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1 **Seasonal availability of inoculum**
2 **of the Heterobasidion root disease pathogen in central Wisconsin**
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1 Abstract

2 After deposition of airborne basidiospores, the root disease pathogen *Heterobasidion*
3 *irregulare* infects fresh conifer stumps and spreads through root grafts or by root contact to
4 adjacent trees. Infection can be prevented, however, by borate application. Because the need
5 for stump protection depends on inoculum availability, spore trapping was conducted
6 (usually biweekly) from September 2009 through December 2011 in three infested
7 plantations of predominantly red pine (*Pinus resinosa*) in central Wisconsin. A semi-
8 selective medium in Petri plates was exposed for 1 hour in daylight at each of four locations
9 in each plantation. After 7-10 days incubation at 20 °C plates were examined and presence
10 and abundance of colonies of the *Spiniger* asexual stage were recorded. *H. irregulare* was
11 detected on most dates during the two growing seasons, but colonies were most abundant
12 during late summer, fall, and early winter. Relatively fewer colonies developed on medium
13 exposed during periods of coldest winter temperatures, but colonies of the pathogen did
14 develop frequently on medium exposed at ≤ 5 °C and occasionally on medium exposed at ≤ 0
15 °C. Biologically based guidelines for stump treatment require additional studies of seasonal
16 factors influencing inoculum availability, *in situ* spore germination, infection, and
17 establishment of the pathogen.

18

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

20

1 Introduction

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

17
18 Detection or quantification of basidiospore inoculum of *Heterobasidion* species in
19 forests is commonly accomplished by exposure of conifer stem disks or culture media. After
20 incubation, confirmation of the presence of these fungi is possible due to production of
21 conidiophores with conidia of their asexual *Spiniger* Stalpers stage (Brefeld 1888; Stalpers
22 1974). These methods have been employed by many previous researchers for pathogen
23 detection and investigations of seasonal and spatial variation in presence of inoculum of

1 *Heterobasidion* species in both Europe and North America (e.g.: Rishbeth 1951; Kallio 1970;
2 Stambaugh et al. 1962; Boyce 1963; Sinclair 1964; Möykkynen et al. 1997; Ross 1970;
3 Edmonds et al. 1984; Gonthier et al. 2005).

4

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

23

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

13
14 **Materials and methods**

15 Spore trapping was attempted (usually biweekly except as noted in Fig. 1) during
16 September 2009 – December 2011 in mature, commercial red pine plantations in central
17 Wisconsin counties of Waushara (44.20, -89.24), and Marquette (43.67, -89.35) and
18 November 2009 – December 2011 in Portage County (44.58, -89.53). Sites were flat or
19 gently sloping at elevations of 240-340 m above sea level with well-drained sand, loamy
20 sand, or sandy loam soils (USDA Natural Resources Conservation Service 2013). These
21 soils are relatively infertile and prone to drought in the summer and were historically often
22 occupied by xeric pine forests and scrub oak communities (Curtis 1959). The climate is
23 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 Table S1.

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 ITS sequence for the well-characterized *H. irregulare* strain TC32-1 (GenBank accession 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 semi-selective medium (20 g agar, 5 g Bacto-peptone, 0.5 g KHPO₄, 0.51 g MgSO₄, 1000 ml H₂O, 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 hour 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,

1 and after 7-10 days incubation at approximately 20 °C were examined using a dissecting
2 microscope at approximately 30 x magnification. If necessary, slides were prepared and
3 examined using a compound microscope at up to 400 x magnification.

4
5 The number of plates deployed at each site on each date on which *H. irregulare* was
6 identified after incubation was recorded. These data were plotted by date and correlations of
7 these data among sites were tested using Minitab Release 14.1 (Minitab Inc., State College,
8 PA). Numbers of colonies of this fungus per plate were recorded as 0, 1-10, 11-20, or ≥ 21 ,
9 and these data also were plotted by date.

11 Results

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

18
19 The number of plates for each date on which *H. irregulare* was identified were
20 correlated among the three sites (values of r for pairwise comparisons ≥ 0.65). Further, due
21 to similarity among sites in the number of colonies per plate on respective dates, a pattern is
22 evident (Fig. 1). Spores were frequently trapped and were often most numerous in late
23 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 hour in a 9-cm-diameter Petri plate (area of approximately 64 cm²) is equivalent to approximately 160 or 3200, respectively, viable spores deposited m⁻² •hour⁻¹. Colonies of *H. irregulare* also developed, though less abundantly than 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 (Figure 1 and Table S1). 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 winter when temperatures recorded during exposure remained ≤ 5 °C or even ≤ 0 °C (Table 1, Table S1).

