

Spring 2019

DISTRIBUTION, PRESENCE, ECOLOGY,
AND HARVEST DYNAMICS OF THE
CHAGA FUNGUS (INONOTUS OBLIQUUS)
IN THE WHITE MOUNTAIN NATIONAL
FOREST

Rhys Thomas Brydon-Williams
University of New Hampshire, Durham

Follow this and additional works at: <https://scholars.unh.edu/thesis>

Recommended Citation

Brydon-Williams, Rhys Thomas, "DISTRIBUTION, PRESENCE, ECOLOGY, AND HARVEST DYNAMICS OF THE CHAGA FUNGUS (INONOTUS OBLIQUUS) IN THE WHITE MOUNTAIN NATIONAL FOREST" (2019). *Master's Theses and Capstones*. 1269.

<https://scholars.unh.edu/thesis/1269>

This Thesis is brought to you for free and open access by the Student Scholarship at University of New Hampshire Scholars' Repository. It has been accepted for inclusion in Master's Theses and Capstones by an authorized administrator of University of New Hampshire Scholars' Repository. For more information, please contact nicole.hentz@unh.edu.

DISTRIBUTION, PRESENCE, ECOLOGY, AND HARVEST DYNAMICS OF THE CHAGA
FUNGUS (*INONOTUS OBLIQUUS*) IN THE WHITE MOUNTAIN NATIONAL FOREST

BY

RHYS BRYDON-WILLIAMS
BA, WARREN WILSON COLLEGE, 2014

THESIS

Submitted to the University of New Hampshire in Partial Fulfillment of the Requirements for the
Degree of

Master of Science

In

Natural Resources

May 2019

This thesis was examined and approved in partial fulfillment of the requirements for the degree of Master of Science in Natural Resources by:

Dr. Heidi Asbjornsen, UNH, Ph.D, Natural Resources

Dr. Isabel Munck, USFS, Ph.D, State and Private Forestry

Dr. Jeffrey Garnas, UNH, Ph.D, Natural Resources

On May 1, 2019

Approval signatures are on file with the University of New Hampshire Graduate School.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS.....	iv
ABSTRACT.....	v
SECTION	PAGE
INTRODUCTION.....	1
I. BACKGROUND.....	1
Biology.....	1
Medicinal Use.....	3
Habitat and Ecology.....	5
White Mountain National Forest: Chaga Harvest.....	7
II. METHODS.....	7
Survey Design.....	7
Survey Methodology.....	10
Tree Cores.....	11
Sampling Materials.....	12
Data Organization.....	13
Univariate Analyses.....	13
Multivariate Analyses.....	15
Spatial Analyses.....	18
III. RESULTS.....	19
Distribution in Relation to Tree Species and Size.....	19
Distribution in Relation to Site Characteristics.....	21
Conk Characteristics.....	21
Presence in Relation to Species Composition and Basal Area: Univariate.....	22

Presence in Relation to Species Composition and Basal Area: Multivariate....	23
Host Tree Damage Trends: Univariate.....	25
Host Tree Damage Trends: Multivariate.....	26
Cluster/Outlier Analysis.....	29
Host Tree Health and Growth Rates.....	29
IV. DISCUSSION.....	31
Trends in Chaga Presence, Distribution, and Ecology.....	31
Management Implications.....	33
2019 Harvest Experiment.....	35
Future Studies.....	36
Conclusion.....	38
V. REFERENCES.....	40
APPENDIX A: Plot Summary Data.....	42
APPENDIX B: Prism Plot Data.....	45
APPENDIX C: Tree Damage.....	47
APPENDIX D: Conk Data.....	48
APPENDIX E: Tree Core Data.....	48

ACKNOWLEDGEMENTS:

Funding for this project was made possible through the University of New Hampshire College of Life Science and Agriculture (COLSA) and the United States Forest Service, grant number 17-CA-11420004-063. The researcher wishes to thank Drs. Heidi Asbjornsen, Isabel Munck, and Jeffrey Garnas for their leadership and assistance in bringing this project to fruition. The researcher would also like to thank Hannah Callahan, Bronwyn Williams, Michael Bohne, and Matthew Vadeboncouer for their assistance in the field, in the office, and in the lab.

ABSTRACT

DISTRIBUTION, PRESENCE, ECOLOGY, AND HARVEST DYNAMICS OF THE CHAGA FUNGUS (*INONOTUS OBLIQUUS*) IN THE WHITE MOUNTAIN NATIONAL FOREST

by

Rhys Brydon-Williams

University of New Hampshire

Inonotus obliquus is a fungal infection of birch trees that produces a large sterile conk, known colloquially as Chaga. When dried, Chaga has medicinal value as an anti-mutagen and for gastro-peptic relief. With the growth of the natural remedies market over the last decade, Chaga has increasingly become the target of harvest in the White Mountain National Forest (WMNF). Forest managers of the WMNF have asked USFS Forest Health Protection staff whether special use permitting for Chaga as a Non-Timber Forest Product (NTFP) should be allowed. However, it is difficult to make management recommendations or best management practices for harvesting Chaga because the abundance and ecology of the Chaga resource in the WMNF is currently unknown. This project sought to quantify the Chaga resource in the WMNF and determine incidence of Chaga by tree species, habitat type, and other variables. Two surveys were conducted in the 2017 and 2018 field seasons, with a total of 66 sites and 2,611 birch trees sampled across the WMNF. These surveys found positive correlations between Chaga presence and birch tree age, diameter at breast height, and site elevation. Chaga was also disproportionately associated with yellow birch. Chaga frequency in WMNF birch trees was low: only 2% of trees sampled had a visible Chaga conk. However, Chaga was present in 56% of stands surveyed.

In addition, Chaga infections were seen to cluster together in four separate areas surveyed. There was no clear correlation between Chaga presence and either stand-level species composition or annual basal area increment. Additional damages to infected trees only associated with Chaga presence insofar as said damages resulted from Chaga presence. In summation, Chaga, while comparatively rare, is widely distributed across the WMNF and tends to prefer older, large-diameter yellow birches at higher elevations as hosts. These results will ultimately be used to craft a series of Best Management Practices (BMPs) for Chaga harvest with a better understanding of the fungus' preferred habitat and potential for cultivation.

INTRODUCTION

This project is intended to quantify the Chaga resource in the WMNF; determine incidence of Chaga by tree species, habitat type, past silvicultural management, elevation, stand age, and other correlating variables; and from this data develop best management practices (BMPs) for Chaga harvest in the WMNF. The U.S. Forest Service crafted the following three desired objectives for this project.

- Quantify the Chaga resource presence and volume in the WMNF
- Determine possible effects of Chaga presence on birch tree health
- Monitor any possible effects of harvesting Chaga conks on birch health
- Determine whether the WMNF should be considering special use permitting for chaga as a non-traditional wood product

I. BACKGROUND

Biology

Inonotus obliquus, source of the sterile conk commonly known as Chaga (from the Russian “чaгa,” or “mushroom”) and formerly known as *Poria obliqua*, is a basidiomycetous fungal pathogen of the family Hymenochaetaceae. The Chaga fungus is found almost exclusively on birch trees, to which it is considered parasitic (Sinclair et al, 2005). It is easily identified by the sterile conk extruded through the bark of the infected tree and, due to an abundance of melanin, takes on an appearance similar to that of charred wood, leading to the colloquial name “Tinder Conk.” This conk is not a fruiting body but instead a sclerotium, or compact mass of congealed mycelium containing nutrient reserves (Szczepkowski et al, 2013).

The Chaga fungus enters the host tree through existing wounds, such as old Nectria cankers, branch stubs, and wound scars, via airborne basidiospores transmitted by insects. The infection court for Chaga infestation is typically the mid- to lower trunk, but can occur



Image 1: Advanced Chaga infection in yellow birch tree. Photo taken by researcher, 10/28/2017.



Image 2: Advanced Chaga infection in white (paper) birch tree. Photo taken by researcher, 11/19/2017

wherever existing wounds are present. Once within the tree, it causes a white fungal rot to spread throughout the heartwood of the host tree; only the sterile conk penetrates the sapwood during the initial infection period (Lee et al, 2008). The fungus will continue to cause decay within the living tree anywhere from ten to 80 years; while the tree is still alive, only conks will be produced (Shigo, 1965).

After the tree has been killed, the sexual stage begins as fertile fruiting bodies emerge from under the tree's bark. These fruiting bodies are usually unseen, only rarely viewed as a resupinate fruit either around the conk or further up the trunk towards the canopy, and produce basidiospores which spread the infection to other vulnerable trees (Szczepkowski et. al, 2013). It is unclear whether Chaga is a direct cause of affected tree mortality or whether this mortality is caused by related, opportunistic organisms. If an afflicted tree falls, its mycelium is usually replaced by secondary saprobes; this is why the fruiting body is generally found on standing birch trees, not logs (Niemelä et al., 1995).

The Chaga fungus is a primary colonist of sapwood and does not obviously require a prior infestation of other micro-organisms (Pilz, 2004). Diseased trees usually contain from one



Image 3: Fruiting bodies of I. obliquus.
Photo credit: Tuomo Niemala, 2011.

to six conks; six or more conks are usually a reliable indicator of a large decay column within the affected tree. (Sinclair et al., 2005). After each successive instance of Chaga fungal expansion, the affected tree will react with compartmentalizing responses, resulting in nested columns of decayed wood delimited by discolored wood containing old

barrier zones. However, the fungus spreads from the infection court in a dense mycelial wedge that re-damages surrounding cambial tissue, sustaining the infection long term decay columns caused by Chaga can elongate by as much as 14 inches annually (Pilz, 2004). In addition, the sterile conk both splits the bark of the tree and kills the cambium, leading to canker formation and spread if the conk does not grow from an existing canker (Shigo, 1965).

Possible management of Chaga infections has been tested using such methods as infected tree felling, girdling, fungicide, and cutting the trunk into bolts. None of these methods have had sustained success in preventing the eventual formation of Chaga conks (Sinclair et al, 2005). However, some birch trees have been seen to reject the Chaga infection, causing the conk to ultimately fall off, though the decay column is left intact (Spahr, 2009).

Medicinal Use

Chaga has an extensive history as a folk remedy for stomach ailments and cancer, particularly in Russia and Northern Europe. Chaga is mentioned in Alexander Solzhenitsyn's

landmark 1967 novel *The Cancer Ward* in which a main character suffering from cancer ventures into birch forests to harvest the conks to brew tea as a natural remedy. Russian legend holds that the famous 12th century prince Vladimir Monomakh cured a tumor on his lip through regular ingestion of Chaga tea (Perevedentseva, 2013). Moreover, the Norwegian term for Chaga is “kreftkjuke,” which literally translates as “cancer polypore.” (Lee et. al, 2008). Among the Khanty people of Western Siberia, Chaga has been documented as a natural remedy for stomach ailments (Lee et. al, 2008); among the Skolt Sami people of Northern Scandinavia, Chaga has been used both as a natural remedy for cold, flu, and stomach ailments, and also as an energetic or recreational tea in place of other teas or coffee (Magnani, 2016)



Image 4: Preparation of Chaga tea. Photo taken by researcher, 11/30/2018.

The nutrient reserves of the Chaga conk contains triterpenoids, which have anti-mutagenic and anti-tumeric properties, such as growth inhibition of lung carcinoma cells via inotodiol triterpenoid (Zhong et al, 2015). One study, which used aqueous extract of Chaga on chemically immunosuppressed mice, found that it caused an increase in Interleukin-6 levels, possibly making it useful as an immune enhancer during chemotherapy, and that Chaga extract may also suppress TNF- α , a cell signaling protein involved in inflammation, thus making it beneficial for autoimmune diseases such as psoriasis (Kim, 2005). In addition, Chaga also contains large amounts of betulinic acid, extracted from the host tree, which has gastro-peptic qualities.

