production, stump infection, and saprophytic activity in stumps. Ann. Bot. (Oxford, U. K.), **15**(1): 1–22.

Ross, E.W. 1973. *Fomes annosus* in the southeastern United States: relation of environmental and biotic factors to stump colonization and losses in the residual stand. USDA Forest Service, Technical Bulletin 1459.

Ross, E.W., and Hodges, C.S., Jr. 1981. Control of *Heterobasidion annosum* colonization in mechanically sheared slash pine stumps treated with *Peniophora gigantea*. USDA Forest Service, Research Paper SE-229.

Sinclair, W.A. 1964. Root- and butt-rot of conifers caused by *Fomes annosus*, with special reference to inoculum dispersal and control of the disease in New York. Memoir 391 of the Cornell University Agricultural Experiment Station, New York State College of Agriculture, Ithaca, New York.

Stalpers, J.A. 1974. *Spiniger*, a new genus for imperfect states of Basidiomycetes. Proc. Koninkl. Nedl. Akad. Wetensch. (Ser. C) **77**: 402–407.

Stambaugh, W.J., Cobb, F.W., Jr., Schmidt, R.A., and Krieger, F.C. 1962. Seasonal inoculum dispersal and white pine stump invasion by *Fomes annosus*. Plant Dis. Rep. **46**: 194–198.

Stanosz, G.R., Guthmiller, M.A., and Kearby, W.H. 1995. Root rot of red pine caused by *Heterobasidion annosum* in Wisconsin. Plant Dis. **79**: 859. doi:10.1094/PD-79-0859A.

Thor, M., and Stenlid, J. 2005. *Heterobasidion annosum* infection of *Picea abies* following manual or mechanized stump treatment. Scand. J. Forest Res. **20**(2): 154–164. doi:10.1080/02827580510008338.

USDA Natural Resources Conservation Service. 2013. Web Soil Survey [online]. Available from http://websoilsurvey.sc.egov.usda.gov/App/HomePage.htm [accessed 8 March 2016].

Wisconsin Department of Natural Resources. 2013. A risk-based guide for the fungicide treatment to prevent annosum root rot in Wisconsin [online]. Available from http://dnr.wi.gov/topic/foresthealth/documents/AnnosumTreatmentGuide.pdf [accessed 8 March 2016].

Wisconsin Department of Natural Resources. 2015. Wisconsin DNR Forest Health 2015 Annual Report [online]. Available from http://dnr.wi.gov/topic/ForestHealth/documents/AnnualReport2015.pdf [accessed 8 March 2016].

Wisconsin State Climatology Office. 2016. Climate data from National Weather Service Reporting Stations [online]. Available from http://www.aos.wisc.edu/~sco/clim-history/acis_stn_meta_wi_index.htm [accessed 26 April 2016].