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), they were 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 *Heterobasidion* species spore deposition 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 than those reported in two studies of deposition of *H. irregulare* inoculum in the northeastern US (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 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 semi-selective 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 $> 45 \times 10^6$ spores $\cdot$ m⁻² $\cdot$ hour⁻¹ on agar exposed for just 5 seconds 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

1 forests of eastern North America is much more limited, early spring fertility of basidiocarps
2 was reported by Sinclair (1964). Of those collected at various sites in New York state from
3 mid-April to mid-June, 23% bore mature basidia and basidiospores. Additionally, red pine
4 stem disks were exposed as spore traps in an infested plantation of Scots pines (*P. sylvestris*
5 L.) and red pines near Varna, New York (42.46, -76.44) from late June to mid-October 1960
6 and early June to mid-November 1961. Although the highest deposition rates occurred in
7 late summer and fall, deposition of over 1000 viable spores m⁻²•hour⁻¹ occurred on two
8 different dates in June in one of the two years of that study. Thus, *H. irregulare* inoculum
9 from basidiocarps produced the previous fall and that have survived relatively harsh winter
10 conditions is likely to occur and occasionally be abundant, even in very early spring.

11
12 Development of colonies from spores deposited on plates exposed at low and
13 subfreezing temperatures also indicates survival of mature basidia with basidiospores with at
14 least potential for periodic dissemination during winter. Abundant discharge of *H. annosum*
15 *sensu lato* basidiospores after a period of cold weather during which minimum air
16 temperatures ranged from -3 °C to -7 °C was reported by Rishbeth (1951). Kallio (1970)
17 noted an occasion on which basidiospores were trapped at -1.0 °C. Basidiospores trapped at
18 subfreezing temperatures in our study may have matured during an earlier, warmer period
19 and were simply dislodged by air currents during the hour of subfreezing exposure of spore
20 traps. Alternatively, incident solar radiation may warm a basidiocarp sufficiently to allow
21 discharge.

1 Although winter dissemination and subsequent basidiospore germination and conifer
2 stump infection by *Heterobasidion* pathogens in northern forests may be less frequent when
3 harvesting is accomplished in winter, it does occur. For example, Thor and Stenlid (2005)
4 investigated efficacy of materials and methods used to treat *Picea abies* (L.) Karst stumps
5 from both summer and winter thinning in stands infested by *H. annosum sensu lato* in
6 southern to central Sweden. Several weeks after trees were cut, disks were cut from stump
7 surfaces and incubated in the laboratory. In spite of development of substantially smaller
8 *Heterobasidion* colonies on most disks from the winter-thinned stumps, the probability of
9 infection of these stumps was approximately 0.1. Comparable studies are lacking in northern
10 portions of eastern North America with severe winters. In a more southern site, however,
11 Stambaugh et al. (1962) investigated infection of eastern white pine stumps resulting from
12 winter thinning in the Tuscarora State Forest (40.30, 77.59) of Pennsylvania. Frequency of
13 stump infection was $\geq 70\%$ for stumps from the December, January, and February thinnings,
14 even when maximum recorded temperatures were usually $\leq 5\text{ }^{\circ}\text{C}$, sometimes did not exceed
15 $0\text{ }^{\circ}\text{C}$, and when minimum recorded temperatures were well below $0\text{ }^{\circ}\text{C}$.

16
17 The prolonged availability of *H. irregulare* inoculum is relevant in the context of the
18 response to its detection and current proliferation in Wisconsin, and also the very recent
19 report of *Heterobasidion* root disease in neighboring Minnesota (Blanchette et al. 2015). The
20 Wisconsin Department of Natural Resources “risk-based guide for the fungicide treatment”
21 to prevent *Heterobasidion* root disease (Wisconsin Department of Natural Resources 2013)
22 has been developed using very limited knowledge of *H. irregulare* biology or host and
23 environmental influences on disease development in the Great Lakes region. Presumably

1 due to the practical difficulty in application of a water-borne chemical in winter, treatment is
2 recommended from April 1 to November 30, but not after November 30 “except under
3 unusual weather patterns.” Examples provided include “prolonged unusually warm weather
4 during the winter period (Dec 1- Mar 31).” Dissemination of viable basidiospores during this
5 period, including at subfreezing temperatures, is the first requirement for infection of fresh
6 stumps when left untreated in winter. The potential for survival of either winter-deposited *H.*
7 *irregulare* inoculum or incipient colonies of the pathogen in stumps resulting from winter
8 thinning in the Great Lakes region, and contribution to continued proliferation, is a subject of
9 ongoing studies.