Many of Chaga's medicinal compounds, particularly betulinic acid, are found only in the chitinous cell walls of the organism, which cannot be digested by humans. For the bio-active components in Chaga to become available, an extraction process is required. Thus, after removal Chaga is most commonly dried, ground, and used as the basis of a tea or tincture. When brewed in tea, Chaga can usually be steeped three times before most of its medicinal compounds are expended. Chaga can also be used as a replacement for hops, due to its tannic qualities, and dried Chaga can be used as incense (Spahr, 2009). Due to its medicinal applications, Chaga has recently become popular as a natural remedy in the United States, at a 2017 value of roughly \$50 per dry pound (Carpenter et. al, 2017).

Habitat and Ecology

Chaga is best described as a “circumboreal” organism as its distribution spans throughout the Northern Hemisphere, typically with dense birch growth interspersed with coniferous boreal forest. Significant Chaga proliferation has been reported in forests in Russia (both European and Asian), Scandinavia, Poland, France, China, Korea, Japan, and Canada. In the United States, Chaga has been reported in the Northeast (Maine through Pennsylvania), the Great Lakes region (Michigan, Wisconsin, and Minnesota), Alaska, and high-altitude areas of the southern Appalachian Mountains with extensive yellow birch growth, particularly western North Carolina (Sinclair et. al, 2005). However, no intensive studies have been conducted into the habitat and distribution of the Chaga resource in the United States. Furthermore, accurate estimates of Chaga fungal habitat worldwide are difficult to determine as the fungus is not usually included in forest inventory data in Europe and Russia (Pilz, 2004).



Image 5: Chaga conk presence on white birch in northern hardwood stand, WMNF. Photo taken by researcher, 10/28/2017.

Chaga is almost exclusively found on birch trees, particularly in white birch species (*B. papyrifera*, *B. pendula*, *B. pubescens*) and in yellow birch (*B. alleghaniensis*), though instances have been found in black birch (*B. lenta*), water birch (*B. occidentalis*), and grey birch (*B. populifolia*). Chaga fungal growth has also been found in red alder (*Alnus rubra*), American and European beech (*Fagus grandifolia* and *sylvatica*), oak (*Quercus spp.*), red maple (*Acer rubrum*), hop-hornbeam (*Ostrya virginiana*), and poplar (*Populus*

spp.), but in these species it appears as a buried stem canker. Only in birch does Chaga produce a sterile conk (Lee et. al, 2008). Reports of Chaga presence on maple, sycamore, and other hardwood species are likely due to misidentification of either different fungi, such as birch polypore on grey birch, or unrelated burls (Spahr, 2013).

A 2015 study by Balandaykin and Zmitrovich in birch stands (mainly *B. pendula* and *B. pubescens*) in the Ulyanovsk region of Russia found that Chaga seemed to prefer sprout coppiced trees to those with seedling coppice, mature stands to younger stands, and oligotrophic soils to eutrophic soils. Furthermore, anthropogenic disturbance in the birch stands had a positive correlation with incidence of Chaga infestation (Balandaykin et al, 2015). In a study conducted in seven regional forests in Poland from 1995-2011, Chaga was seen at higher incidence in stands aged 60 years or older and to prefer mixed birch-coniferous forest and bog forest to wet broadleaved forests, with an estimated total Chaga volume of 46 metric tons (Szczepkowski et al,

2013). However, these studies apply only for European South Boreal forests, and thus have a limited relevance to Chaga distribution in North America (Balandaykin et al, 2015).

White Mountain National Forest: Chaga Harvest

As a result of its emerging economic value, Chaga has become the target of harvest in the White Mountain National Forest (WMNF) where birch trees are common. Birch stands (stands where >40% basal area is in birch species), account for 25,787 hectares or about 8% total area of the WMNF. Forest managers of the WMNF have asked USFS Forest Health Protection staff whether it is possible to harvest Chaga sustainably without adversely affecting the WMNF birch resource; this would allow the managers to consider arranging special use permitting for Chaga as a Non-Timber Forest Product (NTFP). However, it is difficult to make management recommendations or best management practices for harvesting Chaga because the proliferation, distribution, and volume of the Chaga resource in the WMNF has hitherto been unknown.

II. METHODS

Survey Design

The Chaga assessment in the WMNF was undertaken in two parts: The Distribution Survey in 2017, and the Ecology Survey in 2018. The goal of the Distribution Survey was to obtain baseline data on Chaga distribution across diverse sites within the WMNF to inform the design of a more detailed survey during the next year's field

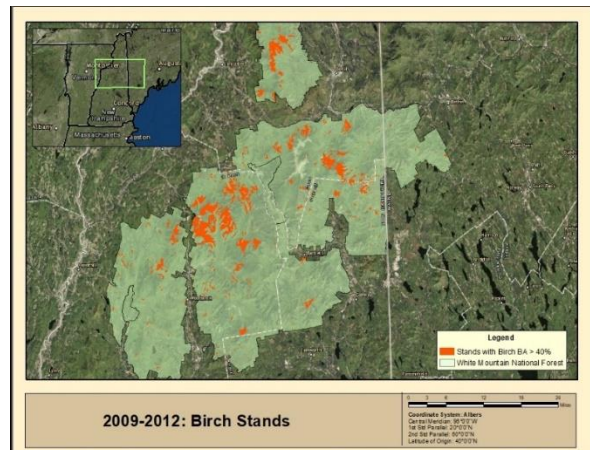


Image 6: All USFS inventory stands with birch basal area of >40%, 2009-2012.

season. The sample plots were populated from data aggregated during a 2009-2012 U.S. Forest Service stand inventory of the WMNF, from which a spreadsheet and a shapefile of all stands with greater than 40% birch basal area were derived. As this was an initial survey to ascertain Chaga incidence, the sites examined were in stands located no further than 500 meters from a road, either public or Forest Service-access only.

A total of 41 birch stands were surveyed between August and December 2017 from an original shortlist of 75, with a number of stands proving too difficult to access given available time. Stands were stratified by total species DBH as reported in the 2009-2012 survey data, based on U.S. Forest Service terminology: regeneration (mean DBH < 11 cm), poletimber (mean DBH >11 cm but < 28 cm), and sawtimber (mean DBH > 28 cm). As Chaga is a sterile conk fungus which derives most of its nutrition from interior rot, external symptoms are not season specific. Conventional wisdom in fact encourages Chaga monitoring and harvest during winter months due to the high contrast between dark conks and snow cover, which aids visibility and accurate identification. Thus, seasonality was not considered a worthwhile factor to account for in this project.

Logistic regression analyses were performed on the Distribution Survey dataset following the end of the field season in order to determine potential Chaga site preferences which could then be used to stratify the 2018 plot frame. These analyses showed strong positive correlations between Chaga presence and mean plot DBH ($\chi^2 = 18.35$ at $p < 0.0001$), stand age ($\chi^2 = 10.30$ at $p < 0.001$), and a

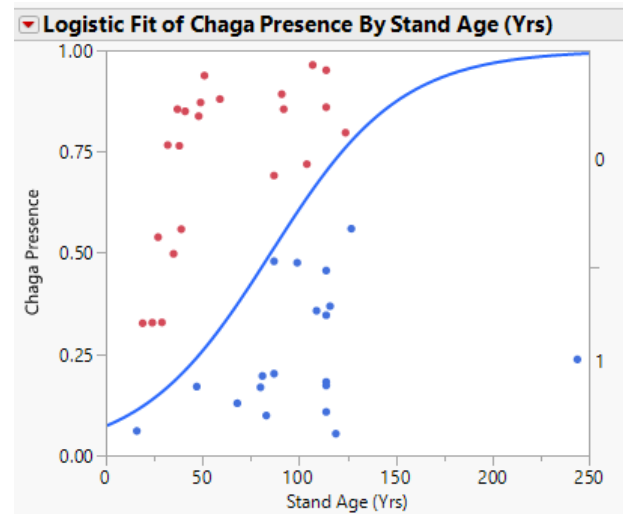


Figure 1: Logistic regression fit of Chaga presence by stand age, 2017

moderate correlation with site elevation, ($\chi^2 = 3.91$ at $p < 0.0479$). 2018 plots were thus selected based on stand age (over 75 years), DBH (mean plot DBH > 28 cm) and elevation (selected along a gradient of 342 to 1029 meters). 2018 stands were not required to be 500 meters from a road, with many being over two miles from the nearest roadway. A total of 25 plots out of 30 possible stands were sampled in the Ecology Survey, and data collection ran from June to September 2018. In neither survey were stands visited in any nominal or geographic order, barring convenience of access.

Survey Methodology

As the sampled stands varied in size and shape, and as Chaga assessment requires only visual confirmation of fungal/conk growth and recording of other possible correlating damages, a systematic model utilizing belt transects was employed for both field seasons. Each stand

Date of collection
Transect (Aspect)
% Broken Branches (2018 only)
% Dieback (2018 only)
Birch species (listed as YB, WB, BB, or GB)
DBH (cm)
Chaga Absence/Presence (0/1)
Live Crown Ratio (%)
Indication of previous Chaga harvest (0/1)
Latitude/Longitude (only Chaga-positive trees in 2017, all trees in 2018)

Table 1: Variables recorded for transect trees

delineated by the 2009-2012 inventory (GIS data provided by the WMNF) has a central waypoint with ascribed geographic coordinates. These waypoints were made the center of the sample plot for the stand. From the waypoint, four 10-meter wide belt transects were laid out along the four cardinal directions (North, South, East, West). Within each of these transects, ten birch trees were sampled. Each of these trees was then surveyed for a number of variables, outlined in Table 1. Live crown ratio was

included as a method of estimating overall tree health, the logic following that poor tree health is generally associated with a decreasing percent of live crown

Each tree was also surveyed for sixteen discrete damages common to birch trees. These damages are listed in Table 2 in the Appendix. Latitude and longitude were originally exclusive to Chaga-positive trees in 2017 in order to ascertain preferred Chaga habitat. In 2018,

coordinates were expanded to all sampled transect trees in order to determine possible clustering patterns. There were also three variables specific to Chaga-positive trees: conk size, conk height, and number of conks.

Variable-radius (“prism”) plots were taken at each stand visited in order to provide a “neighborhood sample” of species composition and basal area by stand. Prism plots were organized differently between 2017 and 2018. In 2017, if Chaga was found on a tree within a sample transect, that tree became the center of a new variable-radius prism plot, to aid in the “neighborhood sample”. No more than three prism plots were taken per site. Any tree with Chaga within a prism plot was not counted as the center of a new prism plot. In 2018, a more consistent prism plot to determine species basal area was utilized. Three prism plots were recorded per stand, one at the end of each sample transect, each at least 10 meters away from the waypoint. This was expected to provide much more accurate, comprehensive data than the more limited 2017 prism plot protocol. In both years, the same variables were recorded for each prism plot tree: species, DBH (cm), Chaga presence/absence, and live crown ratio.