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13 of Natural Resources for access to infested plantations, and the USDA Forest Service Special
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20

- 1 **Table 1.** Detection of *H. irregulare* (x) on semi-selective agar medium in at least one of the
 2 four Petri plates exposed for approximately 1 hour during which temperatures did not exceed
 3 5 °C or 0 °C on various dates over 28 months at three sites in central Wisconsin.

Temperature	Site	Date													
		05 Nov 09	02 Dec 09	16 Dec 09	06 Jan 10	20 Jan 10	16 Feb 10	01 Dec 10	15 Dec10	28 Dec10	03 Nov 11	16 Nov 11	30 Nov 11	15 Dec 11	28 Dec 11
$\leq 5^{\circ}\text{C}$	Portage Co.	x	x					x			x	x	x	x	
	Waushara Co.		x			x		x	x			x	x		x
	Marquette Co.		x	x	x		x	x	x	x		x	x	x	x
$\leq 0^{\circ}\text{C}$	Portage Co.							x						x	
	Waushara Co.					x		x	x						
	Marquette Co.			x	x		x	x	x	x					

4

Fig. 1. Numbers of Petri plates of semi-selective agar medium exposed for 1 hour 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 Co. on 23 June and 8 July 2010 and Portage Co. on 13 July and 26 July 2011 due to harvest activity. Shading of bars indicates the numbers of *H. irregulare* colonies on each plate.

Table S1. Temperatures during the 1-hour exposure of spore traps at three sites in Wisconsin, and available weather data recorded for the 14 days prior to spore-trapping date at nearby weather stations. Asterisks indicate that weather station records for the respective 14-day period were incomplete.

Spore-trapping site, weather station location: Portage County, Stevens Point , WI										
Date of spore trapping	Temperature during spore trapping (°C)		Data for 14 days prior to spore trapping							
			Minimum temperature range (°C)		Maximum temperature range (°C)		Liquid precipitation total (mm)	Snowfall total (cm)	Snow depth range (cm)	
	Min	Max	Min	Max	Min	Max			Min	Max
03 Sep 09	-	-	-	-	-	-	-	-	-	-
16 Sep 09	-	-	-	-	-	-	-	-	-	-
30 Sep 09	-	-	-	-	-	-	-	-	-	-
14 Oct 09	-	-	-	-	-	-	-	-	-	-
05 Nov 09	1	4	-4	9	4	18	47	0	0	0
20 Nov 09	8	8	-5	6	7	19	21	0	0	0
02 Dec 09	0	0	-4	6	2	13	29	0	0	0
16 Dec 09	-12	-11	-21	-3	-13	3	21	23	0	18
06 Jan 10	-16	-13	-23	-3	-13	2	38	11	13	15
20 Jan 10	-8	-5	-19	-5	-7	1	5	9	15	18
03 Feb 10	-13	-11	-22	-1	-15	3	5*	7	8	15
16 Feb 10	-5	-5	-18	-7	-4	1	10	16	13	20
04 Mar 10	-4	-2	-14	-3	-2	7	2	4	13	18
16 Mar 10	3	3	-9	4	4	18	16	0	0	13
31 Mar 10	12	14	-9	4	4	19	0	0	0	0
14 Apr 10	8	12	-6	12	3	27	37	0	0	0
28 Apr 10	7	10	-2	8	11	26	22	0	0	0
12 May 10	6	7	-2	12	6	26	64	0	0	0
27 May 10	18	20	3	21	13	32	5	0	0	0
09 Jun 10	22	23	10	18	16	31	75	0	0	0
23 Jun 10	28	29	10	17	17	29	42	0	0	0
08 Jul 10	26	28	8	21	22	30	110	0	0	0
21 Jul 10	20	22	16	19	25	29	111	0	0	0
04 Aug 10	22	24	15	21	19	31	72	0	0	0
18 Aug 10	18	18	12	23	21	31	96	0	0	0
01 Sep 10	19	21	9	22	22	31	35	0	0	0
15 Sep 10	13	13	5	16	15	26	47	0	0	0
30 Sep 10	11	11	3	13	13	23	146	0	0	0
13 Oct 10	6	7	0	9	11	26	1	0	0	0
28 Oct 10	4	5	-2	13	7	18	51	0	0	0
10 Nov 10	10	10	-4	3	4	18	1	0	0	0
01 Dec 10	-7	-7	-11	1	-6	7	32	1	0	0
15 Dec 10	-25	-25	-24	-8	-10	-1	32	32	0	25
28 Dec 10	-7	-7	-24	-4	-10	1	8	9	20	25

Spore-trapping site, weather station location: Portage County, Stevens Point, WI (continued)										
Date of spore trapping	Temperature during spore trapping (°C)		Data for 14 days prior to spore trapping							
			Minimum temperature range (°C)		Maximum temperature range (°C)		Liquid precipitation total (mm)	Snowfall total (cm)	Snow depth range (cm)	
	Min	Max	Min	Max	Min	Max			Min	Max
13 Jan 11	-7	-7	-19	-1	-13	7	6	8	10	13
25 Jan 11	-7	-7	-28	-7	-16	-4	12	22	13	23
09 Feb 11	-21	-21	-23	-6	-13	-1	11	12	20*	30*
22 Feb 11	-9	-9	-26	-2	-13	9	24	42	10	36
08 Mar 11	-1	-1	-18	-3	-11	6	5	6	18	30
24 Mar 11	-10	-10	-12	2	-4	12	49	13	0	23
13 Apr 11	10	10	-8	8	3	24	45	0	0	3
27 Apr 11	5	5	-3	4	2	17	60	11	0	8
10 May 11	14	14	-3	9	4	20	32	0	0	0
01 Jun 11	16	19	1	15	13	31	34	0	0	0
14 Jun 11	18	21	7	23	12	34	12	0	0	0
29 Jun 11	22	24	11	17	17	26	73	0	0	0
13 Jul 11	-	-	12	20	22	33	26	0	0	0
26 Jul 11	-	-	12	23	22	33	108	0	0	0
10 Aug 11	21	33	12	22	22	31	124	0	0	0
24 Aug 11	25	28	12	18	20	29	20	0	0	0
07 Sep 11	12	17	5	19	16	31	51	0	0	0
22 Sep 11	14	14	1	14	12	28	19	0	0	0
05 Oct 11	12	18	-1	8	11	24	45	0	0	0
19 Oct 11	2	7	2	15	10	26	28	0	0	0
03 Nov 11	0	1	-3	3	6	15	18	0	0	0
16 Nov 11	-1	-1	-4*	2*	2	13	36	7	0	5
30 Nov 11	-9	-5	-8	6	-1	11	11	0	0	0
15 Dec 11	1	2	-16	1	-8	8	30	2	0	0
28 Dec 11	-10	-10	-13	1	-4	7	8	4	0	3

Spore-trapping site, weather station location: Waushara County, Hancock, WI										
Date of spore trapping	Temperature during spore trapping (°C)		Data for 14 days prior to spore trapping							
			Minimum temperature range (°C)		Maximum temperature range (°C)		Liquid precipitation total (mm)	Snowfall total (cm)	Snow depth range (cm)	
	Min	Max	Min	Max	Min	Max			Min	Max
03 Sep 09	-	-	4	17	16	26	25	0	0	0
16 Sep 09	-	-	7	13	23	28	0	0	0	0
30 Sep 09	-	-	-2	14	12	26	5	0	0	0
14 Oct 09	2	2	-5	7	3	14	54	0	0	0
05 Nov 09	6	7	-4	10	4	19	40	0	0	0
20 Nov 09	9	9	-6	6	7	20	21	0	0	0
02 Dec 09	3	3	-5	6	2	14	31	0	0	0
16 Dec 09	-7	-6	-21*	-3*	-14*	8*	16*	35*	0*	33*
06 Jan 10	-7	-7	-27	-1	-14	3	45	8	13	23
20 Jan 10	-5	-3	-21	-5	-9	2	3	14	10	25
03 Feb 10	-6	-4	-21*	-2*	-15	3	20*	7	8	18
16 Feb 10	-2	-2	-21	-7	-4	0	8	36	18	33
04 Mar 10	1	1	-19	-3	-4	4	2	5	10	23
16 Mar 10	6	6	-14	4	2	17	16	0	0	10
31 Mar 10	17	19	-9*	2*	4	20	0	0	0	0
14 Apr 10	13	16	-7	8	3*	27*	40	0	0	0
28 Apr 10	13	19	-3	9	11	27	28	0	0	0
12 May 10	8	8	-3*	12*	6	26	58	0	0	0
27 May 10	23	25	2	21	14	33	5	0	0	0
09 Jun 10	22	23	10	17	21*	33*	58	0	0	0
23 Jun 10	22	25	9*	18*	18	28	62	0	0	0
08 Jul 10	23	26	11	22	22	31	161	0	0	0
21 Jul 10	26	32	15	19	26	31	152	0	0	0
04 Aug 10	27	29	13	21	22	32	77	0	0	0
18 Aug 10	21	21	12	23	22	33	133	0	0	0
01 Sep 10	25	25	7	22	22	32	3	0	0	0
15 Sep 10	14	14	5	16	15	26	21	0	0	0
30 Sep 10	15	15	2	12	13	27	75	0	0	0
13 Oct 10	9	10	-2	9	11	27	1	0	0	0
28 Oct 10	6	6	-3	13	8*	19*	31	0	0	0
10 Nov 10	13	13	-7*	7*	3*	18*	3*	0	0	0
01 Dec 10	-5	-5	-11*	1*	-4*	10*	19*	1*	0*	0*
15 Dec 10	-15	-15	-28	-9	-13	-1	13	50	0	46
28 Dec 10	-3	-3	-28*	-6*	-11	-1	8	10	38	48