Tree Cores

As one of the main objectives of the project was to determine possible effects of Chaga on tree growth, cores were obtained from selected trees over the 2018 field season for later ring width analysis. In each stand where Chaga was located, two cores were taken from a transect tree infected with Chaga, and two from an uninfected (control) transect tree. Uninfected trees were selected to be the same species and, if possible, within ± 10 cm DBH of the cored Chaga tree. Cores were taken from the base of each tree using a 5.2 mm increment borer, and bored to a depth of roughly 10 cm. This depth was chosen in order to capture tree rings dating back at least 20 years, in the

interest of observing the Chaga fungus' effect on ring width and basal area increment per year within the estimated natural infestation cycle of the fungus. The cores were then inserted into paper straws and allowed to dry before mounting for tree ring analysis. A total of 51 cores from 29 trees were collected from the field for ring width analysis, excluding 12 cores which rotted or disintegrated prior to analysis. Of these trees, 16 (55%) were infected with Chaga, while 13 (45%) were not. More cores than these were extracted in the field, but these additional cores rotted or disintegrated before they could be properly mounted.

Sampling Materials

The following materials were utilized: a handheld Garmin 60CSx GPS unit determined waypoint location and marked the location of each tree with Chaga. A BAF 20 angle gauge was used to measure prism plots. Diameter at Breast Height (DBH, usually specified as ~4.5 feet) was measured in centimeters using either calipers or DBH tape depending on size. A Nikon Forestry Pro laser clinometer was used to determine the approximate height of Chaga conks above six feet, with binoculars used to detect any conks present in the canopy of the tree. Chaga conk size was recorded in cubic centimeters based on measured width, length, and outward extension from tree. Any conks found above two meters on the sampled tree had size roughly estimated using the clinometer. All data was entered into the Survey123 app from ARCMAP, which allowed recording of data, coordinates, and pictures for each tree sampled. After mounting and sanding of the sample cores, the MeasureJ2X microscope-YUX-Excel interface program in the UNH Eco-Hydrology laboratory was used to measure and input tree core ring widths into an Excel spreadsheet, from which basal area increment (BAI) measurements for each year recorded could be determined.

Data Organization

From the Survey123 app, coordinates from the sampled trees were entered into ARCMAP to be translated to a map of the WMNF. This served as the basis for subsequent clustering analysis. The transect and prism data were then loaded first into Excel spreadsheets, where graphs were made and less complex analyses such as mean and median values, standard deviations, prism plot basal areas etc.) were performed. These results were then loaded into JMP (v.13-.14) statistical software for more advanced analyses such as logistic regression and ANOVA. These analyses were performed on a Windows 10 PC laptop. For multivariate analyses, the program PC-ORD v7 was utilized on a Windows 8 desktop computer located in the UNH Natural Resources Computer Lab.

Univariate Analyses

Once entered into JMP, the “fit X by Y” function was used to perform a number of data analyses between Chaga presence and other variables within the transect and site summary datasets. Logistic regression analyses were primarily used with determining correlations with Chaga presence. Logistic regression models the probabilities of the levels of a categorical Y response variable as a function of one or more X variable effects. As the Chaga presence variable was both categorical (presence/absence) and also the response variable, logistic regression of the categorical “personality” was used when presence was compared with continuous X variables such as DBH and live crown ratio. As the interest in these regressions was specifically Chaga presence, or the 1 value, the Target Level (the level whose probability is to be modelled) was set as 1. The pertinent results from the logistic regression analyses came from the Whole Model Test, which is a specific likelihood ratio Chi-square (χ^2) test that evaluates how well the

categorical model fits the data applied. Of particular interest were the Chi-square values, which denoted whether or not the relationship was due to chance, and Pearson values (p-values) which denote the statistical significance of the Chi-square values (any Chi-square with a P-value greater than $p < .05$ is not considered to be statistically significant).

Other analyses contained ordinal or continuous response variables, such as comparing percent Chaga composition of sampled trees with plot elevation, and so different analyses were performed with regard to the personalities of variables involved. Analyses of Variance (ANOVAs) were employed in instances where the variables were either both continuous (Bivariate), or where the Y variable was continuous but the X variables categorical (One-Way). For instance, comparing conk size by birch species involved a continuous Y variable and a categorical X variable, thus necessitating a One-way ANOVA. The result of greatest interest for the One-way ANOVAs was the F-ratio, a ratio of inter-group variance compared to in-group variance that in turn determines possible differences between groups. For Bivariate ANOVAs, the emphasis was on the correlation coefficient (r^2), a number between -1 and 1 calculated to represent the linear dependence of two variables or datasets. In both instances, p-values were examined to determine statistical significance of the results

If both variables were categorical, such as in the damages dataset or in examining Chaga presence by birch species, then contingency analyses were employed. Contingency analyses fit two categorical variables. This analysis results in a Chi-square value, which indicates the likelihood that the relationship between these variables is independent, and a P-value to determine the statistical significance of the Chi-square values. For all tests, additional results,

such as parameter estimates for logistic regressions and contingency tables for contingency analyses, are included in the JMP analyses result sheets located in Appendix C.

Multivariate Analyses

The prism plot species basal area and damages datasets both had a large number of X variables in comparison to a single response variable. Multivariate analyses were thus performed on these datasets in addition to a large number of univariate analyses as a means of determining whether multiple covarying variables associated with Chaga presence, particularly given multiple types of community data in each sample unit (i.e. multiple tree species per prism plot, multiple damages per transect tree). The datasets in question were loaded into PC-ORD with all community variables listed in the main matrix and all environmental variables placed in the second matrix. The independent variables were then ordinated by Chaga presence, the environmental variable of greatest concern. In analyzing both datasets, the following sequence of transformation, ordination, and analysis was performed:

1. Row-and-Column analyses were performed to assess skew (bias) and kurtosis (peakiness) of the dataset being analyzed. These results then informed the possible application of data transformations and/or relativizations based on whether or not skew or kurtosis values for the dataset were outside of the desirable range (-1 to 1 for skewness, -2 to 2 for kurtosis), variation was high, or if there were a large number of zero values in the dataset. Species or damages were deleted if they were present in less than 5% of prism plots or trees, respectively, following which all rows with entirely zero values were also deleted.

In both datasets, Beals smoothing was utilized due to the large number of zero values in both sets as well as the categorical nature of the damage variables. Beals smoothing is an operation that replaces each value in the community data matrix (i.e. X variables) with a probability of the species represented appearing in a given sample unit. This removes zero values while maintaining the strongest patterns in the data, and is most effective at discerning patterns in noisy or heterogenous community data (McCune et al., 2002).

2. A Non-Metric Multidimensional Scaling (NMDS) ordination was performed. Ordination is a method of data reduction by which a dataset with numerous variables is summarized into a few “synthetic” variables, thereby preserving the strongest patterns in the data while allowing for greater ease of interpretability of an otherwise multidimensional dataset. NMDS ordination is an iterative search for the best positions of n on k axes that minimizes the stress of k -dimensional configurations, stress defined here as “a measure of departure from monotonicity [homogeneity] in the relationship between the distance in the original p -dimensional space and the distance in the reduced k -dimensional space” (McCune et al., 2002). This ordination uses ranked distances to linearize the relationship between species-space and variable-space distances.

NMDS ordination was chosen because the ordination does not assume linearity between variables and can thus use any kind of distance measure between points. Bray-Curtis (Sørensen) distance was used for these ordinations as this measure explores shared abundances, most effectively with binary environmental data such as species presence/absence. The NMS function in PC-ORD was used to run this ordination. This function has a built-in “autopilot” mode that chooses the best solutions at each dimensionality, while also testing for significance. A series of

“runs” were applied to both the real data and with randomized data, a “run” being a series of solutions slowly moving down in dimensionality from the highest number of axes possible given the X variables to just one axis (McCune et al., 2002).

Results from these “runs” were then assessed for interpretability and use in discerning structure based on five results:

- Dimensionality of the data, or the highest number of dimensions possible that meets the criterion of reduced final stress by 5% or more. This is most often two- or three-dimensional.
- Final stress value of the “best solution”: < 2.5 is considered excellent for interpretability, 5 is good, >20 is poor.
- Monte Carlo test results, or the number of randomized runs and probability that similar final stress could have occurred by chance.
- “Stability” of the best solution, where “instability” is the standard deviation in stress over the preceding ten iterations.
- Proportion of variance in the dataset represented by each final axis, derived from the r^2 value between distance in ordination space and distance in original space (McCune et al., 2002).

3. After the NMDS ordination was completed, a Multi-Response Permutation Procedure (MRPP) was performed on the ordinated data. MRPP is a non-parametric procedure for testing the hypothesis of no difference between two or more groups, or to determine quantitatively whether groups of sample units are different from each other (McCune et al., 2002). This is similar to a multivariate ANOVA (MANOVA) in that it explores in-group and inter-group differences. However, unlike MANOVA, MRPP does not require a normal distribution, making

it ideal for ecological community data. MRPP results in a “test statistic” T which describes the separation between groups, the “chance-corrected within-group agreement” value A which describes the effect size of within-group homogeneity as compared with random expectations (i.e. if all items are identical within all groups, $A = 0$), and a p-value to show the statistical significance of T .

4. With the MRPP performed, the final multivariate step was Dufrene and Legendre’s Indicator Species Analysis (ISA). This analysis builds off of MRPP but, instead of testing the difference or lack thereof between groups, instead describes the “indicator value,” or a statistical value of how well each individual species separates across two or more groups of sample units (Dufrene et al., 1997). This analysis is quick and pertinent results include the number of randomizations used in the built-in Monte Carlo test, the statistically significant indicator values for each species in each group, and the p-values denoting this significance.

Spatial Analysis

From Survey123, the transect tree coordinate data was transferred first to an Excel spreadsheet, where it was joined with Chaga presence/absence data for the trees surveyed, and then added to an ARCMAP data frame as XY data before conversion to a shapefile. Following this conversion, a Cluster and Outlier Analysis using the Anselin Local Moran’s I statistic was performed on the transect tree data. This analysis was intended to identify the statistically significant “hot spots” (clusters), “cold spots” (lack of data), and spatial outliers of Chaga presence in the transect data. This indicates whether any apparent similarity (cluster) or dissimilarity (outlier) is more pronounced than could be expected to occur randomly. The Cluster

and Outlier analysis generates several statistics that can then be used to determine that adjacent features have similar values:

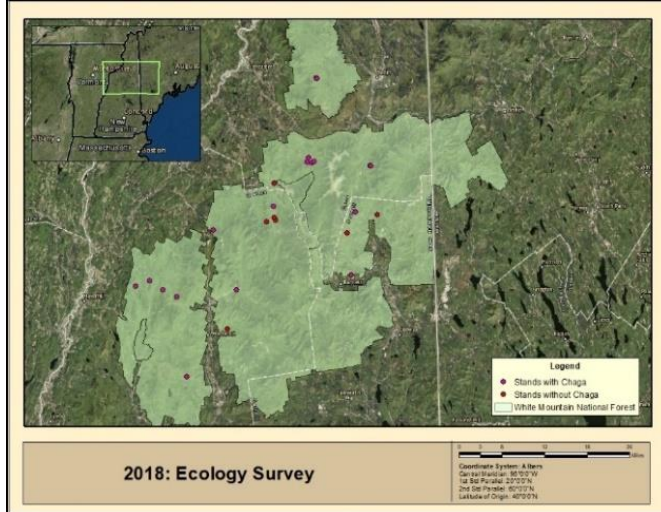
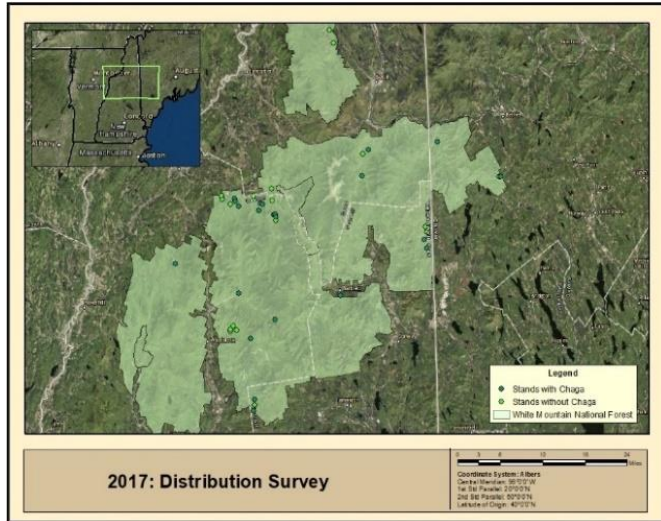
- Z-score: indicates whether surrounding features have similar values, either high or low. High positive Z-scores indicate clusters, low negative Z-scores indicate spatial outliers.
- P-value: statistical significance of Z-score
- Cluster/Outlier Type (COType): indicates statistical significance of clusters and outliers at a 95% confidence interval. For clusters, “HH” denotes significance of high values (“hot spot”), and “LL” significance of low values (“cold spot”).

III. RESULTS

Distribution in Relation to Tree Species and Size

A total of 2,611 trees were sampled over the course of both field seasons, 1,611 in 2017 and 1000 in 2018. Of these transect trees, 1,488 (57%) were white birch, 1,056 (40%) were yellow birch, and 67 (3%) were black birch. No black birch trees contained Chaga infection, and no grey birch trees were sampled in the transect plots at all. Within each sample plot, incidence of Chaga (presence/absence) was fairly rare: only 3.75% (71 total) of birch trees sampled contained any visible Chaga conk presence. However, 37 of the 66 stands sampled contained at least one tree with Chaga presence, accounting for 56% of the total stands surveyed. Thus, while Chaga incidence as a whole was rare, Chaga distribution across the sites sampled was wide.

Among Chaga-positive birches, there was a positive correlation between Chaga presence and yellow birch trees: 63.4% of all Chaga trees were yellow birch, while the remaining 36.6% were white birch, and a contingency analysis of the Chaga positive data in JMP resulted in $\chi^2 =$



Images 7 and 8: All stands surveyed by Chaga presence/absence. 2017 is on top, 2018 on bottom.

17 at $p < .0002$ for Chaga presence between yellow and white birch trees, denoting significant difference in frequency of Chaga presence between species. In a separate logistic regression, Chaga presence also correlated positively with tree DBH across all plots ($\chi^2 = 24$ at $p < .0001$), indicating a moderately strong relationship with clear statistical significance. However, Chaga presence did not correlate with live crown ratio ($\chi^2 = 0.000028$, $p < 0.9958$). Chaga-positive trees had a median DBH of 27.56 centimeters, which was also true of both white and yellow birches respectively, out of a range of 172 centimeters (there were numerous large-diameter outliers in the dataset).

Distribution in Relation to Site Characteristics

In addition to transect data, plot summary data were also entered into Excel, comprising the plot-wide variables listed in Table 4 of Appendix A. Chaga presence/absence, number of trees with Chaga, and percent Chaga presence in transect trees were all analyzed against the remaining variables. These three variables all correlated positively with stand age and mean plot DBH, while only

Response Variable	Test Performed	Stand Age (Years)	Mean Plot DBH (cm)	Elevation (m)
Chaga Presence/Absence	Logistic Regression	$\chi^2 = 16.88,$ $p < .0331$	$\chi^2 = 21.94,$ $p < .0001$	$\chi^2 = 4.53,$ $p < .0331$
Number of Chaga-positive trees	Bivariate ANOVA	$r^2 = .31,$ $p < .0115$	$r^2 = .28,$ $p < .0196$	$r^2 = .19,$ $p < .1094$
Percent Chaga-positive in transect trees	Bivariate ANOVA	$r^2 = .29,$ $p < .0194$	$r^2 = .31,$ $p < .0099$	$r^2 = .14$ $p < .27$

Table 2: Plot summary variables with Chaga presence response variables. Positive correlations are highlighted

Chaga presence correlated with elevation. The tests used and important results therein are listed in Table 2. There were no correlations between any Chaga variable and percent slope of site.

Conk Characteristics

Chaga positive trees had a median conk number of two conks per tree, a median conk height of three meters, and a median conk size of 15.24 cm³. The median stand age for Chaga presence was 109 years. In addition to presence/absence data, information on the conks surveyed on Chaga-positive trees was also analyzed. The three primary variables of conk size, conk

Yellow birch was the only species present in the prism plots whose basal area was significantly greater in stands with chaga ($\chi^2 = 20.72$ at $p < 0.019$). Overall, however, species composition and basal area by stand did not generally appear to associate with Chaga presence. Additionally, there was no significant association in a Bivariate ANOVA between the basal area of Chaga positive trees and the total basal area of prism plots sampled ($r^2 = 0.001$, $p < 0.9842$).

Presence in Relation to Species Composition and Basal Area: Multivariate

The logistic regression analyses described above provided information as to how individual tree species associated with the presence of Chaga in a given prism plot. However, WMNF forests are usually not monocultures, and thus it was necessary to explore cumulative associations of groups of tree species against Chaga presence. The entire basal area dataset was thus subjected to the multivariate process explained in Methods (Beals Smoothing-NMDS – MRPP – ISA). First, the data were subjected to a Row-and-Column to determine skew and kurtosis (peakiness of data). Mean values for both were not within the ideal range of (-1, 1) and (-2, 2), and so to solve this the data were subjected to a series of transformations and relativizations to improve the distance measures (measurements of dis-/similarity) between the data points and to remove outliers. Rare species were deleted, as well as prism plot W617-1, which following the species deletion contained only zero values. Beals smoothing was applied to the dataset to eliminate all remaining zero values while preserving strong patterns. This reduced the number of species from 19 to 10.

The real and randomized data were subjected to 250 runs, concluding in a final minimum stress of 10.48 for a three-dimensional solution. According to the NMDS evaluation guidelines mentioned in the Methods section, this final stress is considered an acceptable ordination that should be able to yield a usable interpretation with low risk of false inferences, though there is potential to misread the results at upper values (McCune et al., 2002). The final

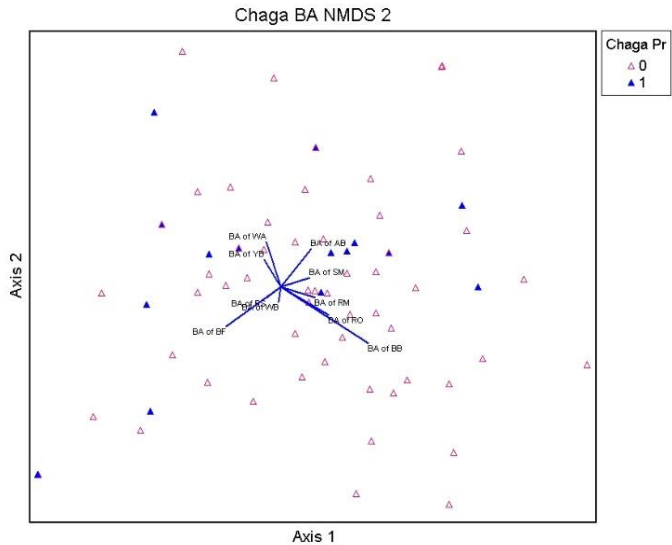


Figure 3: Scatterplot of best solution of prism data NMDS with Chaga presence overlay. Axes 1 and 2 selected due to greatest total variance.

instability of this NMDS, or standard deviation in stress over last 10 iterations, was 0.0001 at 83 iterations. The Monte Carlo test of 250 runs resulted in $p = 0.004$ for all axes, indicating that the NMDS extracted stronger axes than possible through chance. The three axes of the solution cumulatively accounted for 91.2% of total variance in the dataset, with Axis 1 representing 43% of all variance, Axis 2 representing 34.1%, and Axis 3 representing 14.2%. The two-dimensional scatterplot of the resulting ordination can be seen in Figure 3, with Axes 1 and 2 displayed as these two axes represented the two greatest amounts of variance.

Following the NMDS, the ordinated dataset was subjected to an MRPP with Chaga presence as the grouping variable. This process resulted in $T = 0.548$, $A = -0.0015$, and $p = 0.65$. With a non-negative T and a negative A value, this test appeared to show comparatively little separation between groups with a very small “effect size” of within-group homogeneity, and the

high p-value denoted that the sample units of each group were not more similar to each other than would be expected if they belonged to a different group. In essence, plots with and without Chaga presence were not more heterogenous in terms of species composition than would be expected through chance, and did not specifically differ in species composition between each other.

The NMDS provided a visual indication of the lack of association between species basal area and Chaga presence, while the MRPP provided statistical support for this indication. In spite of this apparent lack of difference in species composition between Chaga-present and Chaga-absent plots, an Indicator Species Analysis (ISA) was still performed to determine which species, if any, were most associated with the difference between the groups. Of the ten species remaining in the dataset, the species' basal areas most closely associated with Chaga presence were yellow birch, balsam fir, sugar maple, and American beech, all of which an Indicator Value (IV) around 50. However, none of these species' IVs had a p-value of $p < .05$, which meant that these results were not statistically significant. Thus, the ISA confirmed the inferences made possible through the NMDS and MRPP that species composition and basal area did not significantly associate with Chaga presence.

Host Tree Damage Trends: Univariate

In total, 16 other distinct forms of damage were reported in the transect trees surveyed, represented in Table 2. These damages, and their relationship to Chaga presence, were subjected to logistic regression analyses in JMP. In 2017, dieback and broken branches were listed as nominal variables; however, in 2018, these variables were represented in percentage of live

crown. All damage variables were recorded as presence/absence (0/1) due to the difficulty of ascertaining discrete volumes of some damage types, such as Bronze Birch Borer.

First, contingency analyses were performed between each damage variable and Chaga presence in order to discern correlations between individual damages and Chaga presence. Contingency analyses were utilized due to both X- and Y-axes being comprised of categorical (presence/absence) variables. Statistically significant correlations between Chaga presence and damage variables are listed in Table 3. Out of a total of 17 additional damages, only four directly correlated with Chaga presence: mechanical wounds (i.e. human- or weather-related tree

Transect Tree Damage: Chaga-Positive Correlation	x²-value	P-Value
Mechanical wounds	12.46	<i>p</i> <0.0004
White Fungal Rot	25.22	<i>p</i> <0.0001
Target Canker	46.75	<i>p</i> <0.0001
<i>Trametes versicolor</i> (Turkey Tail)	8.865	<i>p</i> <0.0029

Table 3: Transect tree damages correlated with Chaga presence

damage), target canker, *T. versicolor* (Turkey Tail, a common saprophytic fungus), and white fungal rot. White fungal rot is here classified as any

fungal rot that consumes lignin, leaving lighter cellulose behind. It is an intrinsic result of Chaga infection, but also resultant from other forms of fungi as well, including *A. mellea* (Honey Mushroom) and *G. applanatum*, or Artist’s Conk (Sinclair et al., 2005)

Host Tree Damage Trends: Multivariate

Due to the large number of independent variables in the damages dataset, another series of multivariate analyses were performed to examine relationships between tree damages as

potentially associating with or possibly even driving Chaga presence. As with the basal area multivariate suite, rare species (present in < 5% of trees) were deleted first, as were all trees with entirely zero values following the deletion. This brought the total number of tree damages in the dataset down from 17 to 8. Beals smoothing was then applied to the dataset as the variables listed were entirely categorical. A generalized logarithmic transformation was also performed in order to expand smaller values and compress higher ones. This resulted in acceptable skew ($s = 0.726$) and kurtosis ($k = -0.325$) for ordination.