Spore-trapping site, weather station location: Waushara County, Hancock, WI (continued)										
Date of spore trapping	Temperature during spore trapping (°C)		Data for 14 days prior to spore trapping							
			Minimum temperature range (°C)		Maximum temperature range (°C)		Liquid precipitation total (mm)	Snowfall total (cm)	Snow depth range (cm)	
	Min	Max	Min	Max	Min	Max			Min	Max
13 Jan 11	-2	-2	-22	-7	-12	6	4*	8*	3	13
25 Jan 11	-4	-4	-30*	-7*	-16	-3	8*	22*	8	25
09 Feb 11	-15	-15	-22*	-7*	-9*	-1*	10*	28*	20	38
22 Feb 11	-4	-4	-29*	-1*	-12*	11*	19*	34*	8	41
08 Mar 11	2	2	-21	-3	-7	6	6*	8*	20	43
24 Mar 11	-5	-5	-12*	2*	-3	11*	48*	10*	0	20
13 Apr 11	18	18	-6	8	4	24	49*	0*	0*	3*
27 Apr 11	7	7	-4	5	4	17	63*	14*	0*	10*
10 May 11	24	24	-4	9	6	20	36	0	0	0
01 Jun 11	23	27	2	18	9	31	54*	0	0	0
14 Jun 11	23	26	4	25	12	35	25*	0	0	0
29 Jun 11	26	27	8	16	16*	28*	68*	0	0	0
13 Jul 11	23	27	12	22	23	36	11	0	0	0
26 Jul 11	22	28	12	24	23	36	32	0	0	0
10 Aug 11	19	33	11	26	23	33	61*	0	0	0
24 Aug 11	25	27	9	18	24	31	18*	0	0	0
07 Sep 11	16	21	4*	19	17*	32*	76*	0	0	0
22 Sep 11	20	20	1	14	14	29	29*	0	0	0
05 Oct 11	16	22	-1*	11*	13*	24*	66*	0	0	0
19 Oct 11	9	10	1	14	11	27	31	0	0	0
03 Nov 11	5	6	-3	6	7	16	31*	0	0	0
16 Nov 11	0	2	-6	3	2	14	47*	10	0	10
30 Nov 11	0	2	-9	7	2	11	8	0	0	0
15 Dec 11	0	1	-15	1	-7	7	23	1	0	0
28 Dec 11	-7	-7	-14	-3	-3	7	5	4*	0*	5*