After the transformations, NMDS was performed on the data with 250 runs, both real and randomized. This resulted in a final minimum stress of 9.23 at two dimensions and final instability of 0.00001 at 101 iterations. Taken along with consistent Monte Carlo p-values of $p < 0.0196$, these results indicated a dataset with solid interpretability which was unlikely to

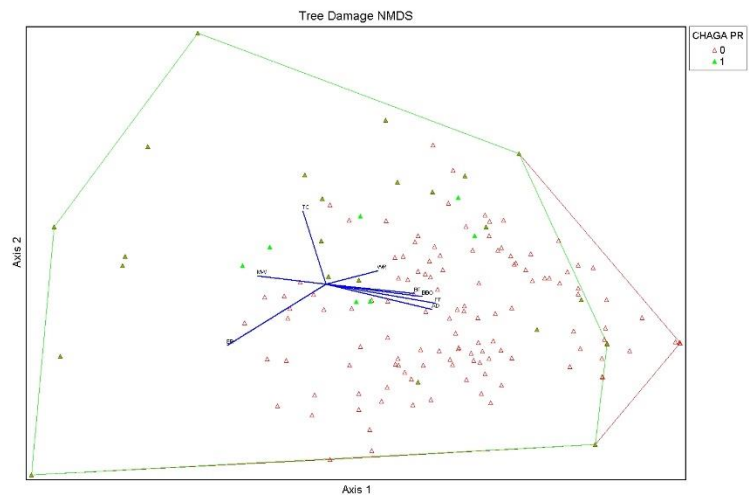


Figure 4: Scatterplot of the tree damage NMDS ordination with joint plots pictured.

be replicable by chance. The two axes present in the two-dimensional solution represented 95% of the total variance of the dataset, further pointing to the inferential possibility of the ordination. Axis 1 represented 84.5% of the total variance, while Axis 2 represented 10.3%. This ordination was graphed and joint plots were applied, as can be seen in Figure 4. These joint plots indicated the relationship between variables and ordination axes, with length and direction showing the strength of the relationship. In this instance, mechanical wounds, target canker, and white fungal

rot all appeared to have a much stronger relationship with Chaga presence than other damage variables. This apparent association would now have to be tested statistically through MRPP.

The MRPP resulted in $T = -5.45$, $A = 0.0024$, and $p = .002$. The negative T-value and the very small p-value implied that the two groups (Chaga-positive and Chaga-negative trees) were strongly separated, while sample units of each group were statistically unusually more similar to each other than could be expected had they belonged to different groups. This was in keeping with the results of the NMDS ordination, which showed three damages moving towards a different association than the bulk of the other damages. However, the low A-value denoted that sample units within the groups were roughly as heterogenous as could be expected through chance ($A = 1$ is considered total homogeneity). Thus, while Chaga presence did select some types of damage, it seemed less likely that any one type correlated with Chaga presence.

To determine if this assumption of a lack of “drive” among damages was accurate, an ISA was then performed. As with the individual contingency analyses, this ISA revealed that mechanical wounds, target canker, and white fungal rot were most closely associated with Chaga presence, each with IVs of approximately 50. Both target canker and white fungal rot had a p-value lower than $p < 0.05$, which meant that these species were statistically significant indicators of the “Chaga present” group. Target canker’s p-value of $p < 0.008$ meant that it was more indicative of Chaga presence than any other damage type in the dataset (for contrast, the p-value of white fungal rot was $p < 0.0176$). While there was not a clear driver of difference between groups that could be discerned from the MRPP alone, the ISA implied an association between Chaga presence and target canker and white fungal rot, which supported the general assertion of difference between groups as evaluated in the MRPP.

Cluster/Outlier Analysis

The results of the Cluster-Outlier analysis showed strong clustering among Chaga-present trees: all nine trees listed in the COType results as HH (a statistically significant cluster of high values) were Chaga-present, accounting for 24% of the 2018 total for Chaga-positive trees. There were four discrete groupings which at substantial distance from one another: the two closest were about 6.5 miles apart, while the greatest distance between clusters was 25 miles.

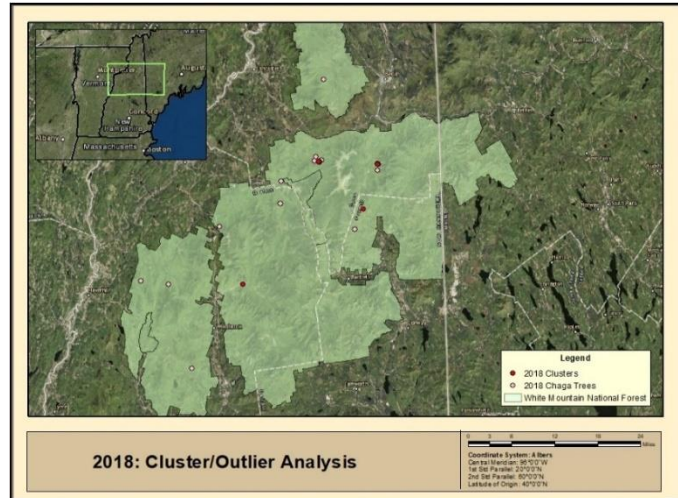


Image 9: Cluster-Outlier Analysis for Chaga trees in WMNF

These clusters also did not appear to be directly associated with elevation,

occurring over a gradient of 511 meters (342 to 853 m, specifically). These clusters are shown alongside 2018 sample plots in Image 9. In addition, all significant spatial data outliers were Chaga-absent trees; a lack of Chaga-present trees in terms of outliers would appear to denote a significant, if not universal clustering effect.

Host Tree Health and Growth Rates

Tree ring width data was analyzed using the Basal Area Increment (BAI) method to determine the impact of Chaga infection on wood production over the course of a standard incubation/infestation period (e.g. 10 to 60 years). Using the ring width data transferred to Excel from the MeasureJ2X system, basal area indices were calculated for each tree sampled: these indices provided an estimation of the amount of basal area put on by each tree in every year

accounted for in the core. The oldest core recorded 144 years, while the youngest recorded only 11 years: as the timeframe that most concerned the project was between 10 to 60 years, this disparity was considered inconsequential

Mean BAIs were calculated for each core for all years; these were then placed in a logistic regression against Chaga presence by sample, with a result of no correlation ($\chi^2 = 0.344$, $p < 0.5572$). Mean BAIs were then calculated by tree (different BAIs for cores from the same tree were averaged) and again placed in a logistic regression equation, yet again with no correlation.

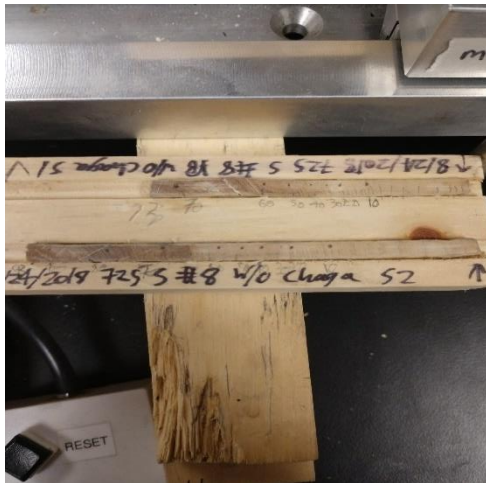


Image 10: A mounted tree core taken during the 2018 field season. Dots on core mark ten-year increments for ease of ring mensuration.

Logistic regressions of both mean core and mean tree BAI for the decade 2007-2018 also showed no correlation, as did mean ring width by core when compared with Chaga presence. Mean ring width by tree did correlate with Chaga presence, but this association was not particularly strong ($p < .02$), and mean ring width by tree for 2007-2018 also showed no correlation.

In addition, two ANOVA tests were run with the variables reversed, thereby analyzing mean BAI for both trees and cores by Chaga presence. These results also showed no correlation: mean core BAI by Chaga presence was $r^2 = 0.006$ at $p < 0.56$, while mean tree BAI by Chaga presence was $r^2 = 0.0099$ at $p < 0.6068$. It thus appeared that birch tree wood production had no significant impact on Chaga presence, nor did Chaga presence appear to impact basal area increment by year.

IV. DISCUSSION

Trends in Chaga Presence, Distribution, and Ecology

From the data analyzed during this project, a number of trends become apparent. On a single tree level, Chaga presence correlates positively with tree DBH, stand age (and therefore average tree age), and with yellow birch as a host. Yellow birch could well be a more suitable host for the Chaga fungus due to its comparative hardiness, able to withstand damage more readily and growing to larger diameters and greater heights than other birch species (Burns et al., 1990). The Chaga fungus may therefore prefer yellow birches as a host as it stands a greater chance at a longer infestation period and a larger, more consistent nutrient flow. It is unclear, however, that this is a causal relationship.

At the stand level, Chaga presence correlations with site elevation and with yellow birch support the findings at the transect level. Yellow birch prefers to grow in the transitional zone between low-elevation northern hardwood forests and high-elevation spruce-fir forests, particularly at elevations above 3,000 feet. Consequently, with greater proportions of yellow birch at higher elevations comes greater probability of Chaga presence at those elevations. Additionally, conditions that are favorable for yellow birch, such as higher levels of humidity in valley ecosystems, may also be favorable for Chaga fungal infestations. However, it remains unclear whether this correlation is causative, as opposed to merely circumstantial, especially given that the multivariate analyses did not report any association between species basal area composition and Chaga presence.

It can thus be asserted that Chaga prefers yellow birch as a host, and its site specificity is the same as that of birch in general and yellow birch in particular. Given that birches in general and yellow birch in particular have a very broad distribution across the WMNF, it is unsurprising that Chaga also has a wide distribution. It is also unsurprising that, when Chaga is present in more than one tree per stand, it has a tendency to cluster. The Chaga fungus is transmitted through spores carried by wind and insect vectors (Lee et al, 2008), and in older stands with large yellow birch basal area, there should be an expectation of substantial numbers of large diameter, densely grown, mature yellow birch with suitable vectors for infection (such as the mechanical damage also correlated with Chaga presence), thereby facilitating multiple Chaga infections in close proximity. However, this dataset is only part of the greater project dataset as not all trees were geographically recorded in 2017. Thus, conclusions drawn from the clustering analysis are, for now, qualified by this limitation in representation.

There was also no clear indication of whether or not Chaga is a primary or ancillary driver of birch tree mortality that could be derived from the dataset. Chaga presence did not correlate with live crown ratio and both the median and modal live crown ratios for all Chaga-positive trees was 25% with a mean value of 23%, indicating comparatively few deceased trees but a large quantity with sub-optimal live crown ratios. The association between Chaga presence and target canker/white rot, as found in the multivariate analysis, is also not particularly substantive, nor are any of the contingency correlations. This is because there is a strong probability that the associated damages are by-products of Chaga presence itself. A type of white fungal rot is generated by *I. obliquus* as part of its naturally produced decay column, while target canker often follows from a Chaga conk piercing the tree cambium. In addition, mechanical

wounds are a common vector of Chaga, often appearing after significant ice and wind-throw events that can cause wounding leading to Chaga infestation, and *T. versicolor* is a saprophyte that often infests decay columns in trees, with fruiting bodies appearing on the bark above. Thus, it is doubtful that the associations with damage experienced by Chaga-positive trees is causative. These results are in keeping with Pilz' assertion that the Chaga fungus is a primary fungal pathogen that does not have any association to existing damages (apart from infection vectors). The lack of mortality among Chaga-infected trees would also appear to indicate that it is not a singularly strong driver of mortality.

Management Implications

One of the primary objectives of this project was to determine whether special-use permitting for Chaga harvest in the WMNF was the best immediate course of action with regard to Chaga harvest. It is intriguing that, out of all trees surveyed during this project, only one displayed signs of previous harvest. Part of this is doubtless due to the remoteness of many sites sampled; however, even in stands less than 500 meters from a roadway, and even in those abutting trails there was no indication of prior Chaga harvest. A further deterrent to harvest is potential conk height: foragers and harvesters are unlikely to pursue harvest of a conk over two meters up the tree trunk, and an average conk height of 4.9 meters places many conks out of effective reach. The relative scarcity of Chaga would seem to imply a low overall volume of the Chaga resource, which would necessitate a special use permitting protocol. However, virtually nonexistent harvest activity in large-quantity Chaga-present stands, combined with a wide geographic distribution and large variation in conk sizes and heights, would appear to indicate a Chaga resource in little risk of exhaustion or even depletion, thereby obviating a permit system

for the time being. Future surveys and monitoring of harvest activity will be required to determine whether these circumstances hold, however.

In addition, the relative scarcity of Chaga, and the varying size and number of conks on birch trees when compared to the estimated value of dried Chaga per pound appears to indicate that the Chaga resource would most likely not produce a substantial value-added to a harvest site. For example, Carpenter et al.'s 2017 letter estimated Chaga value at roughly \$50 per dried pound, while the Maine-based Chaga supplier MainelyChaga is currently vending dried Chaga conks for \$30 per pound. With a sum total of 7,285 cm³ of Chaga surveyed during this project, it is possible to convert the cubic centimeters to pounds with an estimated result of 17.22 pounds of Chaga surveyed in 2017-18. Even if this weight applied to dried conks (not the case in reality), and even if the Carpenter et al. value of \$50/dried pound were used for valuation, the resulting value of all Chaga surveyed in this project would amount to only \$861, which would be a comparatively miniscule value-added in even one stand given typical costs of harvest.

Conk variable results also pointed to a few clear trends with possible impact on future management. Conk size correlated negatively with the number of conks present on a given birch tree, thus signifying that a tree with more conks general means smaller conk volumes. In addition, white birch correlated positively with increased numbers of conks, though the greater frequency of Chaga presence in yellow birches would seem to make this correlation less important for management purposes. More important for understanding the potential of the Chaga resource for management and cultivation, however, is the negative correlation between number of Chaga conks and stand age: older trees can expect to see fewer, larger conks while younger trees can expect more smaller conks. This result is especially noteworthy for

management when taken into account with the species conk size data: white birch tends to be more prone to damage and stress effects than yellow birch, and thus tends to have a short lifespan (Burns et al., 1990).

Furthermore, there was association between species presence and stand age, with yellow birch experiencing a positive correlation with increasing stand age ($\chi^2 = 4.58$ at $p < .0323$). Thus, a convincing argument could be made that, if one is selecting areas for Chaga management, it is preferable to explore inventory data in order to locate stands that are both comparatively older in age (i.e. time since previous management) and have significant yellow birch basal area (for instance, if a stand has been site indexed for yellow birch). However, while this may indicate a preference of *I. obliquus* towards older trees, it does not prove any sort of causative relationship between individual tree age and number of conks present. There was also no clear assessment of whether overall Chaga volume differs between fewer large conks or several smaller conks, and the lack of correlation between conk size and tree species makes singling out birch species for possible management and cultivation tenuous at best until more research is performed.

2019 Harvest Experiment

While the presence, distribution, and ecology of the Chaga fungus in the WMNF has been thoroughly examined at this point, the harvest dynamics of Chaga have yet to be explored. However, the 2019 field season provides the opportunity to explore this third goal via the monitoring of any possible effects of harvesting Chaga conks on birch health and fungus regeneration. Toward this end, an experimental manipulation is proposed involving a random selection of fifteen Chaga-positive at random from the 2017-18 dataset, excluding trees cored in the 2018 field season. These trees will be revisited and 20 of them will undergo some form of

Chaga harvest using a hatchet (to match current harvesting technique). The remaining ten will act as controls. The harvested trees will receive the following treatments:

- Conk fully removed, “clean”: the Chaga conk will be removed using a handsaw, with minimal damage to the tree cambium.
- Conk fully removed, “rough”: in the process of conk removal, hatchet damage will be performed on the tree cambium in the form of five swift cuts, simulating fast, imprecise harvest technique.

Stand age, tree DBH, live crown ratio, and size of conks removed will all be recorded the time of harvest. These 15 trees will be monitored over four successive growing seasons in an ongoing study of the effect of Chaga harvest on conk production and on tree health, the last year of which two cores will be taken from each tree to examine effects on ring widths and wood production.

Future Studies

Beyond the 2019 harvest experiment, there are a number of follow-up projects that might be performed in order to fill remaining knowledge gaps about Chaga presence, biology, and ecology in the WMNF. First, there could be more focused research on grey and black birches. Grey birch was not sampled at all in this project and only 67 black birch trees were recorded, most in one stand sampled in early 2017. It is possible that the absence of these two species is circumstantial due to the growing conditions of many of the stands sampled (often higher elevation with shallow, drier soils more suitable for yellow birch) and perhaps as a result of lower black and grey birch concentrations in the WMNF as a whole, this absence still cannot be

ruled out. A future survey exploring species impact on Chaga presence in greater depth might specifically sample all birch species present in the WMNF, potentially including other birch species such as river birch (*B. nigra*).

There is also the issue of Chaga fungus presence in trees without visible conks. There is scant research on identifying Chaga presence without conks, fruiting bodies, or a canker present, as the fungus forms a column of white fungal rot that to the untrained eye is essentially indistinguishable from other white rot agents. A possible future project might seek to identify indicators of Chaga infection sans conk and concordantly create protocols for determining Chaga presence even in the absence of conks. There may be many times the number of trees with visible conks that are latently infected, and being able to ascertain this condition could fundamentally shift the understanding of how many trees truly carry Chaga in the WMNF.

A third type of study might use the survey protocol applied in this project, but for other variables. Understory composition was noted in the 2017-18 dataset, but results were sparse and nominal. A future survey could well examine understory plants in and around Chaga-positive trees, as well as taking soil samples near infected trees to examine potential soil effects. Sap flow monitoring of Chaga-present trees could provide information on how *I. obliquus* spreads its decay column through the tree, as well as how Chaga conk presence affects water movement through the tree. Light regimes of Chaga-positive trees may also be worth exploring.

Finally, a fourth option exists that would simply involve a modification of this study, but at entirely random points throughout the WMNF, not limited to those areas of 40% of greater birch species basal area. Birch trees can be found in many different ecosystems across the WMNF, thus making a more comprehensive, randomized sample a possible benefit to truly

parsing out possible environmental correlations with *I. obliquus* presence. Such a project would doubtless be a major undertaking in terms of time and labor invested, and would require a modification of the transect system to accommodate much more sparse concentrations of birch trees. However, the potential information to be gained about Chaga presence outside of birch heavy stands could have significant implications for the understanding of Chaga ecology and the crafting of BMPs for Chaga harvest and possible cultivation.

Conclusions

The Chaga resource in the WMNF is rare in incidence but wide in distribution, and can be wherever there are birch trees in general and yellow birch in particular. The resource is most closely associated with higher elevation, yellow birch dominated stands of comparatively advanced age, though it does not appear to otherwise associate with any other species presence or to tree damage apart from those that it directly promotes. Considering the wide range of the resource, the wide variance in size and number of conks, the remoteness of much of its habitat, its occasional presence outside of the reach of foragers, and the lack of much observed previous harvest in the stands sampled, it does not at this point appear that there needs to be a special permitting process for the resource in the WMNF.

However, information on how the Chaga resource and its host tree reacts to harvest is scant and requires exploration, and there are many other potentially correlating variables that could provide a much more in-depth perspective on the more minute details of Chaga presence in the WMNF. This project has shown where, in general, Chaga may be found in the WMNF, and what it tends to associate with when it is present. What is necessary now is to explore how Chaga responds to harvest, as well as potential reasons for why *I. obliquus* selects the hosts that it does.

This will provide the framework for informed, sustainable Best Management Practices for Chaga management and/or cultivation, which will ensure that this remarkable non-timber forest product can be utilized by all who seek it out well into the future.

V. REFERENCES

- Balandaykin, M. E., & I.V. Zmitrovich. (2015). Review on Chaga Medicinal Mushroom, *Inonotus obliquus* (Higher Basidiomycetes): Realm of Medicinal Applications and Approaches on Estimating Its Resource Potential. *International Journal of Medicinal Mushrooms*, 17(2): 95-104.
- Blanchette, R.A. (1982). Progressive stages of discoloration and decay associated with the canker-rot fungus, *Inonotus obliquus*, in birch. *Phytopathology*, 72: 1272-1277.
- Burns, R. M., & B.H. Honkala. (1990). *Silvics of North America*. Vol 2: U.S. Department of Agriculture, Forest Service: 298-309.
- Campbell, W.A., & R.W. Davidson. 1938. *A. poria* as the fruiting stage of the fungus causing sterile conks on birch. *Mycologia*, 30: 553-560.
- Carpenter, C., & R. Crawford. (2017). Chaga Birch Canker assessment conducted by Northeastern Area State and Private Forestry (NA S&PF) Staff in cooperation with University of New Hampshire for the National Forest System (NFS). Received by J.S. Barresi, K. Atkinson.
- Dufrêne, M.P. & P. Legendre. (1997). Species assemblages and indicator species: the need for a flexible asymmetrical approach. *Ecological Monographs*, 61: 345-366.
- Géry, A., C. DeBreule, V. Andre, J. Rioult, V. Bouchart, N. Heutte, P.E. de Pecoulas, T. Krivomaz, & D. Garon. (2018). Chaga (*Inonotus obliquus*), a Future Potential Medicinal Fungus in Oncology? A Chemical Study and a Comparison of the Cytotoxicity Against Human Lung Adenocarcinoma Cells (A549) and Human Bronchial Epithelial Cells (BEAS-2B). *Integrated Cancer Therapy*, 17(3): 832-843.
- Gilbertson, R.L., & L. Ryvarden. (1986, 1987). North American polypores. Vol. 1: *Albatrellus-Lindtneria*. Vol. 2: *Megasporaporia-Wrightoporia*. Fungiflora, Oslo: 885
- Ginns, J.H. (1986). A compendium of plant disease and decay fungi in Canada. Canadian Government Public Center, Ottawa: 416.
- Kim, Y.R. (2005). Immunomodulatory Activity of the Water Extract from the Medicinal Mushroom *Inonotus obliquus*. *Mycobiology*, 33(3): 158-162.
- Lee, M., H. Hur, K. Chang, T. Lee, K. Ha, & L. Jankovsky. (2008). Introduction to Distribution and Ecology of Sterile Conks of *Inonotus obliquus*. *Mycobiology*, 36(4): 199-202.
- Magnani, N. 2016. Reconstructing Food Ways: Role of Skolt Sami Cultural Revitalization Programs In Local Plant Use. *Journal of Ethnobiology*, 36(1): 85-104
- McCune, B., & J.B. Grace. (2002). *Analysis of Ecological Communities*. 3rd ed., MJM Software Design: 135-148, 188-204.
- Niemelä, T., P. Renvall, & R. Pentilla. (1995). Interactions of fungi in late stages of wood decomposition. *Annales Botanici Fennici* 32: 141-152.