Spore-trapping site, weather station location: Marquette County, Montello , WI										
Date of spore trapping	Temperature during spore trapping (°C)		Data for 14 days prior to spore trapping							
			Minimum temperature range (°C)		Maximum temperature range (°C)		Liquid precipitation total (mm)	Snowfall total (cm)	Snow depth range (cm)	
	Min	Max	Min	Max	Min	Max			Min	Max
03 Sep 09	-	-	4	15	17	26	31	0	0	0
16 Sep 09	-	-	7	13	22	29	0	0	0	0
30 Sep 09	-	-	-1	15	13	25	27	0	0	0
14 Oct 09	-	-	-6	8	4	15	30	0	0	0
05 Nov 09	9	10	-3*	9	5	19	64	0	0	0
20 Nov 09	9	9	-6	7	7	22	12	0	0	0
02 Dec 09	3	3	-5	6	3	14	30	0	0	0
16 Dec 09	-5	-5	-22	-2	-12	3	42	40	0	36
06 Jan 10	-5	-3	-22	-1	-13	3	43	12	15	23
20 Jan 10	5	5	-23	-4	-8	2	11	10	20	30
03 Feb 10	-4	-4	-22	1	-13	3	16	5	13	20
16 Feb 10	-1	-1	-21	-7	-3	1	14	18	15	28
04 Mar 10	2	2	-16	-2	-2	6	5	5	18	25
16 Mar 10	13	13	-12	4	4	16	12	0	0	18
31 Mar 10	20	24	-8	3	6	19	0	0	0	0
14 Apr 10	22	25	-6	11	4	28	55	3	0	3
28 Apr 10	22	22	-7	9	10	28	41	0	0	0
12 May 10	8	8	-2	12	7	27	71	0	0	0
27 May 10	26	27	3	21	16	33	7	0	0	0
09 Jun 10	17	20	9	18	18	33	54	0	0	0
23 Jun 10	21	22	11	18	20	29	78	0	0	0
08 Jul 10	21	22	8	22	22	30	62	0	0	0
21 Jul 10	26	29	16	21	26	31	107	0	0	0
04 Aug 10	25	25	14	22	23	31	68	0	0	0
18 Aug 10	24	24	12	22	22	32	112	0	0	0
01 Sep 10	23	24	8	21	22	33	49	0	0	0
15 Sep 10	21	21	6	17	16	27	39	0	0	0
30 Sep 10	18	18	1	14	14	25	19	0	0	0
13 Oct 10	15	16	-2	9	12	28	1	0	0	0
28 Oct 10	6	6	-4	13	11	22	48	0	0	0
10 Nov 10	15	15	-6	4	4	19	0	0	0	0
01 Dec 10	-5	-5	-11	2	-3	12	20	0	0	0
15 Dec 10	-12	-12	-27	-7	-11	2	34	38	0	36
28 Dec 10	-3	-3	-27	-6	-11	2	7	7	28	36

Spore-trapping site, weather station location: Marquette County, Montello , WI (continued)										
Date of spore trapping	Temperature during spore trapping (°C)		Data for 14 days prior to spore trapping							
			Minimum temperature range (°C)		Maximum temperature range (°C)		Liquid precipitation total (mm)	Snowfall total (cm)	Snow depth range (cm)	
	Min	Max	Min	Max	Min	Max			Min	Max
13 Jan 11	-5	-5	-21	1	-10	7	7	8	15	20
25 Jan 11	-3	-3	-29	-7	-14	-2	17	24	18	36
09 Feb 11	-13	-13	-23	-6	-11	0	30	30	30	51
22 Feb 11	-4	-4	-28	0	-11	12	28	18	18	46
08 Mar 11	2	2	-18	-2	-9	6	4	9	23	36
24 Mar 11	-2	-2	-11	2	-2	14	60	3	0	30
13 Apr 11	20	24	-7	9	6	27	22	0	0	0
27 Apr 11	7	7	-3	4	2	22	87	13	0	13
10 May 11	24	24	-3	9	6	21	37	0	0	0
01 Jun 11	23	27	1	17	10	32	54	0	0	0
14 Jun 11	25	30	7	24	13	34	45	0	0	0
29 Jun 11	25	26	10	17	16	27	94	0	0	0
13 Jul 11	20	28	14	21	26	34	20	0	0	0
26 Jul 11	25	33	13	24	23	35	31	0	0	0
10 Aug 11	18	33	14	23	23	32	43	0	0	0
24 Aug 11	23	29	9	18	23	31	8	0	0	0
07 Sep 11	17	18	3	20	17	33	24	0	0	0
22 Sep 11	13	13	1	14	14	30	19	0	0	0
05 Oct 11	20	23	-2	10	14	24	43	0	0	0
19 Oct 11	8	9	1	12	12	27	30	0	0	0
03 Nov 11	7	7	-4	4	6	18	34	0	0	0
16 Nov 11	1	4	-6	2	3	15	93	10	0	10
30 Nov 11	1	3	-8	6	0	12	16	0	0	0
15 Dec 11	0	1	-17	2	-6	9	30	0	0	0
28 Dec 11	-4	-4	-13	2	-3	9	7	1	0	3