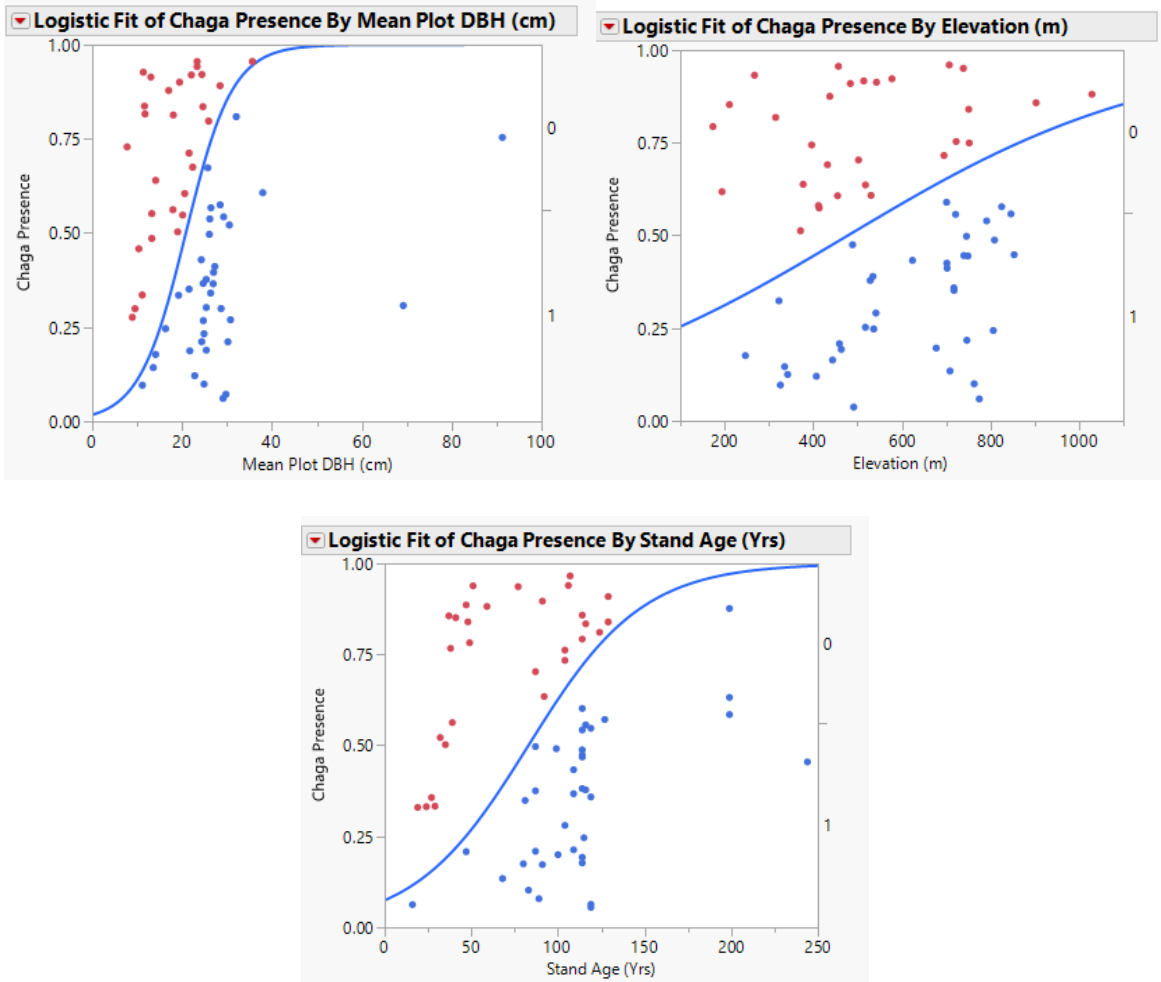
- Perevedentseva, L. (2013). Use of wild-growing mushrooms for therapeutic purposes in the Perm Territory, Russia. *Indian Journal of Environmental Health*, 2: 236-242
- Ryvarden, L., & R.L. Gilbertson. (1993, 1994). European polypores. Part 1: *Abortiporus-Lindtneria*. Part 2: *Meripilus-Tyromyces*. Fungiflora, Oslo: 743.
- Sinclair, W.A., & H.H Lyon. (2005). *Diseases of Plants and Shrubs*. 2nd ed., Cornell University Press: 314-316.
- Shigo, A. L. (1965). The Pattern of Decays and Discolorations in Northern Hardwoods. *Phytopathology*, 55: 648-652
- Shigo, A.L. (1969). How *Poria obliqua* and *Polyporus glomeratus* incite cankers. *Phytopathology*, 59: 1164-1165.
- Spahr, D.L. (2009). *Edible and Medicinal Mushrooms of New England and Eastern Canada*. 1st ed., North Atlantic Books: 185-190.
- Szczepkowski, A., J. Piętka, & A. Grzywacz. (2013). Biology and Medicinal Properties of the Chaga Mushroom *Inonotus obliquus* (Fr.) Pilat. *Sylvan*, 157(3), 223-233
- Szczepkowski, A., J. Piętka, & A. Grzywacz. (2013). Occurrence and Resources of Chaga mushroom *Inonotus obliquus* (Fr.) Pilát in Central and Eastern Poland and Problems of its Protection. *Sylvan*, 157(7): 483-494.
- True, R.P., E.H. Tryon, & J.F. King. (1955). Cankers and decays of birch associated with two *Poria* species. *Journal of Forestry*, 53: 412-415
- U.S. Agricultural Research Service, Systematic Botany and Mycology Laboratory. Fungal Databases. Beltsville, MD. Internet resource.
- Zabel, R.A. (1976). Basidiocarp development in *Inonotus obliquus* and its inhibition by stem treatments. *Forest Science*, 22: 431-437.
- Zhong, X. H., L.B. Wang, & D.Z. Sun. (2011). Effects of Inotodiol Extracts from *Inonotus obliquus* on Proliferation Cycle and Apoptotic Gene of Human Lung Adenocarcinoma Cell Line A549. *Chinese Journal of Integrative Medicine*, 17(3): 218-223.

APPENDICES

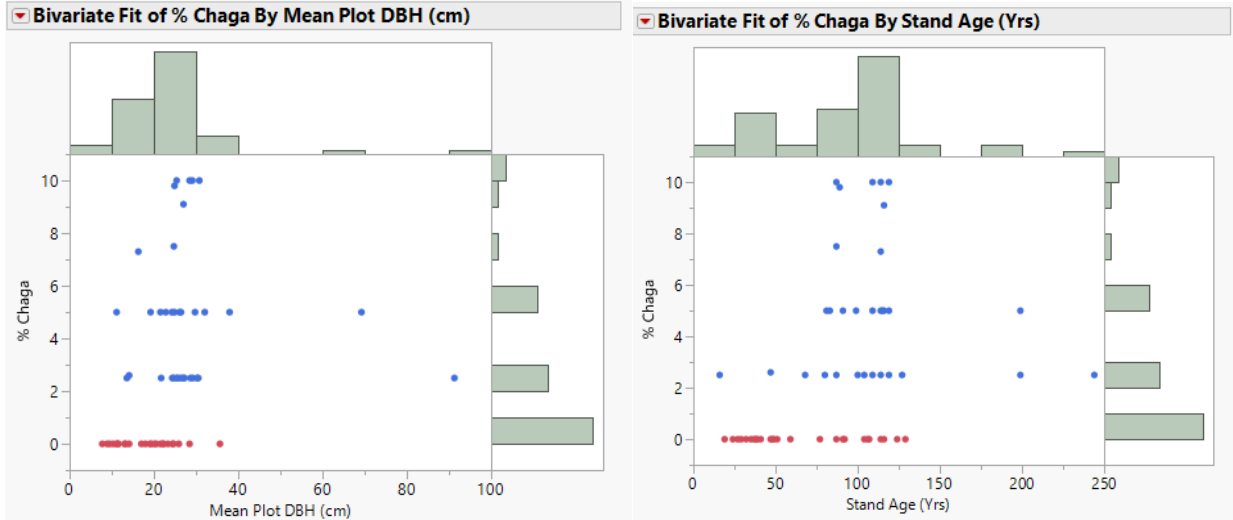
Appendix A: Plot Summary Data

Variable
Waypoint
Date of collection
Latitude/Longitude
Chaga Presence/absence (0/1)
Site Elevation (m)
Percent Slope (collected from 2009-2012 inventory data)
Stand Age (Years, from 2009-2012 data)
Mean Plot DBH (cm)
Total Number of Trees Surveyed
Number of Chaga-positive trees
Number of Chaga-negative trees
Percent Chaga presence in sampled trees

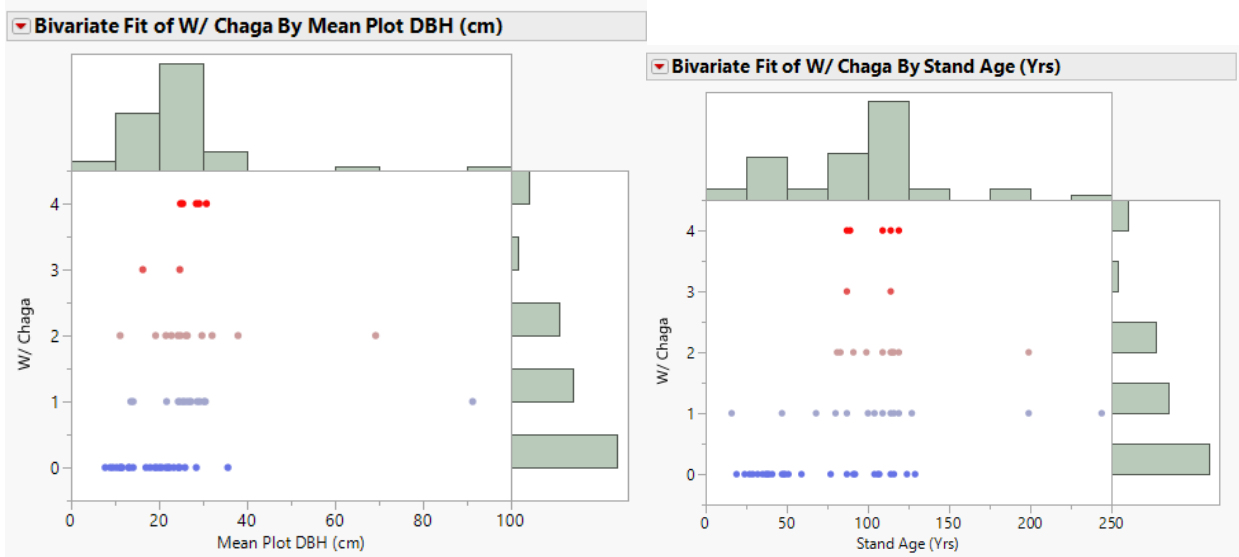
Table 4: Variables used for plot summary analyses



Logistic fits of Chaga presence and mean plot DBH (l), plot elevation (c), and stand age (r).



Bivariate fits of percent Chaga composition of sampled trees with mean plot DBH (l) and stand age (r)

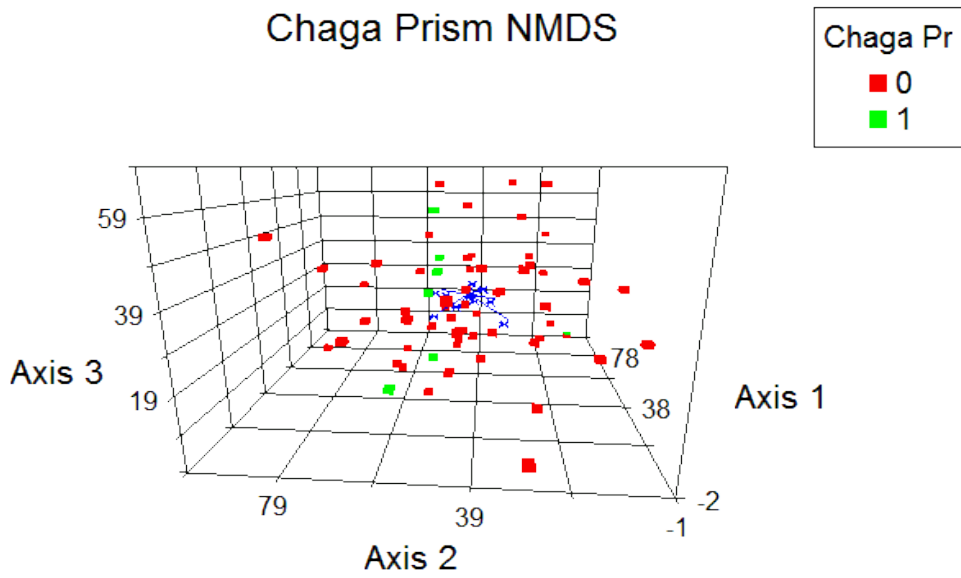
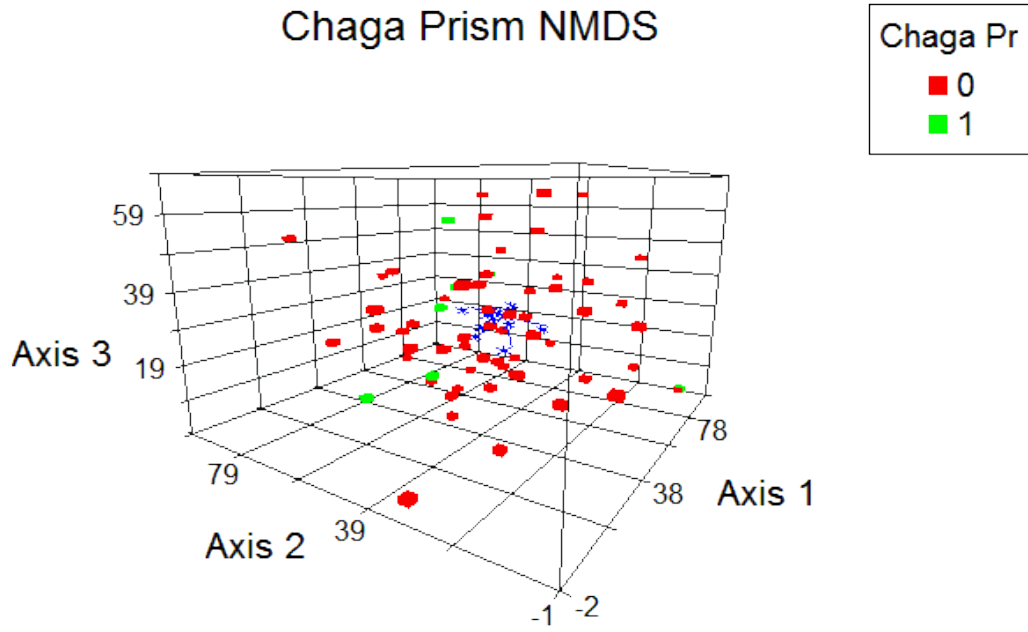


Bivariate fits of number of Chaga-positive trees per stand with mean plot DBH (l) and stand age (r)

Appendix B: Prism Plot Data

Tree Species	Species Code	Mean BA/prism plot (m ²)	Chi-square with Chaga presence	P-value of x ² (bold denotes significance)
White Birch	WB	3.5	1.15	<i>p</i> <.2833
Yellow Birch	YB	2.5	20.72	<i>p</i><.0001
Black Birch	BB	0.31	1.011	<i>p</i> <.3145
Red Spruce	RS	2.43	3.56	<i>p</i> <.0593
Balsam Fir	BF	.6	0.74	<i>p</i> <.3891
Red Oak	RO	.21	1.011	<i>p</i> <.3145
Eastern Hemlock	EH	.14	0.28	<i>p</i> <.5942
Green Ash	GA	.73	0.72	<i>p</i> <.39
Sugar Maple	SM	9.75	2.75	<i>p</i> <.0971
Red Maple	RM	7.35	3.13	<i>p</i> <.0768
Quaking Aspen	QA	.57	.72	<i>p</i> <.40
White Pine	WP	.73	0.86	<i>p</i> <.3527
White Ash	WA	.82	1.310	<i>p</i> <.2524
American Beech	AB	2.20	0.034	<i>p</i> <.85
Norway Maple	NM	.23	0.43	<i>p</i> <.5133
Black Cherry	BC	.39	0.42	<i>p</i> <.5133
Black Oak	BO	.07	0.14	<i>p</i> <.71
Black Spruce	BS	.09	0.14	<i>p</i> <.71
Striped Maple	STM	.39	0.72	<i>p</i> <.40

Table 5: correlations between Chaga presence and species basal area



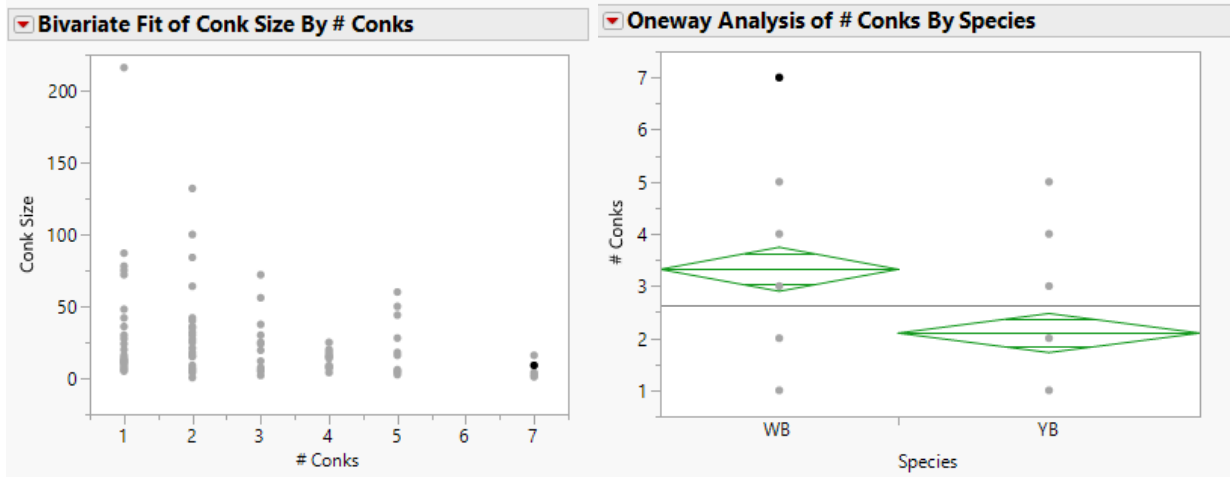
Three-dimensional NMDS graph for prism plot basal area data. Species joint plots are visible in center. Small lengths of joint plot vectors represent weak association of variables with ordination scores.

Appendix C: Tree Damage Data

Damage Code	Type of Damage	Contingency Chi-Square Value	P-value of Chi-square (bold denotes significance)
PI	<i>Phellinus igniarius</i>	0.032	$p < 0.8575$
MW	Mechanical Wound (i.e. human- or weather- related damage)	12.5	$p < 0.0004$
TC	Target Canker	46.75	$p < 0.0001$
GA	<i>Ganoderma applanatum</i> (Artist's Conk)	0.563	$p < 0.453$
FF	<i>Fomes fometarius</i> (Tinder Conk)	0.049	$p < 0.8243$
AD	Animal Damage (i.e. non-BBO insects, bear damage, etc.)	0.84	$p < 0.36$
WR	White Fungal Rot	25.219	$p < 0.0001$
BR	Brown Fungal Rot	0.126	$p < 0.7223$
BT	Broken Top	0.1865	$p < 0.1721$
BBO	Bronze Birch Borer (<i>Agrilus anxius</i>)	1.500	$p < 0.22$
BRL	Burl	0.192	$p < 0.66$
HU	Hang-Up (caught in sampled tree)	0.189	$p < 0.6641$
ER	Exposed Roots	2.962	$p < 0.2274$
BP	Birch Polypore (<i>Fomitopsis betulina</i>)	0.133	$p < 0.7155$
AM	<i>Armillaria mellea</i> (Honey Mushroom)	1.311	$p < 0.2522$
TV	<i>Trametes versicolor</i> (Turkey Tail)	8.865	$p < 0.0029$
UF	Unknown Fungus (unidentifiable)	0.026	$p < 0.8728$

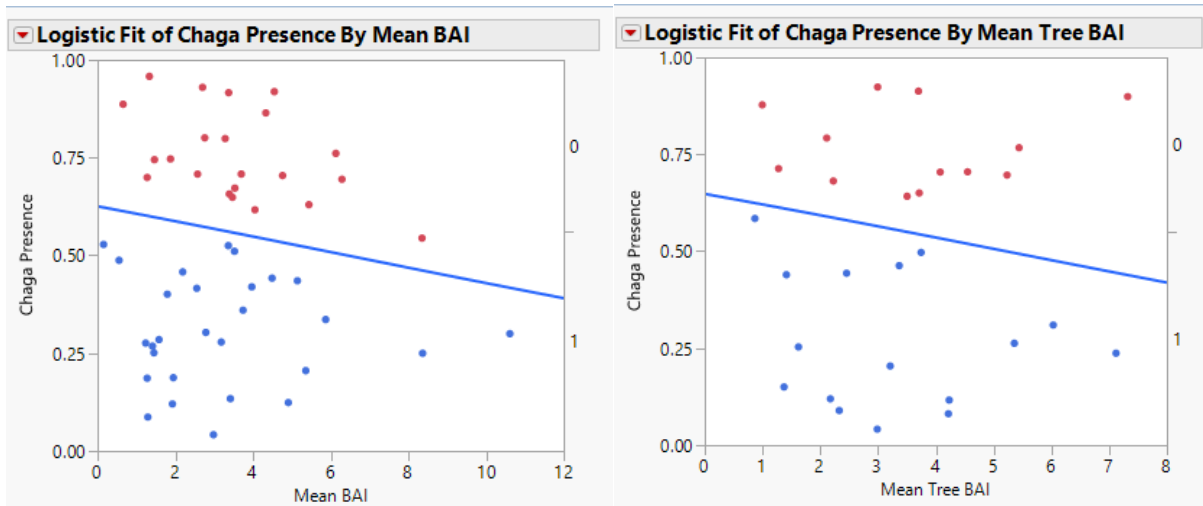
Table 4: Transect tree damages, with Chi-square contingency analyses values for Chaga presence/absence

Appendix D: Conk Data



Bivariate fit of number of conks per tree against conk size (l) and One-way ANOVA of number of conks by species (r)

Appendix E: Tree Core Data



Logistic regression analyses of Chaga presence by average core BAI (l) and average tree BAI (r).